

Bioactivity of Indonesian's Marine Sponge *Xestospongia muta* as Anti-dormant *Mycobacterium smegmatis*

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Abstract

The research of isolation and characterization from the Indonesian's Marine Sponge *Xestospongia muta*, *Clathria* sp. and *Endectyon delaubenfelsi* as anti-dormant against *Mycobacterium smegmatis* has been conducted on May 2018. The bioactive compounds were isolated based on bioassay-guided separation with several steps of chromatography. The result of bioactivity showed different activity from *Xestospongia muta* (MIC₅₀=0.2 µg/mL), *Clathria* sp. (MIC₅₀=0.9 µg/mL) and *Endectyon delaubenfelsi* (MIC₅₀=0.4 µg/mL). The results of FTIR spectrum interpretation of *Xestospongia muta* has vibration at 3435.56 cm⁻¹ indicate as O-H alcohol functional group with the confirmation of the C-O alcohol at 1342.24 cm⁻¹ in the fingerprint region. The active metabolite has C≡N imine at 2365.28 cm⁻¹ with the confirmation of C-N imine at 1637.27 cm⁻¹ in the fingerprint region. Based on MIC₅₀ data shows that *Xestospongia muta* has potential cytotoxic against *Mycobacterium smegmatis*.

Keywords: *Mycobacterium smegmatis*; *Xestospongia muta*; Anti-dormant; Alkaloid

Introduction

Mortality and morbidity infections caused by Tuberculosis (TB) remains a major global with 1.7 million deaths and an estimated incidence of 10.4 million cases by 2016 [1].

Isoniazid is one type of antibiotic that is used to treat tuberculosis (TBC-TB) to be taken for 6 months, every day for the first two months, and three times a week for four months [2]. Drugs also known as isonicotinylhydrazide (INH) are usually combined with other tuberculosis drugs such as ethambutol, pyrazinamide and rifampin to eradicate bacteria. *Mycobacterium tuberculosis* in pulmonary tuberculosis or extrapulmonary disease [3]. But the use of this antibiotic drug gives side effects to the body one of hepatitis disease [4].

In addition to antibiotic drugs, tuberculosis treatment can also be overcome using drugs derived from natural marine materials such as sponge. Currently, the sponge has been widely used for medicinal materials because sponges contain antitumor compounds, antiviral, antibacterial, antifungal, antifouling, and antimalarial.

Methods

MPLC Sepacore X50 HPLC Shimadzu C196-E061R Prominence LC-MS Mariner is used to purify compounds. Instruments FTIR Shimadzu IR Prestige 21.

Biomaterials

Clathria sp., *Xestospongia muta*, *Endectyon delaubenfelsi* taken at Sumur Tiga and Anoi Itam, Sabang Island, Indonesia in 2018 at 20-35 meters. The sponges was dried and stored at the Marine Chemical Laboratory, Marine Science Study Program, Faculty of Marine and Fisheries. The identification and bioactivity of the sponges at the Laboratory of Natural Products for drug discovery, Graduate School/School of Pharmaceutical Science, Osaka University, Japan.

Extraction and isolation

The polar fraction of *Clathria* sp. (32.8 g) partitioned using *n*-Hexane: EtOAc: EtOH (1:1:1 v/v) produce *n*-Hexane fraction (1.43 g), EtOAc fraction (9.64 g), and EtOH fraction (21.73 g). Based on bioactivity test, EtOH fraction (21.73 g (MIC₅₀=2 µg/mL)). The ethanol fraction (21.73 g) fractionated using column of chromatography

(OPN-C18) technique (EtOAc:EtOH:TFA 0.1%) gradient and yielded 4 fractions. The bioactivity from the third fraction shows cytotoxic (7.88 g (MIC₅₀=1.1 µg/mL)). Furthermore, the fraction was purified using HPLC RP-18 column technique (MeCN:H₂O:TFA 0.1%) gradient and yielded 7 fractions. The bioactivity of the fourth fraction (2.74 g (MIC₅₀=0.9 µg/mL)) shows that the active compound has cytotoxic against *M. smegmatis*.

The polar fraction of *Xestospongia muta* (24.5 g) partitioned using *n*-Hexane: EtOAc: EtOH (1:1:1 v/v) produce *n*-Hexane fraction (0.23 g), EtOAc fraction (6.28 g), and EtOH fraction (16.53 g). Based on the bioactivity test, the EtOH fraction (16.53 g (MIC₅₀=0.7 µg/mL)). The ethanol fraction (16.53 g) fractionated using column of chromatography (OPN-C18) technique (EtOAc:EtOH:TFA 0.1%) gradient and yielded 4 fractions. The bioactivity of the second fraction shows activity (9.82 g (MIC₅₀=0.6 µg/mL)). Then, purified using the RP-18 MPLC technique (MeCN:MeOH) gradient and yielded 7 fractions. The bioactivity of the third fraction (4.28 g (MIC₅₀=0.4 µg/mL)) repurified using HPLC RP-18 column technique (MeCN:H₂O) gradient and yielded 5 fractions. The bioactivity of the fourth fraction (0.15 g (MIC₅₀=0.2 µg/mL)) shows that the active metabolite is cytotoxic to *M. smegmatis*.

The polar fraction of *Endectyon delaubenfelsi* (42.85 g) partitioned using *n*-Hexane:EtOAc:EtOH (1:1:1 v/v) obtained *n*-Hexane fraction (3.46 g), EtOAc fraction (27.83 g), and the EtOH fraction (11.58 g). Based on bioactivity test, EtOAc fraction (27.83 g (MIC₅₀=1.9 µg/mL)). The ethyl acetate fraction of 27.83 g was further fractionated using an open column of chromatography (OPN-C18) with EtOAc:MeCN gradient and yielded 6 fractions. The bioactivity of the fourth fraction (12.45 g (MIC₅₀=1 µg/mL)) and purified using HPC 5C-18 HP II column

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with CHCl_3 :MeOH:H₂O low phase gradient and yielded 9 fractions. The bioactivity of the fifth fraction (3.36 g (MIC_{50} =0.6 $\mu\text{g}/\text{mL}$)) re-purified using a 5C-18 MS II HPLC technique (MeCN:H₂O:TFA 0.1%) gradient and yielded 5 fractions. The third fraction (1.13 g (MIC_{50} =0.4 $\mu\text{g}/\text{mL}$)) shows that the active metabolite is cytotoxic to *M. smegmatis*.

Cultivation of bacterial and growth conditions

M. smegmatis were maintained on Middlebrook 7H10 agar supplemented at 37°C with 10% Middlebrook OADC and 0.5% glycerol [5].

Preparation of the extract under aerobic and hypoxic conditions

MTT method used for determined MIC value of *M. smegmatis*. Midlog-phase bacilli (*M. smegmatis*: 1×10^4 CFU/0.1 mL) were inoculated and serially diluted samples added in a 96-well plate [6,7]. Mycobacterial were incubated for 36 hours at 37°C (aerobic conditions) [8]. The protocol of Rustad et al. was used with minor modifications (for hypoxic conditions) [9-11]. Mycobacterial were grown in Middlebrook 7H9 broth under nitrogen atmosphere containing oxygen (0.2%) at 37°C based on OD_{600} =0.8 [12,13]. Mycobacterial were inoculated under aerobic conditions and incubated at 37°C under nitrogen atmosphere containing oxygen 0.2% on a 96-well plate for 96 hours [14]. MTT solution was added to each well after incubation. Then, the well incubated at 37°C for an additional 12 hour under aerobic or hypoxic conditions. Furthermore, the well plate measured to determine MIC value in OD_{560} [15,16].

The curve of death time against *M. smegmatis*

M. smegmatis culture on Middlebrook 7H9 broth was arranged to 1×10^6 CFU/mL. Furthermore, added the extract until $4 \times \text{MIC}$ [17]. and Middlebrook 7H10 agar were diluted to measuring CFU. The numbers of colonies were counted after 36 hours incubation [18].

Results

Compounds that have been isolated from several sponges have different MIC_{50} values (Figure 1). *Clathria* sp. ((2.74 g) MIC_{50} =0.9 $\mu\text{g}/\text{mL}$) was isolated as a colorless solid. Based on the FTIR spectrum shows that the active metabolite has functional groups at 3435.56 cm^{-1} as N-H secondary amines, 2853.39 cm^{-1} as C-H methyl, 2769.64 cm^{-1} as C-H methylene and the fingerprint region of amine at 1637.27 cm^{-1} as C-N imine (Figure 2). The results show that the active compound as an alkaloid.

Xestospongia muta [(0.15 g) MIC_{50} =0.2 $\mu\text{g}/\text{mL}$] was isolated as a colorless solid. Based on the FTIR spectrum shows that the active metabolite has functional groups at 3435.56 cm^{-1} as O-H alcohol, at 2853.39 cm^{-1} as C-H methyl, at 2769.64 cm^{-1} as C-H methylene, at 2365.28 cm^{-1} as C \equiv N imine, the fingerprint region at 1637.27 cm^{-1} (as C-N imine) and 1342.24 cm^{-1} (as C-O alcohol) (Figure 3). Interpretation of FTIR spectrum indicates that the active compound as an alkaloid with oxygenated carbon in the hydrocarbon system.

Endectyon delaubenfelsi [(1.13 g) MIC_{50} =0.4 $\mu\text{g}/\text{mL}$] was isolated as a colorless solid. Based on the FTIR spectrum shows that the active metabolite has functional groups at 3434.6 cm^{-1} as N-H amine, C-H methyl was detected at 3090.46 cm^{-1} , and the fingerprint region at 1637.27 cm^{-1} as C-N imine (Figure 4). Interpretation of data indicates that the active metabolite compound as an alkaloid.

Discussion

Based on the results of extraction and isolation of the three sponges,

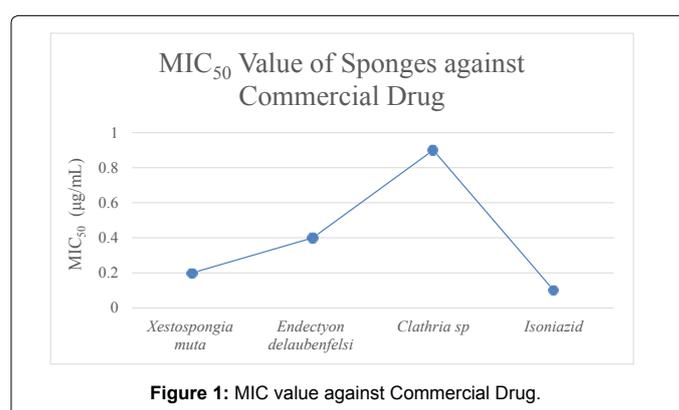


Figure 1: MIC value against Commercial Drug.

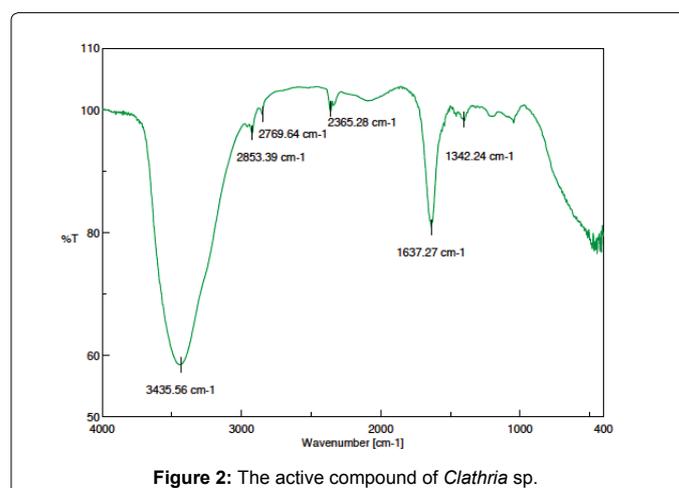


Figure 2: The active compound of *Clathria* sp.

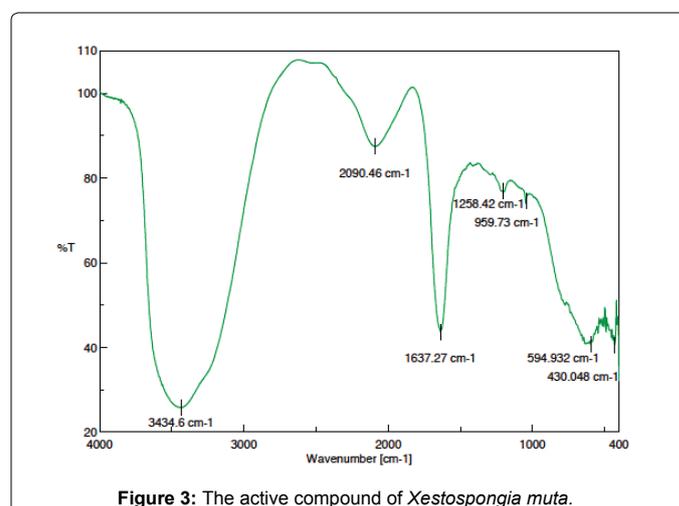


Figure 3: The active compound of *Xestospongia muta*.

obtained MIC₅₀ value of the last fraction of *Clathria* species=0.9 $\mu\text{g}/\text{mL}$, *Xestospongia muta*=0.2 $\mu\text{g}/\text{mL}$, and *Endectyon delaubenfelsi*=0.4 $\mu\text{g}/\text{mL}$. The lowest MIC₅₀ value is the sponge *Xestospongia muta* type of 0.2 $\mu\text{g}/\text{mL}$. The value of MIC₅₀ Isoniazid (INZ) is 0.1 $\mu\text{g}/\text{mL}$ [19], so that the *Xestospongia muta* has potential activity against *Mycobacterium smegmatis*.

The work drugs from alkaloid group are bactericidal that can kill 90% of the germ population after the first few days of use [20]. Isoniazid drugs are highly effective against developing germs. The mechanism of action of isoniazid has an effect on fat, nucleic acid biosynthesis, and

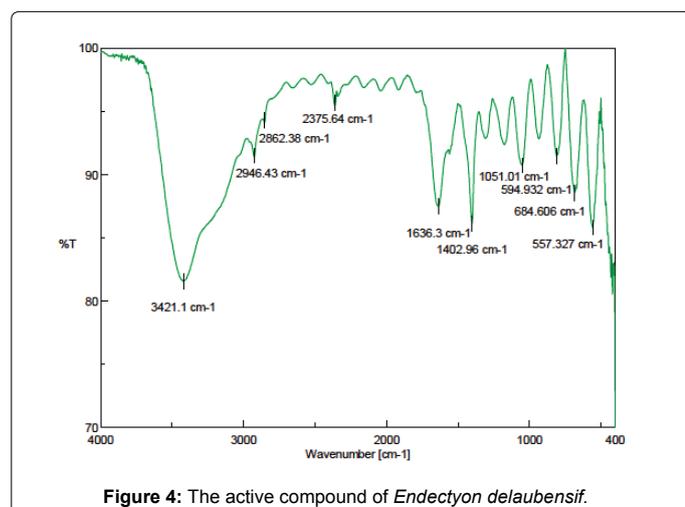


Figure 4: The active compound of *Endectyon delaubensif*.

glycolysis. The main effect of this drug is to inhibit the biosynthesis of mycolic acid (mycolic acid) which is an important element of *Mycobacterium* cell wall. INZ works by inhibiting the synthesis of mycolic acid which is an essential element of *Mycobacterium tuberculosis* cell wall formation [21].

Isoniazid drugs if taken simultaneously with other drugs will cause some interaction. It inhibits anticonvulsant drug metabolism, increases the risk of bleeding if taken with warfarin, reduces the absorption of isoniazid, and increases the risk of peripheral neuropathy.

Conclusion

The alkaloid compounds originating from the Indonesian marine sponges of the *Xestospongia muta* species have activity against *M. smegmatis* with $MIC_{50}=0.2 \mu\text{g/mL}$ which is almost equivalent to the MIC_{50} value of Isoniazid ie $0.1 \mu\text{g/mL}$.

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