

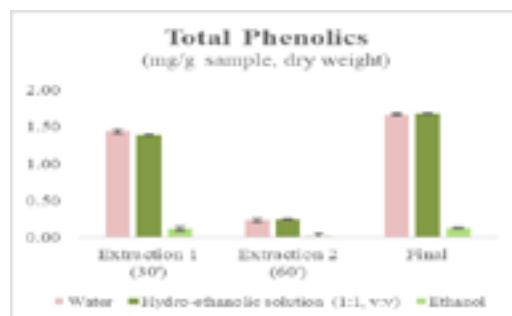
TOTAL PHENOLICS OF *GRACILARIA VERMICULOPHYLLA*: OPTIMIZING EXTRACTION PROCEDURES USING GREEN METHODOLOGIES

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Seaweeds are an important source of natural compounds with recognized health benefits. One of the world's most cultivated and valuable seaweed is *Gracilaria*. This species is mainly cultivated and harvested to extract phycocolloids, providing more than 50% of the world's supply of the agar used by the food and cosmetic industries. The interest in this species goes beyond this, taking into account its composition in secondary metabolites with biological activity, which include phenolics. Overall, these compounds are presumed to protect algal thalli from UV radiation and to act as free-radical scavenging agents. This study aimed to optimize an extraction protocol, using green chemistry principles, for further quantification of the total phenolics of *G. vermiculophylla*. The dried samples, produced in an Integrated Multi-Trophic Aquaculture (IMTA) system (Aveiro, Portugal), were thoroughly ground and homogenized prior to analysis. Samples were submitted to several extraction conditions in which the ratio of sample:solvent:time of extraction were tested, varying one parameter at a time. The extraction procedure was optimized using water as the extraction solvent in the following ratios: 1:30, 1:40 and 1:50 (w:v), during 30, 60, 90 up to 120 minutes, at room temperature. Some of the protocols comprised re-extractions of the samples every 30 minutes, while in others the extraction was continuous for the stipulated period of time. Once the optimum extraction conditions were reached, the same protocol was applied to the samples, using two other solvents: an hydro-ethanolic solution (1:1, v:v) and ethanol. Results of the optimization protocols using water as the extraction solvent show that the total phenolics (TP) varied between 0.88 ± 0.05 and 1.66 ± 0.03 mg/g sample (dry weight). The higher amounts of TP were obtained at a ratio of 1:40 (w:v) for 30 minutes with an additional 30 minutes re-extraction with $\frac{1}{4}$ of the total volume. Continuous extractions did not present advantages over protocols comprising re-extractions over the time. In fact, in most cases, a slight decrease of the TP is observed over time, probably due to the degradation of the compounds. Comparing the water and the hydro-ethanolic solution, in the first extraction, water was more efficient, but, at the end of the process, there were no significant differences between the amounts of TP in both solvents (1.66 ± 0.03 and 1.68 ± 0.03 mg/g sample dw, respectively; $p < 0.05$). Ethanol was the least efficient solvent to extract TP (0.12 ± 0.0 mg/g sample dw). The present work was designed

to investigate the optimum extraction protocol of the TP of *G. vermiculophylla*, using green chemistry principles. The optimum conditions were selected after combining the following parameters: the recovery of the maximum amount of TP, using the eco-friendliest solvent at the lesser amounts possible, during the shortest period of time. In this case, this conducted us to select water as the extractor solvent, using the ratio of 1:40 (w:v) for 30 minutes with an additional 30 minutes re-extraction with $\frac{1}{4}$ of the total volume. This process allows the recovery of natural seaweed-derived antioxidants, which can be safely used for food and cosmetics applications.



BIOGRAPHY

Filipa B Pimentel is a PhD student in Pharmaceutical Sciences (Nutrition and Food Science Speciality) at the Faculty of Pharmacy of the University of Porto, Portugal. Since 2012, she is a researcher of REQUIMTE (Rede de Química e Tecnologia), the largest network in Chemistry and Chemical Engineering established in Portugal, which is focused on the development of Sustainable Chemistry. Her research activities have been developed at the Department of Chemical Sciences of the Faculty of Pharmacy of the University of Porto in the food chemistry and nutrition fields. She has 21 publications, cited over 150 times. Formerly, she completed her degree in Nutrition Sciences at the Faculty of Nutrition and Food Sciences of the University of Porto in 2004, and a Master in Food Service Management from the same Faculty in 2011.

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