Novel mixtures of bioactive compounds from Black Sea sources of fish skin and green seaweed with wound healing properties

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Fish processing industry is responsible for high amounts of solid waste. About 50% of fish tissues including fins, heads, skin, and viscera are discarded causing environmental and economic issues. However, all these by-products represent a valuable source of proteins and lipids (Jafari, 2020). The European bass (*Dicentrarchus labrax*) is found in the North East Atlantic Ocean and the Mediterranean and Black sea, and has a high commercial value from both wild catches and aquaculture production. On the other hand, the green seaweed *Cladophora vagabunda*, which is abundantly found along the Romanian Black Sea shore, especially during the warm season is rich in fatty acids, terpenoids, sterols, phenols, carbohydrates, vitamins and minerals (Horincar *et al* 2014, Sirbu *et al* 2020). The aim of this study was to valorize the discarded skin of European bass and the biomass of *C. vagabunda* as new sources of collagen and bioactive sulfated polysaccharides, respectively, to be further used in the development of wound care products.

The skin of sea bass captured in the Black Sea was provided by local fisheries after filleting the fish. Collagen type I was extracted by incubation in 0.5 M acetic acid solution, at 4 °C, for 48 h and purified by salt precipitation with 2.4 M NaCl. Ash and moisture content of bass collagen (BSSC) was analyzed by weighing the residues obtained at 600 °C and 120 °C, respectively. Hydroxyproline (Hyp) content was determined using specific commercial kit. The purity of fish collagen extract was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Bovine collagen (BC) standard was migrated in the same gel for comparative studies.

Sulfated polysaccharides were obtained from *C. vagabunda* by hot water extraction in a Soxhlet equipment. Methanol and acetone solvents were successively used, in order to remove polyphenols and chlorophyll. Purification of sulfated polysaccharides extract was performed by precipitation with 75% ethanol (PzF75). The total hexose content was analysed by anthrone method, the uronic acid level was estimated by orcinol reaction and sulfate content was measured by barium chloride assay (Moldovan *et al* 2018, Sakthivel and Devi, 2015). The lipoxygenase inhibition assay was performed in the presence of different concentrations of PzF75 using linoleic acid as specific substrate.

Mixtures of collagen-sulfated polysaccharides extract (BSSC-PzF75) were prepared in weight ratios of 2:1 and 1:1 (w/w), and allowed to cool at room temperature, to form a gel structure. The cytocompatibility of the samples was assessed *in vitro* on fibroblasts from NCTC clone 929 cell line by extract method, according to SR ISO 10993-5. Different concentrations of each sample extract in the culture medium were added to the adhered cells and incubated in standard conditions for 24 h. Cell viability was assayed using MTT test. *In vitro* scratch assay was performed in a cell monolayer using a pipette tip and cultivation in the presence of each sample extract, for 24 h. ImageJ software was used to calculate the wound closure rate.

Fish collagen extract presented similar values of ash, moisture and Hyp content, as those of bovine collagen standard (Table 1). The migration profiles obtained by SDS-PAGE showed that BSSC presented a doublet corresponding to α_1 and α_2 bands, similar to BC type I. BSSC also displayed several bands above 250 kDa, corresponding to β dimer and γ trimer of collagen chains.

The purified fraction of *C. vagabunda* polysaccharides contained variable proportions of hexoses and uronic acids, characterized by a high amount of sulfate. PzF75 exhibited significantly ($p<0.05$) higher inhibition of lipoxygenase activity with a calculated value of IC_{50} of 427.36 µg/mL, compared to ascorbic acid, used as control (638.55 µg/mL), suggesting its anti-inflammatory potential.

Table 1. Chemical composition of European sea bass collagen extract (BSSC) and *C. vagabunda* polysaccharides fraction (PzF75)

BSSC extract		PzF75 fraction	
Compound	g/100 g dry weight	Compound	g/100 g dry weight
Ash	1.02 ± 0.12	Hexoses	39.77 ± 1.55
Moisture	97 ± 2.91	Uronic acids	26.67 ± 2.96
Hyp content	6.34 ± 0.21	Sulfate	29.44 ± 0.18

In vitro tests in a culture of mouse fibroblasts showed that BSSC-PzF75 variants were cytocompatible in a wide range of concentrations between 100–1500 µg/mL, after 24 h of cultivation. Both samples stimulated the cell metabolism, at concentrations of 375 and 750 µg/mL, as observed from the significantly higher values of cell viability (121.93% and 137.58% respectively), compared to those of untreated control (100%). Phase contrast images taken at the end of the scratch assay revealed that the cells cultivated in the presence of BSSC-PzF75 variants migrated to a higher extent into the created gap, compared to the untreated cells.

In conclusion, the discarded skin of Black Sea European bass represents a valuable source of collagen with similar structural properties to those of mammalian collagen. In addition, the mixtures of bass collagen and bioactive sulfated polysaccharides from *Cladophora vagabunda* seaweed could be useful ingredients for new wound dressings applicable in skin tissue engineering.

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