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# Assessment of anti-inflammatory effect of 5β-hydroxypalisadin B isolated from red seaweed *Laurencia snackeyi* in zebrafish embryo *in vivo* model

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ABSTRACT

5β-Hydroxypalisadin B, a halogenated secondary metabolite isolated from red seaweed *Laurencia snackeyi* was evaluated for its anti-inflammatory activity in lipopolysaccharide (LPS)-induced zebrafish embryo. Preliminary studies suggested the effective concentrations of the compound as 0.25, 0.5, 1 μg/mL for further *in vivo* experiments. 5β-Hydroxypalisadin B, exhibited profound protective effect in the zebrafish embryo as confirmed by survival rate, heart beat rate, and yolk sac edema size. The compound acts as an effective agent against reactive oxygen species (ROS) formation induced by LPS and tail cut. Moreover, 5β-hydroxypalisadin B effectively inhibited the LPS-induced nitric oxide (NO) production in zebrafish embryo. All the tested protective effects of 5β-hydroxypalisadin B were comparable to the well-known anti-inflammatory agent dexamethasone. According to the results obtained, 5β-hydroxypalisadin B isolated from red seaweed *L. snackeyi* could be considered as an effective anti-inflammatory agent which might be further developed as a functional ingredient.



## 1. Introduction

Inflammation is a highly regulated biological process that enables the immune system to efficiently remove the injurious stimuli and initiate the healing process (Masresha et al., 2012). The inflammatory symptoms result from the action of inflammatory agents such as bradykinin, histamine, prostaglandins, and nitric oxide, which can originate locally or from cells that infiltrate in the sight of insult (Mequanint et al., 2011). Although inflammation is a defense mechanism, the complex events and mediators involved in the prolonged inflammation

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can induce various diseases and disorders. Hence, the employment of anti-inflammatory agents may be useful in the therapeutic treatment of those pathologies associated with inflammatory reactions (Yonathan et al., 2006).

Secondary metabolites play an important ecological role in marine organisms. Isolation and characterization of such marine natural products may facilitate the investigation of bioactive metabolites in order to development of the pharmaceutical agents. In contrast, the development of marine organisms-derived compounds into functional ingredients has been attracted attention over the years (Thomas & Kim, 2011).

Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites (de Almeida et al., 2011). Many researchers have focused on red algae as a potential source of bioactive compounds over the past few years. The red algal genus *Laurencia* typically produces halogenated secondary metabolites, and these compounds have been exhibited antimicrobial and cytotoxic effects (Kamada and Vairappan, 2012). Once the structures and functional properties of these biologically active compounds are understood, they may serve as potential active ingredients for the development of nutraceuticals or pharmaceuticals. Hence, understanding of the action pathways of such compounds is critical in the development of effective therapeutic agents for a particular disease.

Zebrafish (*Danio rerio*) is becoming increasingly popular *in vivo* model organism for examining the biological properties of natural products with biomedical relevance (Pyati et al., 2007). In contrast, over the past few years the zebrafish has been manipulated in a large number of studies as a model organism for exploration of biological functions of the natural bioactive components (Rinkwitz et al., 2011). Therefore, in this study, the zebrafish, a common aquatic vertebrate embryo has been used as a tool for the assessment of *in vivo* anti-inflammatory potential of 5β-hydroxypalisadin B, a brominated secondary metabolite isolated from red seaweed *L. snackeyi*.

## 2. Materials and methods

### 2.1. Isolation of 5β-hydroxypalisadin B from red alga *Laurencia snackeyi*.

Red alga, *L. snackeyi* (Weber-van Bosse) Masuda was collected at the depth of 5 m by scuba divers at PulauSulug Island, Kota Kinabalu, Sabah, Malaysia. Collected specimens were cleaned off epiphytes, sand and organic debris, brought to the laboratory under 4 °C in a chiller. In the laboratory the algae were rinsed in three exchanges of double distilled water (DDW) and subjected to air-drying under 24 °C away from direct sunlight. Partially dried algal thallus (220 g) was extracted with MeOH for 5 days. The MeOH solution was concentrated in vacuo and partitioned between $Et_2O$ and $H_2O$. The $Et_2O$ solution was washed with water, dried over anhydrous $Na_2SO_4$, and evaporated to leave a dark green oil (1.9 g). Chemical profiling of the crude extract was done by spotting crude extract on $SiO_2$ gel $F_{254}$ thin layer chromatography and developed in toluene (100%) and hexane:EtOAc (3:1) solvent systems, visualized by UV light (254 nm) and molybdophosphoric acid and heated.

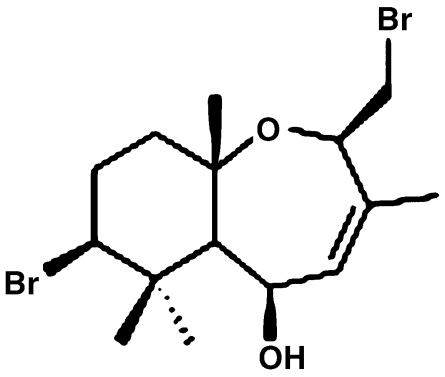


**Fig. 1 – Chemical structure of 5β-hydroxypalisadin B isolated from red seaweed *L. snackeyi*.**

The crude extract (1.0 g) was then fractionated by Si gel column chromatography with a step gradient (hexane/EtOAc in the ratio of 9:1, 8:2, 7:3, 5:5, and 100% EtOAc). Fraction eluted with hexane-EtOAc (5:5) was subjected to preparative TLC with $CHCl_3$ to give 5β-hydroxypalisadin B (Fig. 1). Independent structural elucidation and comparison of spectroscopic data to publish data lead to the identity of the isolated metabolite as 5β-hydroxypalisadin B (de Nys et al., 1993; Tan et al., 2011). The final confirmation of the compound was made based on the HRMS and optical rotation values and was similar to the ones in the published documents. In addition, the purity of the compound was determined via HPLC and $^1$H-NMR to be >99%.

### 2.2. Origin and maintenance of parental zebrafish

Adult zebrafishes were obtained from a commercial dealer (Seoul aquarium, Korea), and 10 fishes were kept in 3 L acrylic tank with the following conditions; 28.5 °C, with a 14/10 h light/dark cycle. They were fed three times a day, 6 d/week, with tetramin flake food supplemented with live brine shrimps (*Artemiasalina*). Embryos were obtained from natural spawning that was induced in the morning by turning on the light. Collection of embryos was completed within 30 min.

### 2.3. Measurement of the toxicity of the isolated compound

Sample toxicity was determined by means of survival rate and the heart beat rate of the zebrafish embryos. Briefly, the embryos ($n = 15$) were transferred to individual wells of 12-well plates containing 950 μL embryo media from approximately 3 to 4 h post-fertilization (3–4 hpf), 5β-hydroxypalisadin B was introduced to the embryos up to 24 hpf. The survival rate was measured every day. Then, the zebrafish embryos were rinsed in fresh embryo media. The heart-beating rate of both atrium and ventricle was measured at 24 hpf to determine the sample toxicity. Counting and recording of atrial and ventricular contraction were performed for 3 min under the microscope, and the results were presented as the average heart-beating rate per min (Cha et al., 2011). The survival rate of zebrafish embryos exposed to 5β-hydroxypalisadin B was determined until 5 day post-fertilization (5 dpf).