RJC

RASĀYAN *J. Chem.*
Vol. 12 | No. 3 |1463 - 1469| July - September | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

CYTOTOXIC AND ANTIBACTERIAL ACTIVITIES OF MARINE SPONGE-DERIVED FUNGUS *Aspergillus nomius* NC06

Muh. Ade Artasasta[1], Yanwirasti[1], Muhammad Taher[2], Akmal Djamaan[3] and Dian Handayani[3,*]

[1]Departement of Biomedical, Faculty of Medicine, Andalas University, 25163 Padang, Indonesia
[2]Faculty of Pharmacy, International Islamic University Malaysia,
25200 Kuantan, Pahang, Malaysia
[3]Laboratory of Sumatran Biota, Faculty of Pharmacy, Andalas University,
25163 Padang, Indonesia
*E-mail: dianhandayani@phar.unand.ac.id

ABSTRACT

Sponge-derived fungi have attracted recent attention due to its important source of interesting biologically active compounds. In our previous study, we have obtained 13 fungi from marine sponge *Neopetrsiachaliniformis*. Among them, only *Aspergillus nomius* NC06 showed cytotoxic activity with the percentage of viability113.9 % and 70.31 % of Vero cell and WiDr colon cancer cell, respectively. This study aimed to isolate the cytotoxic compound from the ethyl acetate extract of *N. nomius* NC06 using chromatography method. A total of 5 fractions of the extract obtained using vacuum liquid chromatography. These fractions were tested against HCT 116 colon cancer cell and ten human pathogenic bacteria. Fraction II, III, IV, and V showed cytotoxic activity with IC_{50} of 5.28, 15.82, 10.27, and 45.57 µg/mL, respectively. In antibacterial testing, fraction II and III were potential because of their ability to inhibit the growth of ten pathogenic bacteria with the diameter of inhibition zone more than 12 mm.

Keywords: Sponge-derived fungi, *Aspergillus nomius*, Cytotoxicity, Antibacterial

INTRODUCTION

The sponge is known as the host for various microorganisms such as bacteria and fungi.[1,2] But the association relationship and ecological function of microorganism in sponge body are remaining unclear[3]. However, microorganism associated with a sponge is known as natural resources to produce the potential bioactive compound. Especially marine sponge-derived fungi repeatedly show the potential bioactive compounds against various diseases such as cancer and pathogenic microbial infection.[3-5]

Thiel *et al*., (2007) figured out that the sponge-derived microorganisms mostly exist in cortex and endosome layers of sponge nevertheless the location of associated-fungi in the sponge is still unknown[6]. In addition, Kjer*et al*., (2010) published a protocol to isolate marine fungi from sponges and other marine macroorganisms. This protocol focused to isolate fungal to the marine sponge[7]. However, there are a lot of unknown interactions between sponges to its associated fungi. Furthermore, several studies have suggested that sponge-derived fungi have shown to exhibit interesting new bioactive sources that were previously unknown to originate from terrestrial starins[8].

Aspergillus nomius was successfully isolated from marine sponge *N. chaliniformis* in our previous study. This fungus showed selectivity between cell normal Vero and WiDr colon cancer cell with the percentage of viability of 113,9 % and 70,3 %, respectively.[11] It is potential fungus to be continued to explore its bioactive compound.

EXPERIMENTAL

Sponge Material, Isolation, Cultivation, and Extraction of Secondary Metabolites from Marine Sponges-Derived Fungi

These stages have been conducted due to our previous study[12]. Marine sponge *N. chaliniformis* was taken at Mandeh Island, West Sumatra, Indonesia using scuba diving method. Furthermore, isolation of fungi

http://dx.doi.org/10.31788/RJC.2019.1235284

from *N. chaliniformis*was conducted using Sabouraud Dextrose Agar as a medium and incubated at a temperature of 27-29 °C for 5-7 days then purified by using the scratch method. We obtained *A. nomius* from this sponge and cultured in big scale by using rice as a medium for 4 to 8 weeks[7]. After this fungus overgrown on rice, then it was extracted using ethyl acetate (1:1).

Fractionation of Secondary Metabolites

Isolation compounds of 27.53g of crude extract from *A. nomius* were done by vacuum liquid chromatography with n-hexane, ethyl acetate, dichloromethane, and methanol as mobile phase and silica gel 60(0.063-0.2 mesh) as the stationary phase. Thin-layer chromatography (TLC) was used for monitoring every vials-collected and combining as one fraction that has the same spot pattern on TLC. In this study, we successfully collected 5 fractions. Furthermore, these fractions were submitted to the HPLC to be characterized by every compound in one fraction.

MTT Assay

Sample Screening

Cytotoxic activity of HCT 116 as a colon adenocarcinoma cell line was conducted using MTT assay. These cell lines were obtained from the Laboratory of Biotechnology and Cell Culture Pharmacy Faculty, International Islamic University Malaysia. HCT 116 was cultured in DMEM Gibco™. This cell was seeded in 96-well plates (density: 6x103 cells/well) and incubated at 37ºC, 98% relative humidity with 5% CO_2. After overnight incubation (confluence), the fraction of Ethyl acetate *A. nomius* extract was added with concentrations of 100 µg/mL, 10 µg/mL, 1 µg/mL and 0.1 µg/mL.Then 100 µLMTT (5 mg/mL) was added and incubated for 4 hours. The absorbance was measured using Tecan Microplate at 560 nm using DMSO as blank and Doxorubicin as a positive control. The absorbance of each fraction against HCT 116 colon cancer cells was expressed as viability percentage.[13]

Antibacterial Activity

Salmonellatyphosa, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Escherichiacoli* as Gram-negative bacteria and *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcusmutans*, *Bacillus subtilis*, *Micrococcusluteus*, *Staphylococcusepidermidis* as Gram-positive bacteria had been prepared for this study. Every fraction was diluted being 5 % with DMSO as a test solution. One piece of sterile disk paper (6mm) was soaked in the test solution. DMSO was used for negative control and chloramphenicol disk as a positive control. Zone of inhibition (mm) was measured after incubation at a temperature of 37 ^oC for 24 hours.[14]

High-Performance Liquid Chromatography

Every fraction was diluted with methanol HPLC (1:1), and then pipetted out 50 µL to the HPLC vial and added with methanol HPLC until the total volume of 500 µL. Furthermore, diluted sample on HPLC vial was submitted to UltiMate™ 3000 UHPLC with column C_8, 4.6 x 150 mm, 5 µm, mobile phase A: H_2O with 0.1 % TFA; B: methanol, flow rate: 1 mL/min. Gradient elution from0 to 35 min was 10 - 100 % B while 35 to 60 min was 100 – 10 % B.

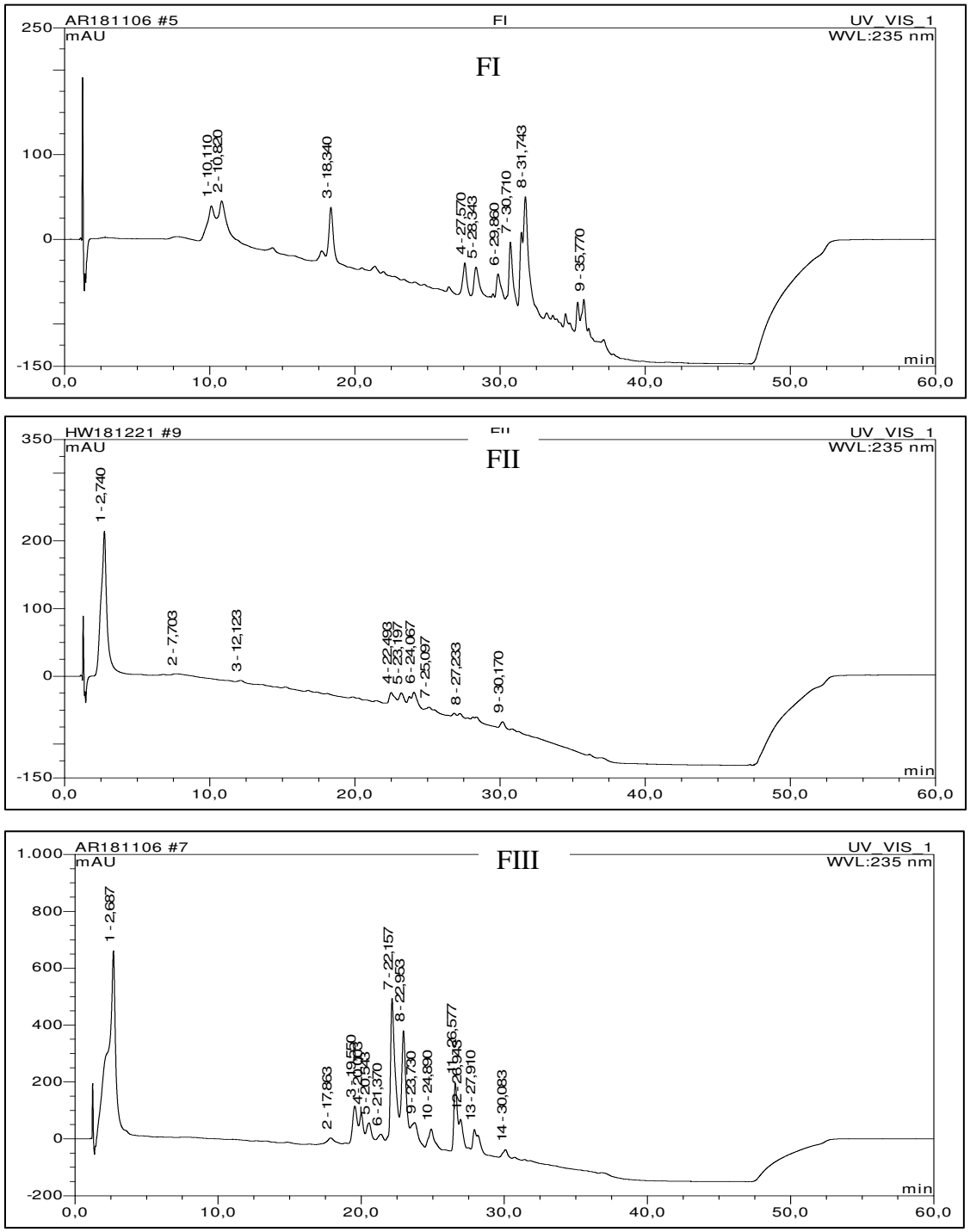
RESULTS AND DISCUSSION

Aspergillus nomius is a species of fungi as a potential source for cytotoxic and other bioactivity[15]. In our study, we successfully isolated. *nomius* from marine sponge *N. chaliniformis*. As much as 27.53 g, crude ethyl acetate extract of *A. nomius* was obtained. The result of VLC column was obtained 5 fractions that we collect based on the same spot pattern on TLC. These fractions were characterized based on its retention time of compounds contained in each fraction by using HPLC. In Figure 1, HPLC chromatogram for every fraction is shown. Every peak that appeared in 235 nm were representative why these fractions were different from each other.

Based on HPLC chromatograms, the same main peak was observed in fraction II to V, with the retention time around 2.67 min. On the other hand, every fraction exhibited different main peak such as a peak in the retention time of 18.34 and 31.74 which were only observed in fraction I. For fraction III and IV there

were also 2 main peaks in retention time around 22.15 and 22.95 min. The peak in retention time around 26.37 min appeared as the main peak in fraction IV and V.

Cytotoxic screening from a natural product is important to be conducted due to the prospecting of potential anticancer agents[16]. In this study, MTT assay was used to evaluate the cytotoxic activity of the fifth fractions. Here, we reported cytotoxic activity of the fifth fractions against HCT 116 colon cancer cell line. Figure 2 showed fraction II was the most potent cytotoxic activity with IC_{50} of 5.28 µg/mL followed by fraction IV, fraction III, fraction V then fraction I.Doxorubicin was used as a positive control against HCT 116 colon cancer cell with IC_{50}of0.47 µg/mL.



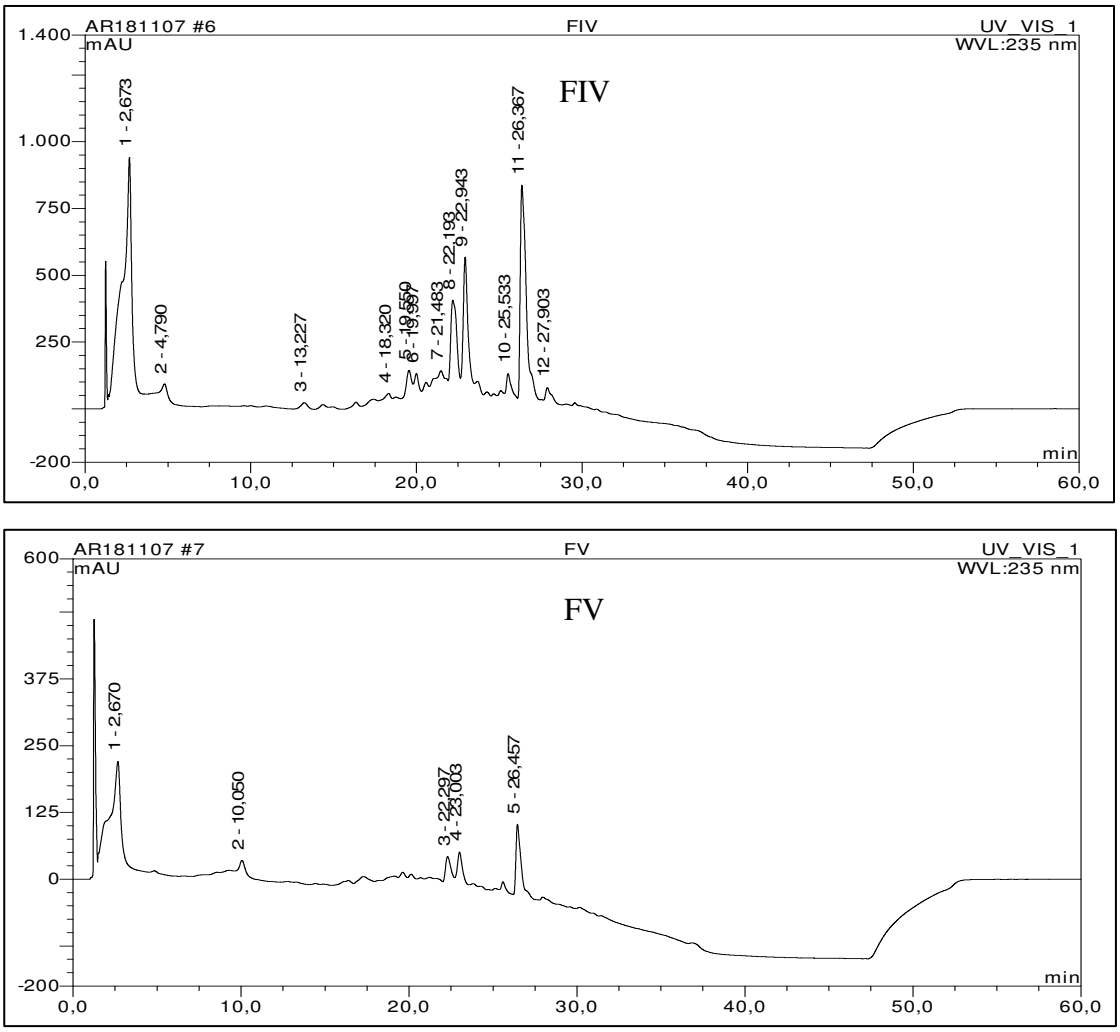


Fig.-1: HPLC Chromatogram of Fraction I to Fraction V; Column C_8, 4.6 x 150 mm, 5 µm, Mobile Phase A: H_2O with 0.1 % TFA; B: Methanol, Flow Rate: 1 mL/min.

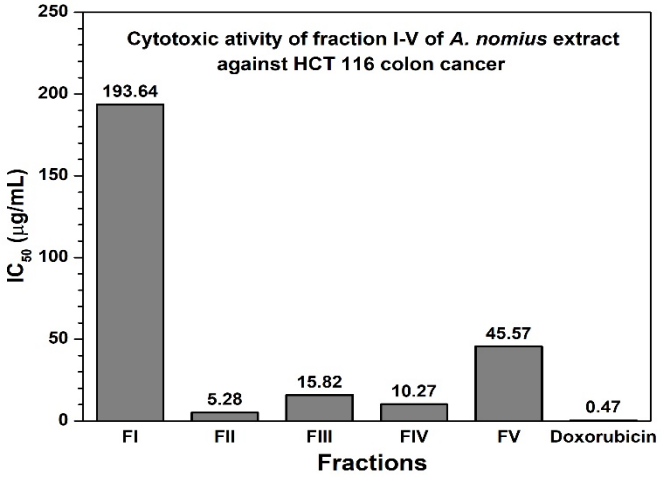


Fig.-2: Cytotoxic Activity of Fraction I-V of *A. nomius* extract against HCT 116 colon Cancer.

Antibacterial activity test is listed in Table-1. In this study, fraction II and III showed potential antibacterial activity that can inhibit ten pathogenic bacteria with inhibition zone more than 10 mm. In Table-1 also showed Fraction III can inhibit Gram-positive and Gram-negative bacteria with inhibition diameter zone (mm) of 15.43±0.27, 15.38±0.33, 16.7±0.55, 15.41±0.28, 15.98±0.29, 13.95±0.58, 14.76±0.94, 15.86±1.5, 17.53±0.62, 15.71±0.45against*S. typhosa*, *P. aeroginosa*, *V. cholera*, *E. faecalis*, *S. epidermidis*, *S. aureus*, *E. coli*, *S. mutans*, *B. subtilis*, *M. luteus*, respectively.

Table-1: Antibacterial Activity of Fraction I-V of *A. nomius* extract

Pathogenic Bacteria	Inhibition Zone (mm) ± Standard Deviation (SD)				
	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V
S. typhosa	10.83±1.41	13±0.7	15.43±0.27	-	7.28±0.12
P. aeroginosa	8.2±0.08	12.66±0.79	15.38±0.33	-	7.85±0.56
V. cholerea	12.75±0.55	14.31±0.20	16.7±0.55	-	-
E. faecalis	9.78±0.54	14.86±0.57	15.41±0.28	-	-
S. epidermidis	9.55±0.35	12.4±1.01	15.98±0.29	7.28±0.18	8.18±0.11
S. aerus	8.33±0.15	12.16±0.05	13.95±0.58	-	-
E. coli	10.73±1.31	13.38±1.32	14.76±0.94	7.38±0.12	8.4±0.26
S. mutans	-	10.6±0.43	15.86±1.5	-	-
B. subtilis	9.55±1.3	12.85±1.58	17.53±0.6	-	7.4±0.57
M. luteus	10.21±0.45	14.05±1.21	15.71±0.45	6.83±0.32	7.53±0.15

The result of cytotoxic and antibacterial activity showed that fraction II and III were potential fractions to be furtherly studied. Due to of HPLC chromatogram in these fractions, there was some peak that showed as main peak such us retention time of 2.74 min, 22.16 min, 22.95 and 26.58 min. Based on library hits of UV spectra of this main peak were probably eudesmic acid (2.74 min), spongiciadin E (22.16 min), and Notoamide E (26.58 min). Another main peak of these fractions in the retention time of 22.95 min was undetected or no spectra library hits found (Figure 3). Further study is needed to prove what compounds are contained in these fractions using the spectroscopy method. However, these main peaks might be responsible for the potential cytotoxic and antibacterial activity of fraction II and III.

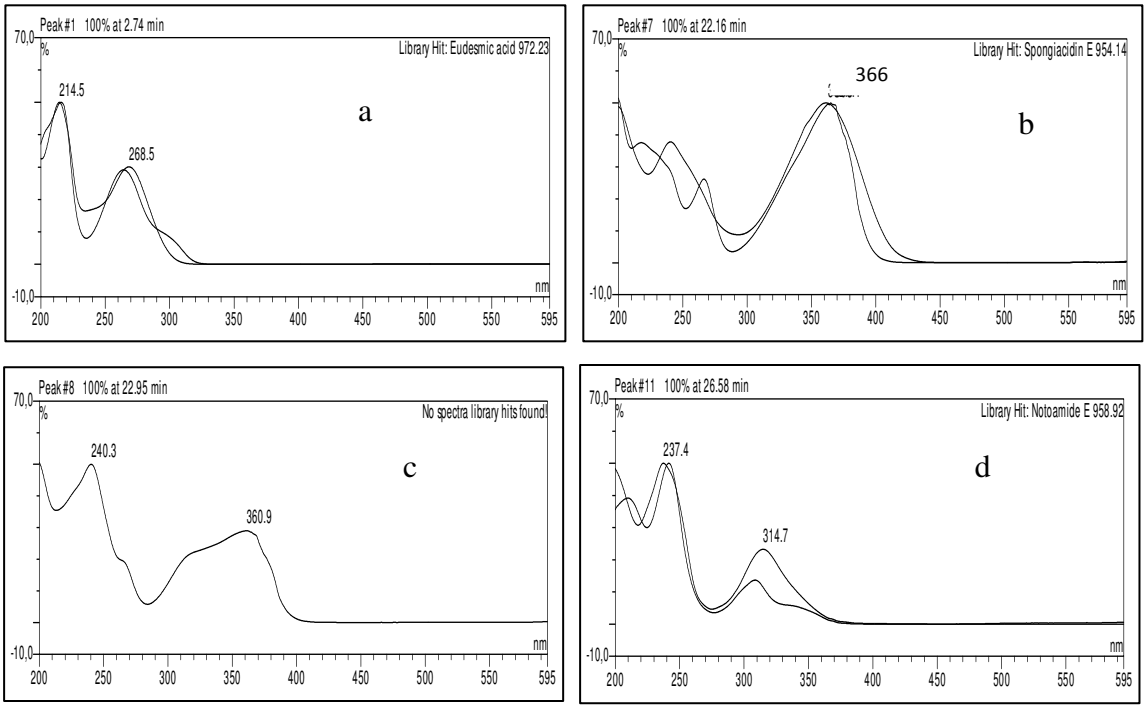


Fig.-3: UV Spectra of Main Peaks from Fraction II and III. (a) Library Hit of UV Spectra of the Compound in Retention Time 2.74 min (eudesmic acid), (b) 22.16 min (spongiacidin E), (c) 22.95 min (no spectra library hit), (d) 26.58 min (notoamide E).

Lack of information on secondary metabolites of *A. nomius*as cytotoxic and antibacterial activity. However, the study of Gloer*et* al., (1989) and Staub *et al*., (1992) reported bioactive compounds from *A. nomius*, isolated from pine sawfly *Dipriionsimilis*, such us nomine, and aspernomine which are indole diterpenoids. This study reported that aspernomine exhibits cytotoxicity against solid tumor cell such us A-549 lung carcinoma, MCF-7 breast adenocarcinoma, and HT-29 colon adenocarcinoma with IC_{50} values of 3.09, 4.93, and 3.08 µg/mL, respectively[17,18]. Compared with *A. nomius* that we isolated from marine sponge *N. chalinifromis* may produce different secondary metabolite due to the different environment[19].

Antibacterial from *Aspergillus* genus repeatedly showed strong activity. Study of Li *et al*., (2012) successfully reported aspergiterpenoid A, sydonol, and sydonic acid from *Aspergillus sp.* had potential antibacterial activity towards *S. albus*, *B. subtilis*, *B. cereus*, *S. lutea*, *E. coli*, and *M. tetragenus* with MIC range values of 1.25 to 5 µM[20]. Moreover, a new cyclic hexapeptide and a new isocoumarin isolated from sponge-associated fungus *A. similanensis* showed potential antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* with MIC value of below2.56 µg/mL[21].

CONCLUSION

*Aspergillus nomius*which was isolated from marine sponge *N. chaliniformis* showed potential cytotoxic and antibacterial activities. Fraction II and III were found most cytotoxic against HCT 116 colon cancer (IC_{50}< 20 µg/mL) and antibacterial activity (inhibition zone > 12 mm) against *S. typhosa*, *P. aeroginosa*, *V. cholera*, *E. faecalis*, *S. epidermidis*, *S. aureus*, *E. coli*, *S. mutans*, *B. subtilis*, *M. luteus*. Further study is recommended to identify the active compound that is responsible for cytotoxic against HCT 116 colon cancer and ten pathogenic bacteria.

ACKNOWLEDGMENT

Authors thank Prof. Dr. Peter Proksch and Mrs. Ni P. Ariantari (Institute of Pharmaceutical Biology and Biotechnology, Heinrich Heine University Düsseldorf, Germany) for HPLC analysis of fractions. This research was funded by The Ministry of Research and Technology, Indonesia through PMDSU Research, 059/SP2H/LT/DRPM/IV/2018.

REFERENCES

1. U. Hentschel, K. M. Usher , and M. W. Taylor, *FEMS Microbiology Ecology*, **55(2),**167(2006), **DOI:** 10.1111/j.1574-6941.2005.00046.x
2. G. Wang, *Journal of Industrial Microbiology and Biotechnology*, **33**, 545(2006),**DOI:** 10.1007/s10295-006-0123-2
3. J. Wiese, B. Ohlendorf, M. Blumel, R. Schmaljohann and J. F. Imhoff, *Mar. Drugs*, **9(4)**, 561(2011), **DOI:** 10.3390/md9040561
4. P. Bhadury, B. T. Mohammad, and P. C. Wright, *J. Ind. Microbiol. Biotechnol*, **33**, 325(2006), **DOI:** 10.1007/s10295-005-0070-3
5. V. Vasanthabharathi, and S. Jayalakshmi, *African Journal of Biotechnology*, **10(54)**, 11224(2011), **DOI:** 10.5897/AJB11.296
6. V. Thiel, S. Leininger, R. Schmaljohann, F. Brummer, and F. Johannes, *Microbial Ecology*, **54(1)**, 101(2007), **DOI:** 10.1007/s00248-006-9177-y
7. J. Kjer, D. Abdessamad, H. A. Amal, P. Peter, *Nature Protocols*, **5,** 479(2010), **DOI:** 10.1038/nprot.2009.233
8. P. Proksch, and R. E. Edrada, *Appl. Microbiol. Biot.*, **59(2)**,125(2002), **DOI:** 10.1007/s00253-002-1006-8
9. Y. M. Lee, H. Li, J. Hong, H. Y.Cho, K. S. Bae, M. A. Kim, D. K. Kim, and J. H. Jung, *Archives of Pharmacal Research*, **33(2)**, 231(2010), **DOI:** 10.1007/s12272-010-0207-4
10. Q. Wu, H. L.Long, D. Liu, P. Proksch, and W. H. Lin, *Journal of Asian Natural Products Research*,**17(12)**, 1137(2015), **DOI:**10.1080/10286020.2015.1119127
11. M. A. Artasasta, Yanwirasti, A. Djamaan, D. Handayani, *Journal Applied of Pharmaceutical Science*,**7(12)**, 174(2017), **DOI:**10.7324/JAPS.2017.71225

12. D. Handayani, M. A. Artasasta, *J. App. Pharmaceutical Science*, **7(05)**, 066(2017), **DOI:** 10.7324/JAPS.2017.70512
13. B. Yamini and R. Nanthini, *RASAYAN Journal Chemistry*, **11(2)**, 440(2018), **DOI:** 10.31788/RJC.2018.1121992
14. D. U. C. Rahayu, Hartono, and P. Sugita, *RASAYAN Journal Chemistry*, **11(2),**762(2018), **DOI:** 10.31788/RJC.2018.1122076
15. G. M. Staub, J. B. Gloer, D. T. Wicklow and P.F. Dowd, *Tetrahedron Letters*, **34(16)**, 2569(1993), **DOI:** 10.1016/S0040-4039(00)77627-1
16. N. G. M. Gomes, F. Lefranc, A. Kijjoa and R. Kiss, *Marine Drugs*, **13(6)**, 3950(2015), **DOI:** 10.3390/md13063950
17. J. B. Gloer, B. L. Rinderknecht, D. T. Wicklow and P. F. Dowd, *J. Org. Chem*, **54(11)**, 2530(1989), **DOI:** 10.1021/jo00272a012
18. G. M. Staub, K. B. Gloer and J. B. Gloer, *J. Am. Chem. Soc.*, **114(3)**, 1015(1992), **DOI:** 10.1021/ja00029a033
19. S. Romano, S. A. Jackson, S. Patry and A. D. W. Dobson, *Marine Drugs*,**16(7)**, 224(2018), **DOI:** 10.3390/md16070244
20. D. Li, Y. Xu, C. L. Shao, R. Y. Yang, C. J. Zheng, Y. Y. Chen, X. M. Fu, P. Y. Qian, Z. G. She, N. J. Voogd and C. Y. Wang, *Marine Drugs*, **10(1)**, 234(2012), **DOI:** 10.3390/md10010234
21. C. Prompanya, C. Fernandes, S. Cravo, M. M. M. Pinto, T. Dethoup, A. M. S. Silva and A. Kijjoa, *Marine Drugs*,**13(3)**, 1432(2015), **DOI:** 10.3390/md13031432

[RJC-5284/2019]