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Introduction

Extensive work has been done in the field of determining bioactivity of marine organisms with a tendency towards its application in various industries, such as food, pharmaceutical, and cosmaceutical industries. Polysaccharides with marine origin have already proved to have numerous properties beneficial for human health. These properties depend on the species, but also various biotic and abiotic characteristics of the ecosystem. The extreme variety regarding abiotic conditions of the Adriatic sea has forced marine organisms to develop unique biochemical and physiological properties, thus representing a valuable source of novel bioactive compounds. This study was designed to evaluate the antioxidant activity of polysaccharide fractions from five brown macroalgae Cystoseira barbata, Cystoseira compressa, Fucus virsoides, Halopteris scoparia, and *Padina pavonica*. The highest protective effect against H₂O₂-induced oxidative stress in zebrafish embryos was observed during exposure to Cystoseira and Halopteris species (fluorescence intensity decreased from 38.97% (for C. *barbata*) up to 60.27% (for *H. scoparia*), comparing to the group on H_2O_2), which corresponds well with the high amount of polysaccharide groups. Tested polysaccharide fractions showed no embryotoxic, cardiotoxic and/or neurotoxic potential. Collectively, the results obtained suggest that polysaccharides isolated from the Adriatic Sea macroalgae might be a potent source of natural antioxidants.

Goals

- \triangleright evaluate the toxic potential and novel *in-vivo* antioxidant activity determination of polysaccharide fractions
- > develop a robust platform for the high-throughput screening of novel marine compounds with antioxidant properties

Methods

Five brown macroalgae were sampled – C. barbata, C. compressa, F. virsoides, H. scoparia and P. pavonica



Algal fraction + H₂O₂ **Protection against ROS** \rightarrow

Results

Tested polysaccharide fractions did not show embryotoxic, cardiotoxic, or neurotoxic potential. In order to examine the protective effect of macroalgal polysaccharide fractions on survival rate, zebrafish embryos were exposed to polysaccharide fractions in the presence and absence of 5 mM H_2O_2 . A significant increase in mortality was observed upon exposure to 5 mM H_2O_2 (66.25 ± 4.79%), compared to the non-treated control group (0.00%; Fig. 1). Pretreatment with C. compressa and H. scoparia fractions significantly reduced the mortality rate at all tested concentrations (0.25-1.00 mg/mL). Pre-treatment with *C. compressa* fraction reduced mortality by 23.75% at 0.25 mg/mL (p = 0.0086), 33.75% at 0.50 mg/mL (p = 0.0007) and by 32.92% at 1.00 mg/mL (p = 0.0015), relative to individuals treated with 5 mM H_2O_2 . Pre-treatment with H. scoparia caused a similar effect, with a more pronounced reduction in the pre-treatment mortality rate of 1 mg/mL (36.25%, p < 0.0001).

Fractions of brown macroalgae C. barbata, C. compressa and H. scoparia showed a protective effect on the occurrence of oxidative stress induced by H₂O₂. C. barbata fraction (1.00 mg/mL) reduced the fluorescence intensity by 38.97% compared to the positive control (p = 0.0232; Fig. 2). C. compressa fraction (1.00 mg/mL) reduced the fluorescence intensity by 57.10% (p <0.0001). *H. scoparia* fraction significantly reduced the fluorescence intensity at all tested concentrations. At 0.25 mg/mL the fluorescence intensity decreased for 42.51%, while at 1.00 mg/mL decreased for 60.28% (p <0.0001).





Fig 1. Effects of polysaccharide fractions on the survival rate in H_2O_2 -treated zebrafish.



Fig 2. In vivo evaluation of antioxidant potential of polysaccharide fractions. ROS production is visualized with DCF-DA staining. a) artificial water, b) H₂O₂, c) C. barbata, 1 mg/mL, d) C. compressa, 1 mg/mL, e) H. scoparia, 1 mg/mL.

Conclusion

The highest antioxidant activity was recorded during embryos exposure to C. barbata, C. compressa and H. scoparia fractions, which manifested in a decrease of fluorescence intensity relative to the positive control (H_2O_2) was observed. This research showed that the investigated Adriatic sea macroalgae represent a potent source of natural antioxidants that could be sustainably utilized in industrial applications. These have created excellent preconditions for the development of functional products and dietary supplements based on algae, individually or even by combining different species.

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