Sakinah et al., Biochem Pharmacol (Los Angel) 2018, 7:4
DOI: 10.4172/2167-0501.1000262

Biochemistry & Pharmacology: Open Access

Research Article Open Access

Isolation of Bioactive Compounds from Indonesian Marine Sponge *Clathria* sp., *Xestopongia muta*, and *Endectyon delaubenfelsi* as Antibacterial *Escherichia coli*

Rauzatul Sakinah[1], Nanda Muhammad Razi[1], Syarifah Keumala[1], Guntur Febrian Illahi[1], Khairunnisa[1] and Viqqi Kurnianda[2]*

[1]*Department of Marine Science, Marine and Fisheries Faculty, Syiah Kuala University, Banda Aceh, 23111, Indonesia*
[2]*Marine Chemistry Laboratory, Marine and Fisheries Faculty, Syiah Kuala University, Banda Aceh, 23111, Indonesia*

Abstract

The isolation of bioactive compounds from the sponge *Xestopongia muta*, *Clathria* sp. and *Endectyon delaubenfelsi* against *Escherichia coli* as an antibacterial has been conducted on May 2018. Active metabolite isolated based on bioassay-guided separation of several steps of chromatography. The result of bioactivity showed sponges of *Xestopongia muta* and *Endectyon delaubenfelsi* had the same MIC_{50} values, MIC_{50} = 0, 2 µg / mL, *Clathria* sp. has a value of MIC_{50} = 0, 6 µg/mL. Interpretation of the FTIR spectrum indicates that *Xestopongia muta* has an O-H functional group at 3435,56 cm^{-1} and a C ≡ N imine functional group at 2365,28 cm^{-1}. The sponge of *Endectyon delaubenfelsi* showed a functional group of N-H of amine 3434, 6 cm^{-1} with confirmation of C-N imine 1637,27 cm^{-1} group in fingerprint region. The result of the active metabolite compound is a group of alkaloid compounds.

Keywords: *Escherichia coli*; *Clathria* sp.; *Xestopongia muta*; *Endectyon delaubenfelsi*, Antibacterial; Alkaloid

Introduction

Bacterial infection *Escherichia coli* is one of the diseases that attack human digestive organs. Diarrheal disease is still a major public health problem with severe cases of illness and death. Mortality and morbidity caused by this bacteria are 1,7 million children every year, generally attacking children under 5 years of age [1,2].

To cope with this disease caused by bacteria, metronidazole antibiotics are used. Metronidazole is a class of antimicrobial drugs used to treat various infections caused by protozoan microorganisms and anaerobic bacteria. The use of this drug has side effects on the body such as changes in taste on the tongue [3-5].

One alternative that has been done in the prevention of this disease is to use marine natural materials obtained from waters Sabang sourced from sponges. But for the time being the sponge distribution in Sabang is still very limited. The sponge has been widely used for various research studies and can also be used as a medicine for various types of diseases [6,7].

Material and Methods

Compound purification using the MPLC Sepacore X50 HPLC Shimadzu C196-E06IR Prominence LC-MS Mariner by using a detector. IR spectra were obtained from FTIR Shimadzu IR Prestige 21.

Tools and materials

Methanol, ethyl acetate, chloroform, dichloromethane, n-hexane, dragendorff reagents, cerium sulfate reagents, nutrient agar medium (NA), bacterial resistant *E. coli*, chloramphenicol, and H_2O.

Biomaterials

There are three types of *Clathria* Sp., *Xestospongia muta*, and *Endoctycon delaubenfelsi* obtained from the location of the three wells and Anoi Itam, Sabang Island Indonesia in 2018. The third type of sponge is taken at a depth of 20 to 35 meters. Sponge Storage at Marine Chemical Laboratory, Marine Science Study Program, Faculty of Marine and Fisheries. Sponge analysis was performed at the Laboratory of Natural Products for drug discovery, Graduate School / School of Pharmaceutical Science, Osaka University, Japan.

Extraction and isolation

Crude methanol fraction *Clathria* sp. used as much as 32,8 grams of crude 3x partitioned using n- Hexane: EtOAc: EtOH (1: 1: 1 v/v) yields n-Hexane fraction (1,43 g), EtOAc fraction (9,64 g), and EtOH fraction (21,73 g). Based on bioactivity, EtOH fraction yields [21,73 g (MIC_{50} = 10 µg/mL)]. The ethanol fraction as much (21,73 g) will then be fractionated using an open chromatography column (OPN-C180 with a gradient eluent EtOH: TFA 0,1% to produce 4 fractions. The activity of the third fraction [7,88 g (MIC_{50} = 0,75 µg / mL)] and purified using RP-18 HPLC column with MtCN eluent: H_2O: TFA 0,1% gradient resulting in 7 fractions. Activity from the fourth fraction [2,74 g (MIC_{50} = 0,6 µg/mL)] showed cytotoxic activity of *Escherichia coli*. (MNZ MIC_{50} = 0,1 µg/mL).

Xestospongia muta (24,5 grams of crude) was partitioned 3x using n-Hexane: EtOAc: EtOH (1: 1: 1 v / v) resulting in n-Hexane fraction (0,23 g), EtOAc fraction (6,28 g), and the EtOH fraction (16,53 g). Based on the results of bioactivity, obtained EtOH fraction [16,53 g (MIC_{50} = 0,9 µg / mL)]. The ethanol fraction (16,53 g) was further fractionated by using open chromatographic column (OPN-C18) with EtOA eluent: EtOH: 0,1% TFA resulting in 4 fractions. The activity of the second fraction [9,82 g (MIC_{50} = 0,6 µg/mL)] and purified by using the RP-18 MPLC column with the Acetonitrile eluent: MeOH gradient

***Corresponding author:** Kurnianda V, Marine Chemistry Laboratory, Marine and Fisheries Faculty, Syiah Kuala University, Banda Aceh, 23111, Indonesia, Tel: +81-956205740; E-mail: viqqikurnianda@yahoo.co.id

Received: December 07, 2018; **Accepted:** December 18, 2018; **Published** December 24, 2018

Citation: Sakinah R, Razi NM, Keumala S, Illahi GF, Khairunnisa V, et al. (2018) Isolation of Bioactive Compounds from Indonesian Marine Sponge *Clathria* sp., *Xestopongia muta*, and *Endectyon delaubenfelsi* as Antibacterial *Escherichia coli*. Biochem Pharmacol (Los Angel) 7: 262. doi: 10.4172/2167-0501.1000262

resulting in 7 fractions. The third fraction activity [4,28 g (MIC_{50} = 0.5 µg/mL)] is then refined using HPP RP-18 column with the MeCN: H_2O gradient eluent and yields 5 fractions. Activity from the fourth fraction [0,15 g (MIC_{50} = 0,2 µg/mL)] showed cytotoxic activity against *Escherichia coli*.

Endectyon delaubenfelsi (42,85 gram of crude) was repeated 3x using n-Hexane: EtOAc: EtOH (1: 1: 1 v / v) resulted in fraction of n-Hexane (3,46 g), EtOAc fraction (27,83 g), and the EtOH fraction (11,58 g). Based on the results of bioactivity, the EtOAc fraction obtained was [27.83 g (MIC_{50} = 0,7 µg / mL)]. The ethyl acetate fraction (27,83 g) was further fractionated by an open chromatographic column (OPN-C18) with EtOAc-graded eluates: Acetonitrile gradient yielded 6 fractions. Activity of the fourth fraction [12,45 g (MIC_{50} = 0,5 µg / mL)] to be purified using a 5C-18 MS II HPLC column with CHCl3 eluent: MeOH: H_2O low-phase gradient yielded 9 fractions. The activity of the fifth fraction [3,36 g (MIC_{50} = 0,4 µg / mL)] was further refined using a 5C-18 MS II HPLC column with the MeCN: H_2O: TFA eluent of 0,1% gradient resulting in 5 fractions. The activity of the third fraction [1,13 g (MIC_{50} = 0,2 µg / mL)] shows cytotoxic activity against *Escherichia coli*.

Bacterial cultivation and growth conditions

Escherichia coli incubated at 37°C on Middlebrook 7H10 added with 10% Middlebrook OADC and 0,5% gliserol, or Middlebrook 7H9 lsolution with 10% Middlebrook OADC, 0,2% gliserol, and 0,05% Tween 80 [8-11].

Antimicrobial activation of the extract under aerobic and hypoxic conditions

MIC values against *Escherichia coli* were determined by using the established MTT method. Midlog-phase bacilli (*Escherichia coli*: 1 x 10^4 CFU/0,1 mL) were inoculated in a 96-well plate, then serially diluted samples were added. For aerobic conditions, bacteria were incubated at 37°C for 12 h (*Escherichia coli*). For the hypoxic conditions, the protocol of Rustad et al. was used with minor modifications [12-16]. Mycobacterial were grown in Middlebrook 7H9 broth at 37°C under nitrogen atmosphere containing oxygen (0,2%) until OD_{600}=0,8. Subsequently, Mycobacterial were inoculated in a 96-well plate (same density) under aerobic conditions and incubated at 37°C under the nitrogen atmosphere containing oxygen (0,2%) for 12 h (*Escherichia coli*). After incubation, MTT solution (50 mL, 0,5 mg/mL) was added to each well and incubated at 37°C for an additional 12 h under aerobic or hypoxic conditions. OD_{600} was measured to determine MIC value [17-19].

The curve of death time from extract to bacteria

OD560 was measured *Escherichia coli* was grown in Middlebrook 7H9 broth at 37°C under anaerobic condition or nitrogen atmosphere containing oxygen (0,2%) until OD600=0,8. *Escherichia coli* culture in Middlebrook 7H9 broth was adjusted to 1 x 10^6 CFU/mL, then extract (4 x MIC) was added. An aliquot (100 mL) was collected at each time point, and serially diluted cultures were plated on Middlebrook 7H10 agar to measure CFU. The numbers of colonies were counted after four-week incubation determine MIC value [20,21].

Results

Based on the test of several isolated sponges obtained different MIC50 values in Figures 1-4.

Clathria sp. [2,74 g (MIC_{50} = 0,6 µg / mL) that has been isolated as a colorless solid The results of FTIR data show that the functional groups

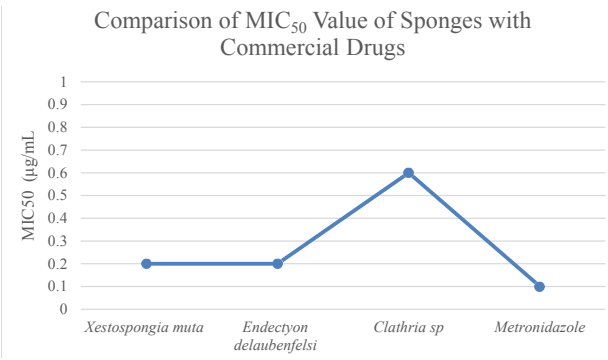

Figure 1: The result of MIC_{50} test.

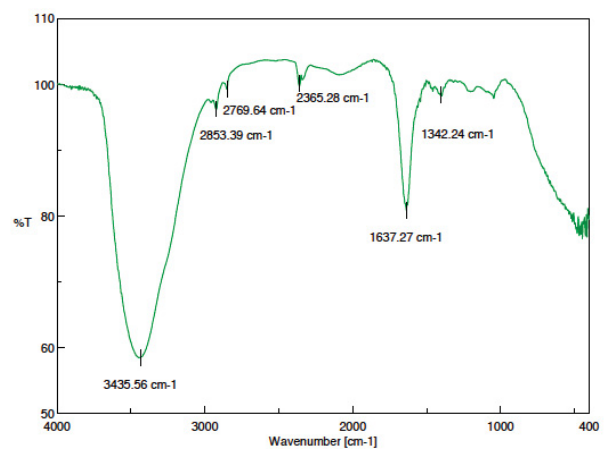

Figure 2: FTIR Spectrum of *Clathria* sp.

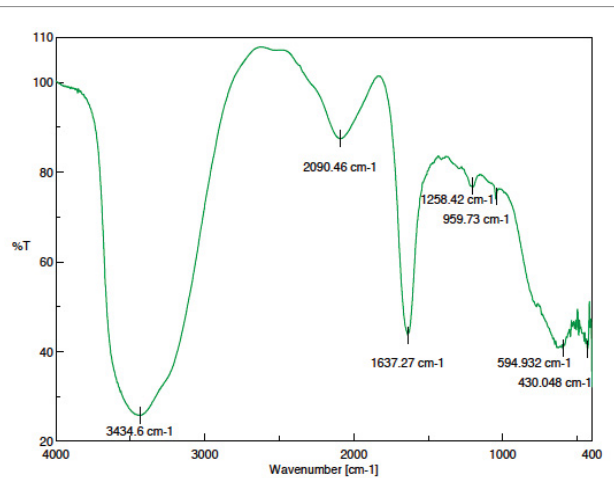

Figure 3: FTIR Spectrum of *Xestospongia muta*.

of secondary amine at 3435,56 cm^{-1}, C-H methyl at 2853,39 cm^{-1}, C-H methylene at 2769,64 cm^{-1} and C-N imine at 1637,27 cm^{-1}. Based on the results of the data interpretation indicating the active metabolite compounds belong to the group of alkaloid compounds.

Xestospongia muta [0,15 g (MIC_{50} = 0,2 µg/mL)] which has been isolated as a colorless solid. The results of FTIR data show that the

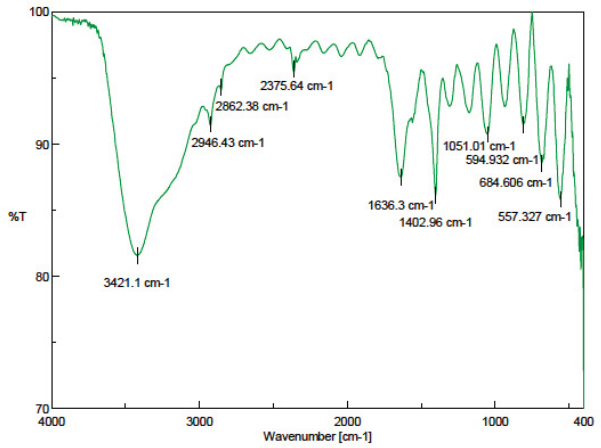

Figure 4: FTIR Spectrum of *Endectyon delaubensif.*

functional group of OH alcohol at 3435,56 cm^{-1}, CH methyl at 2853,39 cm^{-1}, CH methylene at 2769,64 cm^{-1} and C≡N imine at 2365,28 cm^{-1}, CN imine fingerprint in 1637, 27 cm^{-1}, and CO alcohol fingerprint at 1342,24 cm^{-1}. Based on the results of the data interpretation indicates that the active metabolite compounds belong to the group of alkaloid compounds.

Endectyon delaubenfelsi [1,13 g (MIC_{50} = 0,2 µg/mL)] that has been isolated as a colorless solid. The results of FTIR data indicate that the functional group of N-H of amine 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 data interpretation indicating active metabolite compounds belong to the group of alkaloid compounds.

Discussion

The results of the three sponges that have been tested show different MIC_{50}. Sponge type *Clathria* sp. has a MIC_{50} value = 0,6 µg / mL which is very far from MIC_{50} MNZ = 0,1 µg/mL which means this type of sponge is not suitable as an alternative to bacterial infection of *Escherichia coli*. Metronidazole (MNZ) has the highest resistance to antibiotics that is 96,43%. Metronidazole is an effective antiprotozoal and antibacterial agent capable of fighting anaerobic protozoan parasites and anaerobic gram-negative bacilli, including Bacteroides sp., And gram-positive spore-forming anaerobes [22-24].

Metronidazole requires reductive activation of nitro groups by a susceptible organism. Single electron transfer forms a highly reactive nitro radical anion. The anions kill susceptible organisms through the radically mediated mechanism. The sponges of *Xestospongia muta* and *Endectyon delaubenfelsi* have an MIC_{50} value of 0.2 µg/mL which is almost close to the MIC_{50} MNZ value. So that these two sponge can be used as medicine as a substitute for MNZ [25-27].

Metronidazole (MNZ) is not always well consumed by humans. This is due to the resulting negative effects that affect people who take this drug and suffer from certain diseases. Based on the MIC_{50} results, the two sponges can be used as Metronidazole (MNZ) replacement drugs [28].

Conclusion

Bioactive compounds obtained from the Indonesian marine sponge contain two types of *Xestospongia muta* and *Endectyon delaubenfelsi* which have bioactivity against *Escherichia coli* with the same MIC_{50} value that is MIC_{50} = 0,2 µg / mL. The MIC_{50} value of both sponges approximates the MIC_{50} value to MNZ commercial drug with MIC_{50} value = 0.2 µg/mL.

References

1. Gilman, Goodman A (2012) Basic Pharmacology Therapy, ed. 10, EGC, Jakarta.
2. WHO (2017) Global Health Observatory: //.www.who.int/. Access date is July 24, 2017.
3. Kurnianda V, Setiawan A (2015) Bioactivity a poly hydroxyl isocopalane from Callyspongia sp. as antibacterial resistance *Escherichia coli*. Int J Pharm Biol Chem Sci 4: 30-33.
4. Kurnianda V (2017) Bioactivity from Indonesian's marine sponge Xestospongia sp. as antibacterial resistance *Escherichia coli*. Nat Prod Chem Res 5: 265-269.
5. Berer N, Rudi A, Goldberg I, Benayahu Y, Kashman Y (2004) Callynormine A, a new marine cyclic peptide of a novel class. J Org Lett 6: 2543-2545.
6. Mbah JA, Ngemenya MN, Abawah AL, Babiaka SB, Nubed LN, et al. (2012) Bioassay-guided discovery of antibacterial agents: in vitro screening of Peperomia vulcanica, Peperomia fernandopoioana and Scleria striatinux. Ann Clin Microbiol Antimicrob 11: 10.
7. Harborne JB (1984) Phytochemical Method. Chapman and Hall ltd. London.
8. Huang RM, Ma W, De DJ, Feng ZX, Xu T, et al. (2010) A New 1,4-Diazepine from South China Sea Marine Sponge Callyspongia Species. Molecules 15: 871-877.
9. McMurry J (2008) Organic Chemistry. 7th Edn. Graphic World Inc. Pp: 440-469.
10. Sun W, Dai S, Jiang S, Wang G, Liu G, et al. (2010) Culture dependent and culture-independent diversity of Actinobacteria associated with the marine sponge Hymeniacidon perleve from the South China Sea. Antonie van Leeuwenhoek 98: 65-75.
11. Vynne NG, Mansson M, Gram L (2012) Gene sequence based clustering assists in dereplication of Pseudoalteromonas luteoviolacea strains with identical inhibitory activity and antibiotic production. Mar Drugs 10: 1729-1740.
12. Duncan KR, Haltli B, Gill KA, Correa H, Berrué F, et al. (2015) Exploring the diversity and metabolic potential of actinomycetes from temperate marine sediments from Newfoundland, Canada. J Ind Microbiol Biotechnol 42: 57-72.
13. Liu Y, Lai Q, Du J, Shao Z (2016) Bacillus zhangzhouensis sp. nov. and Bacillus australimaris sp. nov. Int J Syst Evol Microbiol 66: 1193-1199.
14. Prieto ML, O'Sullivan L, Tan SP, McLoughlin P, Hughes H, et al. (2012) Assessment of the bacteriocinogenic potential of marine bacteria reveals lichenicidin production by seaweed-derived Bacillus spp. Mar Drugs 10: 2280-2299.
15. Sumi CD, Yang BW, Yeo IC, Hahm YT (2014) Antimicrobial peptides of the genus Bacillus, A new era for antibiotics. Can J Microbiol 61: 93-103.
16. Berrue F, Ibrahim A, Boland P, Kerr RG (2009) Newly isolated marine Bacillus pumilus (SP21): A source of novel lipoamides and other antimicrobial agents. Pure Appl Chem 81: 1027-1031.
17. Ouoba LII, Diawara B, Jespersen L, Jakobsen M (2007) Antimicrobial activity of Bacillus subtilis and Bacillus pumilus during the fermentation of African locust bean (Parkia biglobosa) for Soumbala production. J Appl Microbiol 102: 963-970.
18. Clark GJ, Langley D, Bushell ME (1995) Oxygen limitation can induce microbial secondary metabolite formation: Investigations with miniature electrodes in shaker and bioreactor culture. Microbiology 141: 663-669.
19. Jami M, Ghanbari M, Kneifel W, Domig KJ (2015) Phylogenetic diversity and biological activity of culturable Actinobacteria isolated from freshwater fish gut microbiota. Microbiol Res 175: 6-15.
20. Yoon JH, Kim IG, Kang KH, Oh TK, Park YH (2003) Bacillus marisflavi sp. nov. and Bacillus aquimaris sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea. Int J Syst Evol Microbiol 53: 1297-1303.
21. Yoon JH, Yeo SH, Oh TK, Park YH (2005) Psychrobacter alimentarius sp. nov., isolated from squid jeotgal, a traditional Korean fermented seafood. Int J Syst Evol Microbiol 55: 171-176.
22. Niu L, Xiong M, Tang T, Song L, Hu X, et al. (2015) Aeromicrobium camelliae sp. nov., isolated from Pu'er tea. Int J Syst Evol Microbiol 65: 4369-4373.

23. Yan ZF, Lin P, Chu X, Kook MC, Li CT, et al. (2016) Aeromicrobium halotolerans sp. nov., isolated from desert soil sample. Arch Microbiol 198: 423-427.

24. Magarvey NA, Ehling-Schulz M, Walsh CT (2006) Characterization of the cereulide NRPS -hydroxy acid specifying modules: Activation of -keto Acids and chiral reduction on the assembly line. J Am Chem Soc 128: 10698-10699.

25. Huang T, Li L, Brock NL, Deng Z, Lin S (2016) Functional characterization of PyrG, an unusual nonribosomal peptide synthetase module from the pyridomycin biosynthetic pathway. Chem Biol Chem 17: 1421-1425.

26. Heravi KM, Shali A, Naghibzadeh N, Ahmadian G (2014) Characterization of cis-acting elements residing in the chitinase promoter of Bacillus pumilus SG2. World J Microbiol Biotechnol 30: 1491-1499.

27. Ozcengiz G, Alaeddinoglu NG, Demain AL (1990) Regulation of biosynthesis of bacilysin by Bacillus subtilis. J Ind Microbiol 6: 91-100.

28. Lee DW, Lee SD (2008) Aeromicrobium ponti sp. nov., isolated from seawater. Int J Syst Evol Microbiol 58: 987-991.