

Bioactive Potential and Chromatographic Characterization of Body Mucus from Portugal Coastal Fish *Halobatrachus didactylus*

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The body mucus in fish provides a stable physical or chemical barrier against invading pathogens. In mucus, antimicrobial peptides are secreted as a response to immune stimulation. Studies have shown antimicrobial activity against multidrug-resistant pathogens and low toxicity to eukaryotic cells. Previously, body mucus from five captive *Halobatrachus didactylus* individuals was collected. We aim to explore the mucus studying molecules with bioactive potential. Size exclusion high-performance liquid chromatography (SE-HPLC) analyses were performed on the five body mucus samples showing a similar molecular size distribution with a maximum peak of ca. 800 Da. These five mucus samples were pooled to assess the following bioactivities: antioxidant (ABTS and ORAC), antimicrobial (minimal inhibitory concentration), and cytotoxicity (Caco-2 and HaCaT human cell lines). The protein content in the mucus, determined by the bicinchoninic acid methodology, was 16836 ± 1020 μ g BSA/mL. The antioxidant activity resulted in 268 ± 11 μ mol TE/g mucus protein for ABTS and 306 ± 11 μ mol TE/g mucus protein for ORAC. The antibacterial activity was assessed against five pathogenic bacteria: *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, within minimal inhibitory concentrations of 421 to 105 μ g mucus protein/mL. Moreover, the mucus showed non-cytotoxic for Caco-2 cells in concentrations between 196 to 25 μ g mucus protein/mL, while it showed cytotoxicity for HaCaT cells. In the future, liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis will be performed to determine the molecules behind these bioactivities, namely antimicrobial peptides.