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Proceeding Paper

Characterization of *Codium tomentosum* Phytosomes and Their Neuroprotective Potential †

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Abstract: Due to the increase in life expectancy, promoting active aging is a crucial challenge for the XXI century. Therefore, searching for new neuroprotective drugs is urgent, and marine macroalgae are considered excellent sources of structurally diverse bioactive molecules with recognized pharmaceutical and biomedical potential. Aiming to contribute to finding new neuroprotective drugs, a multi-step subcritical water extraction (SWE) process was applied to the green macroalga *Codium tomentosum* using a gradient of temperatures (from room temperature to 250 °C). Four fractions were obtained and fraction F4 (obtained in the range of 190–250 °C) was the most active against oxidative stress and enzymes linked to neurodegeneration and major depression. Therefore, phytosomes were prepared with F4 for a future food application. A Box-Behnken design with three independent variables was applied and the phytosomes prepared using the optimal conditions were further modified with DSPE-PEG(2000)-maleimide and APoE and characterized by dynamic light scattering (DLS), UV spectrophotometry, octanol-water partition coefficient (Kow), differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR). Results demonstrated that the complex was successfully formed and displayed low particle size and high octanol-water partition coefficient.

Keywords: Box-Behnken; *Codium tomentosum*; phytosomes; seaweeds

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1. Introduction

Codium tomentosum is a green macroalgae with neuroprotective potential. Silva et al. [1] reported that several fractions obtained from the methanolic and dichloromethane extracts of *C. tomentosum* displayed neuroprotective activity in SH-SY5Y cells exposed to the neurotoxin 6-hydroxydopamine (6-OHDA). The authors also investigated which mechanisms were triggered by these fractions, concluding that they mitigated reactive oxygen species (ROS) generation, counteracted mitochondrial dysfunctions and DNA damage, and reduced caspase-3 activity. Recently, a fraction obtained by subcritical water extraction (SWE) in the range of 190–250 °C (F4) was tested against several brain enzymes involved in the etiology of neurodegenerative and neuropsychiatric disorders, namely, cholinesterases, monoamine oxidase A and B and tyrosinase, revealing good inhibition. The same fraction also demonstrated to be a strong superoxide anion radical ($O_2^{\bullet -}$) and nitric oxide radical ($^{\bullet}NO$) scavenger [2].

In order to counteract the low absorption of bioactive phytocompounds, nanosized drug delivery systems, such as phytosomes, can be used to enhance their penetration across biological barriers, such as the blood-brain barrier [3]. Phytosomes are innovative lipid-based nanocarrier that have liposomes-related structure but with some additional

advantages. While hydrophilic biomolecules are just entrapped in the aqueous core of liposomes without chemical bonds being formed, in phytosomes, H-bonds are established between the extracts and the phosphate group of phospholipids, such as phosphatidylcholine, increasing their stability [3].

This study aimed to extend the study developed by Soares et al. [2] by designing and characterizing phytosomes containing the SWE F4 of *C. tomentosum* for its future application in a neuroprotective functional food product suitable for senior citizens, which are prone to develop neurodegenerative and neuropsychiatric disorders. This fraction is rich in phenolic compounds and Maillard reaction products, both classes of compounds with recognized neuroprotective properties.

2. Materials and Methods

2.1. Samples and Extraction

C. tomentosum was produced in an Integrated multi-trophic aquaculture system and supplied in dried form by ALGAplus (Ílhavo, Portugal). The samples were hydrated for 5 min in salted water (35 g NaCl/L) and then washed in ultrapure water to eliminate NaCl. Then, seaweeds were dehydrated at 41 °C (Excalibur, model 4926T, Dublin, Ireland) for 18 h and ground to obtain particles in the 1–2 mm range. The SWE was carried out with c.a. 20 g of sample, at a constant pressure of 100 bar and a constant flow rate of 10 mL/min. Four fractions were obtained: F1—room temperature to 90 °C; F2—90 to 140 °C; F3—140 to 190 °C; and F4—190 to 250 °C. After the extractions, the fractions were liophylized until further use [2].

2.2. Box-Behnken Factorial Design

A Box-Behnken design with three independent variables [A—time (1—4 h), B—temperature (25–60 °C) and C—ratio F4:phosphatidylcholine (1:1–1:4)] was applied to obtain the best conditions to maximize the phytosome complex formation. Fifteen runs were performed, considering 3 central points. The software Design Expert (version 11, Stat-Ease Inc., Minneapolis, MN, USA) was used for experimental design, data analysis and model building. After determining the model equation, a Derringer's desirability function was used to maximize the modelled responses.

2.3. Phytosome Characterization

The phyotosmes produced using the optimal conditions of temperature, time and ratio F4:phosphatidylcholine were further functionalized with ApoE and DSPE-PEG(2000)-maleimide. Afterwards, they were characterized by dynamic light scattering (DLS), UV spectrophotometry, octanol-water partition coefficient (Kow), differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR), according to established procedures [4–7].

3. Results and Discussion

3.1. Box-Behnken Design

The entrapment efficiency of fraction F4 varied according to the set of conditions tested, ranging from 25.2% (A—1 h, B—42.5 °C and C—1:4) and 45.7% (A—2.5 h, B—25 °C and C—1:4; and A—1 h, B—60 °C and C—1:2.5). A reduced cubic model was fitted to the experimental results and two optimal conditions were retrieved from the model to obtain the highest % of complexation: condition 1 (A—1 h, B—59 °C, and C—1:1) and condition 2 (A—4 h, B—25 °C, and C—1:4) achieving 61.76 and 57.63%, respectively.

3.2. Characterization of Phytosomes

Phytosomes were functionalized prior to their characterization. Concerning their size and polydispersity index, phytosomes produced through condition 1 showed to be lower

and less polydisperse than those produced through condition 2 (245.76 ± 49.00 nm and PDI = 0.26 ± 0.06 vs. 261.02 ± 70.46 nm and PDI = 0.32 ± 0.02). Therefore, condition 1 was selected for further characterization.

The study of the solubility in water and in n-octanol of fraction F4 and of the phytosomes produced showed that the isolated extract had high aqueous solubility (95%) and low solubility in n-octanol (5%), which would be expected due to the highly aqueous character of the extract. On the other hand, the complexation of the extract with phosphatidylcholine conferred an amorphous character to the system and moderated its lipophilic character, improving the solubility in n-octanol and dissolution profile. In this way, an increase in lipophilicity to 28% was observed for the phytosomes.

The UV spectra of fraction F4 and phytosomes were analyzed in the range of 200–700 nm. A characteristic absorption peak of fraction F4 (280 nm) remained present in that of the phytosome, suggesting that weak physical interactions between the extract and L-*α*-phosphatidylcholine occurred during the formation of the complex.

The differential calorimetry scans of fraction F4, L-*α*-phosphatidylcholine and phytosome were also assessed. The extract exhibited a first endothermic peak at 49.2 °C characteristic of a first heat absorption, at 377.5 °C it exhibited a sharp and high intensity melting peak, followed by rapid decomposition at 500 °C. L-*α*-phosphatidylcholine presented three low intensity, broad and diffuse endothermic peaks, the first peak at 249.5 °C which is indicative of the melting of the polar region of the phospholipids. The last two peaks at 355.8 °C and 391.4 °C aroused due to the thermal melting of the hydrophobic tails of the phospholipids leading to the phase transition from gel to liquid crystalline state [5,6]. The phytosomal complex did not present any of the characteristic peaks of fraction F4 and of L-*α*-phosphatidylcholine, presenting a new endothermic peak of very low intensity at 462.5 °C. In addition, the sharp melting peak of fraction F4 disappeared, indicating its amorphization and the appearance of new hydrogen bonds between the -OH groups of the phenolic rings of the fraction F4 with the phospholipid [5,6].

The possible interaction between fraction F4 and L-*α*-phosphatidylcholine in the phytosome was also studied by FTIR, showing different patterns among isolated extract, L-*α*-phosphatidylcholine and phytosomal complex (Table 1).

Table 1. Major FTIR bands recorder [4–7].

	Typical Stretching Bands					
	-(OH)	**C-H**	**C=O**	**C-O-C**	**P=O**	**C-O**
F4 fraction	x	x	x	x		
L-*α*-phosphatidylcholine	x	x	x		x	
Phytosomal complex	x	x	x			x

4. Conclusions

Phytosomes complexed with a neuroprotective seaweed fraction was successfully produced. Results of the FTIR and DSC studies confirmed the phyto-phospholipid complex formation, and DLS analysis revealed that phytosomes had low particle size and polydispersity. Moreover, the octanol-water partition coefficient was higher for the complex than for the isolated fraction F4. Future work will cover the potential use of these phytosomes to prepare functional foods for elderly.

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Conflicts of Interest: The authors declare no conflict of interest.

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