

# Polyhydroxy Isocopalane from Indonesian's Marine Sponge *Callyspongia* Sp. as Anti – White Spot Syndrome Virus from *Litopenaeus vannamei*

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## Abstract

The study of Indonesian's marine sponge *Callyspongia* Sp. against white spot syndrome virus has been conducted in June 2017. Bioactive metabolite terpene isolated based on methods of bioassay-guided separation. LCMS-ESI spectrum indicate that molecular formula  $C_{24}H_{44}O_5$  with molecular weight is 412.576 m/z. FTIR spectrum indicated bioactive metabolite has ether C-O group at  $1261\text{ cm}^{-1}$  and hydroxy group O-H at  $3419\text{ cm}^{-1}$ . The spectrum of  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR indicated that metabolite active compound as polyhydroxy isocopalane. The antiviral activity of isolate compound has been conducted against white spot syndrome virus from *Litopenaeus vannamei*. The result antiviral activity showed that the compound activity is strong at concentration 60 mg/L with survivability percentage at 34%. The result indicated that polyhydroxy isocopalane has an activity as anti-white spot syndrome activity from *Litopenaeus vannamei*.

**Keywords:** Bioactivity; Marine sponge; White spot syndrome virus; *Litopenaeus vannamei*

## Introduction

The farming of *Litopenaeus vannamei* is dominant species of global shrimp of aquaculture production. However, this species is often infected by viral diseases. The most deadly pathogenic disease is White Spot Syndrome Virus (WSSV), causing 100% mortality within 3-7 days [1,2].

The synthesis drug believed to have a negative effect on shrimp, so that the drug from natural products is expected to be a solution to combat WSSV diseases because the synthesis drug is very difficult to digest by the body [3,4].

The source of natural products compound to overcome the virus attack is a sponge. Bioactivity from Indonesian's marine sponges potential as cytotoxic, antimicrobial and antiviral properties so that secondary metabolite compounds that is very useful for the development of research and pharmaceutical [5-7]. The study of antiviral from Indonesian's marine sponge which can be applied to cultivating organisms is still very limited [8-16].

This study focuses the use to Indonesian's marine sponge *Callyspongia* Sp. as an anti-virus of white spot syndrome virus.

## Experimental

Purification of metabolite secondary using HPLC Shimadzu C196-E061R Prominence with a reverse phase column (Shim-pack XR-ODS II/ 150 mm L. x 3 mm I.D) using a Photodiode Array detector analyzed in UPT LTSIT, Lampung University (Indonesia), data of FTIR was recorded on a FTIR Shimadzu IR Prestige 21 analyzed in Graduate School of Pharmaceutical Science, Osaka University (Japan). MS-ESI data was measured using LCMS-ESI Mariner analyzed in Indonesian Institute of Sciences (Indonesia).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectrum was measured using Jeol 500 MHz ( $\text{CD}_3\text{OD}$ ;  $\text{H}_2\text{O}$ ) analyzed in Graduate School of Pharmaceutical Science, Osaka University (Japan).

## Materials

Methanol, ethanol, ethyl acetate, chloroform, dichloromethane, n-hexane, Dragendorff reagents, Liebermann-Burchard reagents, cerium sulfate reagents, NaCl, Tris-HCl, EDTA, Thiosulfate citrate bile salts-sucrose, potato dextrose agar, TRIZOL reagent, isopropanol, diethyl pyrocarbonate.

## Biomaterial

Sponge taken at Sabang, Aceh Province, Indonesia on March, 2017 using SCUBA at 32 meters. The wet sponge (50 g) was dried up for a week analyzed in Laboratory of Marine Chemistry, Faculty of Marine and Fisheries, Syiah Kuala University (Indonesia). The sponge identification was carried out by biologist in Department of Biological

Science Graduate, Faculty of Science, Osaka University (Japan) and PCR analyzed in Graduate School of Pharmaceutical Science, Osaka University (Japan).

## Extraction

12 g sponge (dry) was first partitioned using n-Heksana: aquadest (1:1 v/v). The polar extract fractionated using reverse phase column chromatography and mobile phase using aquadest and methanol (1:1 v/v). The purification of final result fraction from methanol extract was separated using reverse phase column chromatography and mobile phase using chloroform: ethanol gradient from 8:2, 7:3, 5:5, 3:7 and 100% of ethanol solution. The result of gradient fraction (5:5) set as the target compound bioactivity against WSSV.

## Collection of *Litopenaeus vannamei*

*L. vannamei* (5-9 g), were collected from Peudada sub-district (Bireuen district, Aceh Province. 5 to 9 g shrimp put into a tank of 30 L with the salinity of 20 ppt sea water with a temperature of 27-30°C. Feeding using artificial feed (Kaiohji feed, Indonesia).

## Viral Preparation

WSSV-infected shrimp are collected from shrimp farms located in Peudada sub-district (Bireuen district, Aceh province). The soft part of the cephalothorax was macerated using NTE 10 mL (0.2 mol/L NaCl, 0.02 mol/L Tris-HCl and 0.02 mol/L EDTA, pH 7.4) and then centrifuged twice at a rotational speed 3000 rpm for 20 minutes and 8000 rpm for 30 minutes to produce a supernatant filtered through 0.4 µm.

## Bioactivity of Polyhydroxy Isocopalane

The terpene compounds were used to test the bioactivity of *L. vannamei* administered intramuscularly with concentrations ranging from concentrations of 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, 30 mg/L, 35 mg/L, 40 mg/L, 45 mg/L, 50 mg/L, 55 mg/L and 60 mg/L. Observation of toxicity was observed for 7 days with regard to body color and food intake [10,11].

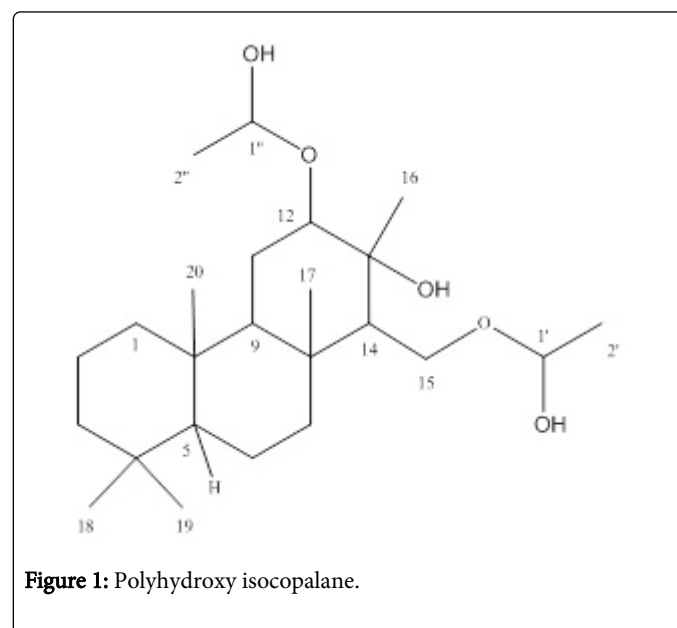
## Results and Discussions

### Structure identification

Polyhydroxy isocopalane was isolated as a amorphous solid with a molecular formula of  $C_{24}H_{44}O_5$  with molecular weight is  $[M+H]^+$  412.576 m/z that determined by MS-ESI spectrum [7]. The result from FTIR spectrum showed functional groups from C-O eter at  $1261\text{ cm}^{-1}$ , C-H methyl ( $CH_3$ ) at  $2854\text{ cm}^{-1}$ , C-H methylene ( $CH_2$ ) at  $2925\text{ cm}^{-1}$  and O-H hydroxyl at  $3419\text{ cm}^{-1}$  (Figure 1).

Spectrum of  $^1H$  NMR showed signals ( $\delta C=0.82, 0.84, 0.85, 1.12, 1.15, 1.17, 1.49\text{ ppm}$ ) that identified as seven methyl ( $CH_3$ ). Proton signals for CH-OH hydroxyl functional groups ( $\delta H=4.74, 4.81\text{ ppm}$ ) identified as secondary alcohol. Spectrum of  $^{13}C$  NMR signals ( $\delta C=12.6, 21.9, 25.2, 27.8, 29.3, 43.9, 45.4\text{ ppm}$ ) identified as seven methyl ( $CH_3$ ), signals ( $\delta C=15.4, 19.6, 25.2, 36.7, 40.1, 41.2, 57.7\text{ ppm}$ ) identified as seven aliphatic methylene ( $CH_2$ ), one oxygenated quaternary carbon signal ( $\delta C=75.7\text{ ppm}$ ), two quaternary carbon signals ( $\delta C=34.1, 37.7\text{ ppm}$ ) which indicated the presence of hydroxyl groups ( $\delta C=43.9, 45.4\text{ ppm}$ ). Base on FTIR, MS-ESI,  $^1H$  and  $^{13}C$

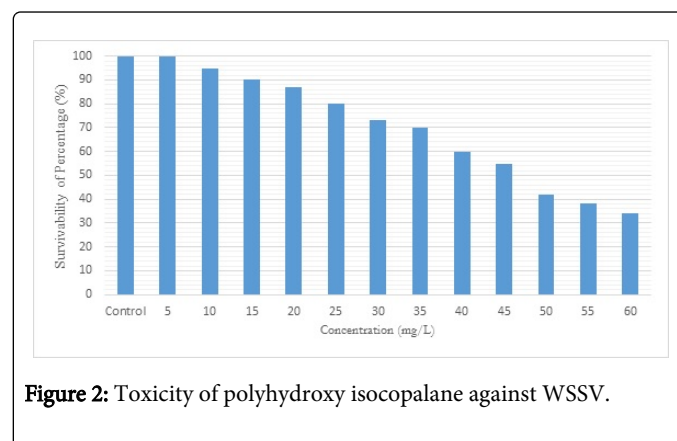
spectrum, terpene compound indicate as polyhydroxy isocopalane (Figure 2) [7-22].



**Figure 1:** Polyhydroxy isocopalane.

### Bioactivity against WSSV

*L. vannamei* (5-9 g) were injected with different concentrations from 5 to 60 mg/L and monitored for 7 days. The mortality at concentration 10 to 60 mg/L and the highest mortality at 60 mg/L (Figure 2).



**Figure 2:** Toxicity of polyhydroxy isocopalane against WSSV.

## Discussion

Polyhydroxy isocopalane from Indonesian's marine sponge indicated as antiviral against WSSV in *L. vannamei*. The terpene compound showed a toxicological effect with a survivability percentage below 40% at a dose of 60 mg/L. Provision of antiviral compounds WSSV showed an effective toxicity effect at concentrations of 10 mg/L having the highest toxicity effect at concentrations of 60 mg/L [12-15].

The hydroxy group mechanism (-OH) from polyhydroxy isocopalane forms a hydrogen bond with the active side of the enzyme that inhibits the Mitogen Activated Protein and causes deactivation of the enzyme [5-9]. So it can inhibit the formation of replication of DNA

and the formation of amino acids on the active side of the transpeptidase enzyme [10-13].

The failure of viral metabolism in the process of breeding in the host organism is due to the interaction between hydroxy groups present in the terpene compound class preventing viral replication of the host organism cell. Giving intramuscular bioactive compounds result in DNA viruses contained in the tissues do not experience replication [14-21].

## Conclusions

Polyhydroxy isocopalane from Indonesian's marine sponge indicated as anti-WSSV *L. vannamei* with survivability percentage below 40% at a dose of 60 mg/L.

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