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Anti-dormant *Mycobacterium bovis* Produced by Indonesian's Marine Sponge *Xestospongia muta*

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Abstract

Mycobacterium bovis is the causative agent for tuberculosis disease. This *Mycobacterium* has an ability to become dormant. Isolation and characterization of bioactive compounds from Indonesian's Marine Sponge *Xestospongia muta*, Clathria sp., and *Endectyon delaubenfelsi* have been done on May 2018. Bioactive compound was isolated based on bioassay-guided separation. The results of Minimum Inhibitory Concentration showed cytotoxic from *Xestospongia muta* is 0.4 µg/mL, Clathria sp. is 0.5 µg/mL, and *Endectyon delaubenfelsi* is 0.8 µg/mL. Interpretation of FTIR spectrum showed that the active compound from *Xestospongia muta* has O-H alcohol functional group at 3435 to 56 cm^{-1} with the fingerprint region of C-O alcohol functional group at 1342 to 24 cm^{-1}. The C≡N imine functional group was detected at 2365 to 28 cm^{-1} with fingerprint region of C-N imine at 1637 to 27 cm^{-1} indicate that the active compound as alkaloid. Based on MIC_{50} indicate that MIC_{50} from *Xestospongia muta* has the highest activity against *Mycobacterium bovis*.

Keywords: *Mycobacterium bovis*; *Xestospongia muta*; Alkaloids; Anti-dormant

Introduction

Tuberculosis is the world's main health problem responsible for the deaths of 1.7 million people each year caused by infection with *Mycobacterium bovis* [1]. The number of mortality and morbidity that has been caused by 1.7 million/year. The requirements for long-term therapy to cure TB make it difficult to treat diseases [2].

Various ways of coping, caused by the bacteria *M. bovis* have been done such as isoniazid antibiotics. The recommended use of the drug for 3 months, but the use of drugs within a period of fewer than 3 months causing these bacteria to be dormant [3].

Dormant conditions caused by the ability of bacteria in maintaining the survival of an antibiotic, so that bacteria reduce cell respiration activity [4]. The bacterium converts the aerobic respiration system into an oxygen deprivation called a hypoxic condition [5,6]. The hypoxic condition can be result dormancy. Dormancy is a period in an organism's life cycle when growth, development, and physical activity are temporarily stopped.

Studies on *M. bovis* have been widely reported, but the results obtained are not as expected. The results that have been widely reported to date are still high concentrations to obtain good bioactivity.

Until now, the utilization of natural materials has been done. Natural materials are known to have potential against bacteria such as sponges. Several studies have reported that the sponge has bioactivity as antibacterial, anticancer, antiviral and antitumor. So in this study will be described the study of bioactivity from Indonesian's marine sponge Clathria sp., *Xestospongia muta*, and *Endectyon delaubenfelsi* against *M. bovis*.

Material and Methods

The purification using MPLC Sepacore X50 HPLC Shimadzu C196-E061R Prominence LC-MS Mariner. Data IR diperoleh dari instrument FTIR shimadzu IR Prestige 21.

Biomaterials

The sponge from Sumur Tiga and Anoi Itam, Sabang Island of Clathria sp., *X. muta* and *E. delaubenfelsi* was collected on 2018 at 20-30 meters. The sponge's storage at Marine Chemical Laboratory, Marine Science Study Program, Faculty of Marine and Fisheries. Analysis of sponges using chromatography technique at Laboratory of Natural Products for drug discovery, Graduate School/School of Pharmaceutical Science, Osaka University, Japan.

The cultivation of *Mycobaterium bovis*

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

Aerobic and hypoxic conditions of antimicrobial activation

MTT method was used for determined MIC values against *M. bovis* [8,9]. Midlog-phase bacilli (*M. bovis*: 1 x 10^4 CFU/0.1 mL) were inoculated in a 96-well plate [10-12]. For aerobic conditions, *M. bovis* were incubated for 36 hours at 37°C (*M. bovis*) [13-15]. For the hypoxic conditions, *M. bovis* were grown on Middlebrook 7H9 broth with OD_{600}=0.8 under nitrogen atmosphere containing oxygen (0.2%) at 37°C [16-19]. Then, *M. bovis* were inoculated in a 96-well plate (aerobic conditions). Furthermore, *M. bovis* was incubated under the nitrogen atmosphere containing oxygen (0.2%) for 96 hours at 37°C [20,21]. After incubation, MTT solution (50 mL, 0.5 mg/mL) was added to each well and incubated at 37°C for an additional 36 h under

aerobic or hypoxic conditions and MIC value was measured with OD_{560} [22].

The death curve time

M. bovis culture on Middlebrook 7H9 broth was controlled to 1 x 10^6 CFU/mL and add the extract (4 x MIC) [23]. Then, an aliquot (100 mL) was collected and diluted cultures on Middlebrook 7H10 agar to measure CFU. The numbers of colonies were counted after four-week incubation [24].

Results

The MIC_{50} values from several sponges are shown in Figure 1.

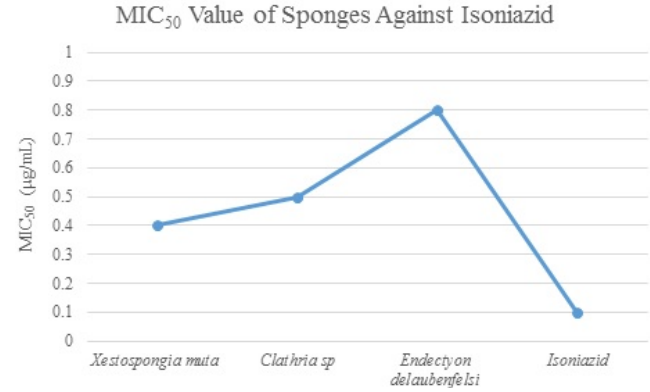

Figure 1: MIC_{50} of Sponges against Isoniazid.

Extraction and isolation

The sponge of Clathria sp. from polar extract (32.8 g) was partition using n-Hexane: ethyl acetate: ethanol (1:1:1 v/v). The result shows that n-Hexane fraction (1.43 g), ethyl acetate fraction (9.64 g), and ethanol fraction (21.73 g). The bioactivity guideline shows that ethanol fraction (21.73 g) has the lowest Minimum Inhibitory Concentration against *M. bovis* [MIC_{50}= 2.5 µg/mL]. Then, ethanol fraction (21.73 g) was separated using open chromatography (OPN-C18) with eluent ethyl acetate: ethanol: 0.1% TFA obtains 4 fractions. The bioactivity guideline show that the third fraction (7.88 g) has cytotoxic [MIC_{50}= 1.9 µg/mL]. Furthermore, the extract was purified using HPLC RP-18 column with eluent acetonitrile: water: TFA 0.1% gradient obtain 7 fractions. The fourth fraction (2.74 g) shows cytotoxic [MIC_{50}= 0.5 µg/mL] against *M. bovis*.

The fourth fraction [2.74 g (MIC_{50}= 0.5 µg/mL) that has been isolated as a colorless solid. The FTIR spectrum results show functional groups of N-H secondary amines at 3435.56 cm^{-1}, C-H methyl at 2853.39 cm^{-1}, C-H methylene at 2769.64 cm^{-1} and C-N imines at 1637.27 cm^{-1}. Based on the results of the data interpretation indicates that the active metabolite compound as a group of alkaloid compounds as depicted in Figure 2.

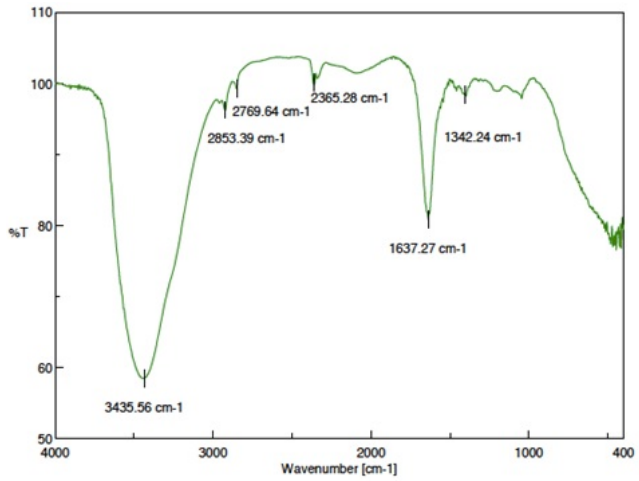

Figure 2: Interpretation FTIR data of Clathria species.

The sponge of *X. muta* from polar extract (24.5 g) was partition with n-Hexane: ethyl acetate: ethanol (1:1:1 v/v). The results show that n-Hexane fraction (0.23 g), ethyl acetate fraction (6.28 g), and ethanol fraction (16.53 g). Based on bioactivity guidelines show that ethanol fraction has potential cytotoxicity [MIC_{50}= 1.5 µg/mL]. Furthermore, ethanol fractions (16.53 g) was separated using open chromatography (OPN-C18) with eluent ethyl acetate : ethanol : 0.1% TFA obtain 4 fractions The bioactivity guidelines show that the third fraction (9.82 g) has the lowest cytotoxic [MIC_{50}= 1.3 µg/mL] and purified using HPLC RP-18 columns with acetonitrile: water: TFA 0.1% gradient obtain 7 fractions. The fourth fraction (4.28 g) shows the lowest activity [MIC_{50}= 0.7 µg/mL]. Then, the fourth fraction was re-purified with HPLC RP-18 column using acetonitrile: water gradient produce 5 fractions. The fourth fraction (0.15 g) has the lowest viability rate (MIC_{50}= 0.4 µg/mL) against *M. bovis*.

The fourth fraction [0.15 g (MIC_{50}= 0.4 µg/mL)] has been isolated as a colorless solid. FTIR spectrum results show the functional group of O-H alcohol at 3435.56 cm^{-1}, C-H methyl at 2853.39 cm^{-1}, C-H methylene at 2769.64 cm^{-1}, C≡N imine at 2365.28 cm^{-1}, C-N imine fingerprint at 1637.27 cm^{-1}, and C-O alcohol fingerprint at 1342.24 cm^{-1}. Based on the results of the data interpretation indicates that the active metabolite compound as a group of alkaloid compounds as depicted in Figure 3.

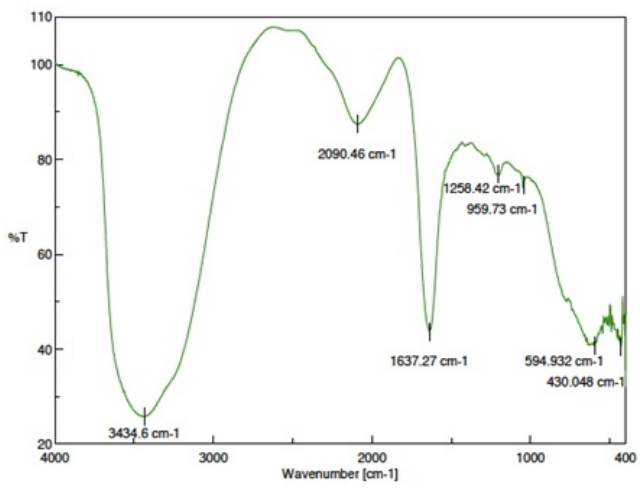

Figure 3: Interpretation FTIR data of *Xestospongia muta*.

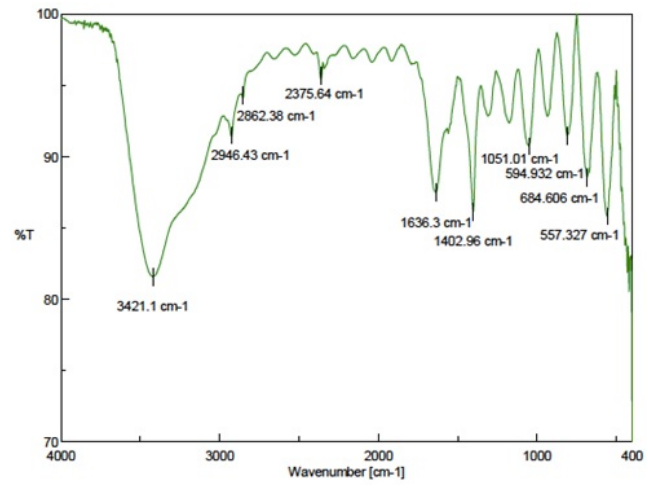

Figure 4: Interpretation FTIR data of *Endectyon delaubensif*.

The sponge of *E. delaubenfelsi* from polar extract (42.85 g) was partition using n-Hexane: ethyl acetate: ethanol (1:1:1 v/v). The results show that n-Hexane fraction (3.46 g), ethyl acetate fraction (27.83 g), and ethanol fraction (11.58 g). Based on bioactivity guidelines show that ethanol fraction (27.83 g) has activity against *M. bovis* [MIC_{50}= 1.6 µg/mL]. Then, ethanol fraction (27.83 g) was separated using open chromatographic columns (OPN-C18) ethanol: acetonitrile gradient produce 6 fractions. The bioactivity guidelines show the fourth fraction (12.45 g) has activity against *Mycobacterium* [MIC_{50}= 1.3 µg/mL]. The fourth fraction (12.45 g) was purified using 5C-18 MS II HPLC columns with chloroform: methanol: water low-phase gradient yield 9 fractions. Based on bioactivity guidelines show the fifth fraction (3.36 g) has cytotoxic against *M. bovis* [MIC_{50}= 1 µg/mL]. The fifth fraction (3.36 g) was re-purified using a 5C-18 MS II HPLC column with the methanol: water: TFA 0.1% gradient yield 5 fractions. The third fraction (1.13g) shows cytotoxic [MIC_{50}= 0.8 µg/mL] activity against *M. bovis*.

The third fraction [1.13 g (MIC_{50}= 0.8 MIC µg/mL)] which has been isolated as a colorless solid. The FTIR spectrum results show the functional groups of N-H amines at 3434.6 cm^{-1}, C-H methyl at 3090.46 cm^{-1}, and C-N imine fingerprint at 1637.27 cm^{-1}. Based on the results of the data interpretation indicates that the active metabolite compound as a group of alkaloid compounds as depicted in Figure 4.

Discussion

An Alkaloid has been isolated from Indonesian's marine sponge *X. muta* as anti-dormant *M. bovis*. Isoniazid (MIC_{50}= 0.1) is an antibiotic drug used in the treatment of tuberculosis diseases. This drug must be combinate with other medicines so that the results are more optimal in the healing process [25]. However, these drugs cause various side effects. The right solution by way of replacing this commercial antibiotic drug with natural antibiotic drugs because natural antibiotic drugs do not cause side effects [26].

Based on the results of the three types of sponges, Clathria sp. has MIC_{50}= 0.5 µg/mL, *X. muta* has MIC_{50}= 0.4 µg/mL, *E. delaubenfelsi* showed MIC_{50}= 0.8 µg/mL. *X. muta* is the lowest viability than another sponge. So, *X. muta* is potential solution to replace isoniazid.

Conclusion

Bioactive compounds from *X. muta* has cytotoxic against *M. bovis* with MIC_{50}= 0.4 µg/mL which is very close to MIC_{50} value against commercial drug Isoniazid MIC_{50}= 0.1 µg/mL.

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