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Sustainable Bioprospecting and Circular Economy Approach: Fractionation and Identification of Bioactive Peptides from Lusitanian Toadfish (*Halobatrachus didactylus*) Mucus

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Bioactive molecules from marine organisms, especially the *Halobatrachus didactylus*, also known as the Lusitanian toadfish, native to the Portuguese coast, remains largely unexplored. It is important to use sustainable bioprospecting practices to protect both the species and its ecosystem (1). Therefore, by adopting a circular economy approach, we can maximize the sustainable use of marine resources by sampling fish in a non-harmful way (e.g. mucus collection) and returning them to their natural habitat, thus allowing these organisms to thrive in their ecosystems. Fish mucus acts as a natural barrier from the surrounding environment, acting as a first line of defense, for instance, against pathogens (2,3). In this study, we carefully collected *H. didactylus* mucus using a synthetic sponge to ensure no physical harm to the fish and to minimize stress by reducing handling time. We evaluated the potential bioactivities of the body mucus and characterized its peptide fraction composition.

The body mucus of *H. didactylus* exhibited promising antioxidant, antihypertensive, and antimicrobial activities (4). The body mucus peptide profile was obtained using size exclusion chromatography, revealing a prominent peak ca. 800 Da. Using LC-MS/MS, we identified several peptide sequences from the mucus peptide fraction and selected five of them based on their predicted bioactivities *in silico.* We then validated the bioactivities through *in vitro* assays. Peptides with the amino acid

sequences EDNSELGQETPTLR (HdKTLR), DPPNPKNL (HdKNL), PAPPPPPP (HdPPP) and VYPFPGPLPN (HdVLPN) showed antibiofilm potential against *P. aeruginosa*. HdVLPN and PFPGPLPN (HdLPN) exhibit significant antioxidant activity, while HdPPP demonstrates antihypertensive and antidiabetic activities.

In conclusion, this study not only underscores the therapeutic potential of *H. didactylus* peptides but also serves as a model for sustainable bioprospecting practices that align with circular economy principles.

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Keywords: fish mucus, peptide fraction, sustainable bioprospecting, antioxidant activity, renewable resources

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Marta Fernandez Cunha is a PhD student in Biotechnology at the Faculty of Biotechnology, Portuguese Catholic University. She holds a Bachelor's degree in Biology-Geology and a Master's in Plant Molecular Biology, Biotechnology, and Bioentrepreneurship from the University of Minho. Since 2021, Marta has been a researcher at the Bioactives and Bioproducts Research Laboratory at the CBQF. Marta has been involved in the international project "FISHMUC" in collaboration with Macau, through which she has published research articles and presented her findings at international conferences. She also completed a one-month internship in Macau, further expanding her research experience. Her PhD work focuses on discovering novel bioactive molecules, using nature as an inspiring model. Through her research, she aims to expand shared knowledge within the scientific community and contribute to enhancing public health.