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

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Introduction

The aim of this study was to valorize the discarded skin of European bass and the biomass of *Cladophora vagabunda* as new sources of collagen and bioactive sulfated polysaccharides, respectively, to be further used in the development of wound care products.

Research methods

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, moisture and hydroxyproline (Hyp) content was determined for bass collagen (BSSC). 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, followed 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. The lipoxygenase inhibition assay was performed in the presence of different concentrations of PzF75 using linoleic acid as specific substrate.
Mixtures of fish collagen-sulfated polysaccharides extracts (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 *in vitro* cytocompatibility of the samples was assessed on fibroblasts from NCTC clone 929 cell line by MTT test, according to ISO 10993-5. *In vitro* wound healing was investigated in L929 cell monolayer with a scratch made using a sterile pipette tip and cultivation in the presence of each mixture, for 24 h. ImageJ software was used to calculate the wound closure rate.

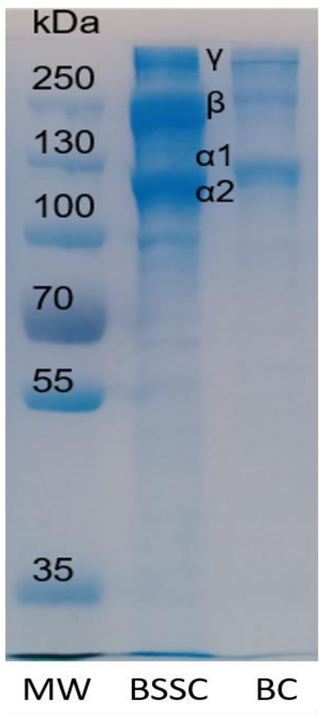
Results & Discussion

Chemical composition of bass collagen and seaweed polysaccharides

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

Chemical analysis showed that marine collagen extract contained 6.34% Hyp, a specific amino acid, also found in terrestrial collagen. Seaweed PzF75 fraction contained 39.77% hexoses and 26.67% uronic acids. Also, sulfated PZ were found.

SDS-PAGE of European bass collagen



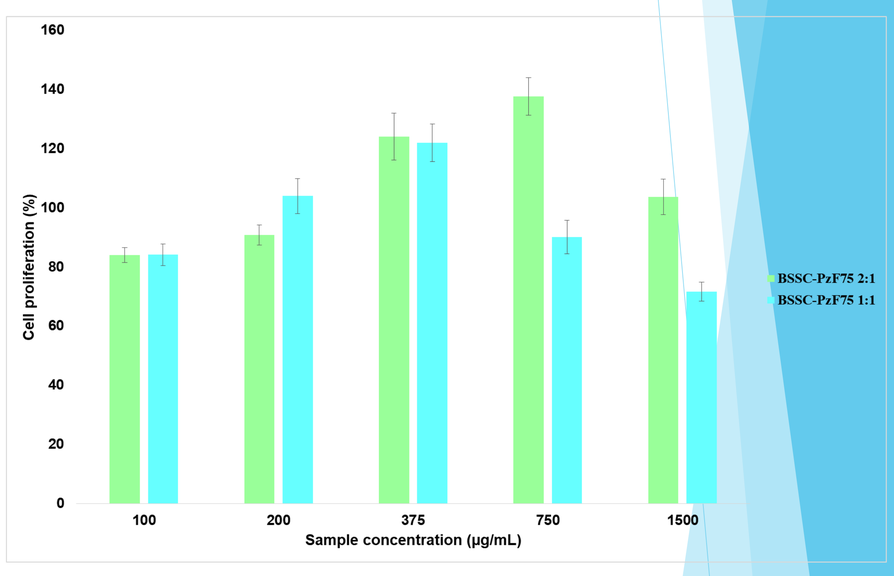
The migration profiles obtained by SDS-PAGE showed that fish collagen (BSSC) presented a doublet corresponding to α_1 and α_2 bands, similar to bovine collagen type I (BC). BSSC also displayed more pronounced bands above 250 kDa, corresponding to β dimer and γ trimer of collagen chains.

Anti-inflammatory potential of *C. vagabunda* polysaccharides

Lipoxygenase inhibition assay		
	PzF75 fraction	**Ascorbic acid**
IC_{50} (µg/mL)	427.36 ± 32.1	638.55 ± 42.98

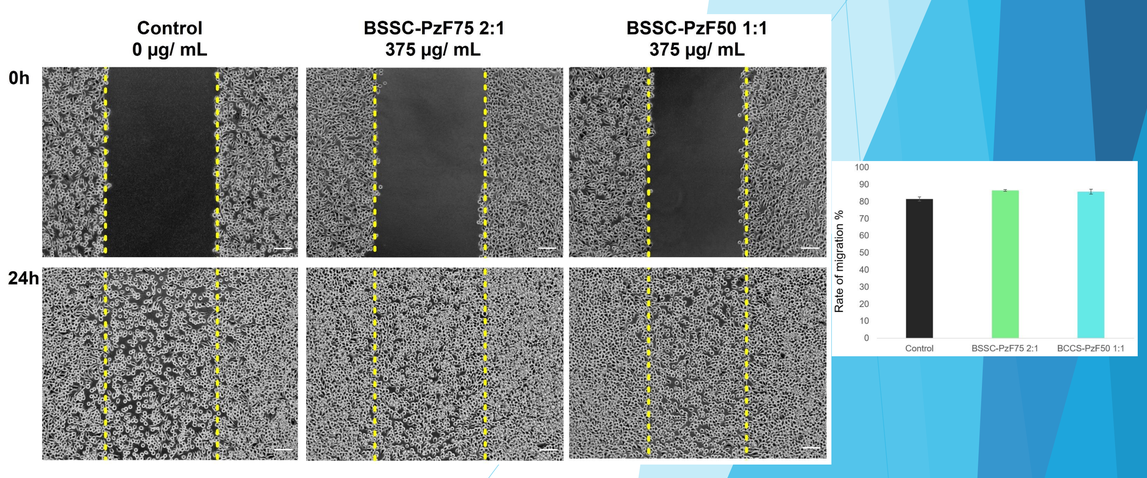
The seaweed PzF75 fraction exhibited higher inhibitory effect on soybean lipoxygenase activity compared to that of ascorbic acid, suggesting high anti-inflammatory potential.

In vitro cytocompatibility of BSSC-PzF75 mixtures



In vitro tests in a culture of L929 fibroblasts showed that BSSC-PzF75 mixture variants were cytocompatible in a wide range of concentrations between 100–750 µg/mL, after 24 h of cultivation. The variant BSSC-PzF75 2:1 stimulated L929 cell metabolism, at concentrations of 375 and 750 µg/mL, as showed by significantly higher values of cell viability (124.02% and 137.59%, respectively), compared to those of untreated control (100%). Similar, the variant BSSC-PzF75 1:1 increased cell growth up to 103.91% and 121.93% at concentrations of 200 and 375 µg/mL, respectively.

In vitro wound healing potential of BSSC-PzF75 mixtures



Phase contrast images and quantitative data of migration rate revealed that L929 cell incubation with mixture variants BSSC-PzF75 could stimulate cell migration into the created gap in a higher extent, compared to the untreated cells.

Conclusions

- The discarded skin of Black Sea European bass represents a valuable source of collagen with similar structural properties to those of terrestrial collagen.
- The polysaccharidic extract from *C. vagabunda* seaweed presented anti-inflammatory potential.
- The bioactive mixtures of BSSC-PzF75 stimulated L929 fibroblasts metabolism, growth and migration, revealing wound healing potential and recommending their use as ingredients of novel skin wound dressings.

Acknowledgment: This work was supported by a grant of the Romanian Ministry of Research, Innovation and Digitization, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2021-0114, within PNCDI III.