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Anti-Inflammatory Activity of *Sargassum cristaefolium* Lipid Fractions: Experimental Evidence and Structure-Based Insight into Cellular Uptake

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Abstract

Brown seaweed is widely recognized for its health-promoting properties, including anti-inflammatory and antioxidant activity. Brown seaweed-derived lipophilic compounds are considered key contributors to these biological effects. This study highlighted the bioactivity of lipid extracts and fractions from *Sargassum cristaefolium*, complemented by a structure-based evaluation of their potential cellular permeability. Crude lipids extracted from S. *cristaefolium* were fractionated using normal-phase column chromatography into dichloromethane (DCM), acetone (ACT), and methanol (MeOH) fractions. Among these, the ACT fraction exhibited the strongest nitric oxide (NO) inhibition in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages under both pre-incubation and co-incubation conditions. The inhibitory effect was more pronounced in the pre-incubation model, indicating a key role of intracellular modulation in the anti-inflammatory response, although other mechanisms cannot be excluded. The ACT fraction also demonstrated strong antioxidant activity, as determined by DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. Metabolite profiling using UHPLC-ESI-ORBITRAP-MS/MS combined with OPLS analysis revealed 14 features associated with bioactivity, of which seven were putatively identified, including porphyrin-derivatives, isoprenoid compounds, and galactolipids (MGDGs) containing ω-3 polyunsaturated fatty acids. To further interpret the potential intracellular action, selected compounds were evaluated based on physicochemical descriptors related to membrane permeability, including log P, molecular weight, hydrogen bonding capacity, and molecular volume. Based on Lipinski's rule of five (Ro5) and beyond rule of five (BRo5), pheophorbide a and fucoxanthin derivatives were predicted to have a higher likelihood of passive membrane permeation, suggesting their role in intracellular modulation. Nevertheless, other mechanisms may also contribute, including indirect effects mediated by extracellular transformation products or alternative pathways demanding further investigation. Overall, these findings support the potential development of S. *cristaefolium* lipid fractions as functional food ingredients.

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