Holothurin

by Teresa W

Submission date: 17-Dec-2020 09:32PM (UTC+0700)
Submission ID: 1477697195
File name: Wargasetia_20,_Anticancer_potential,_AIP.pdf (1.6M)
Word count: 1950
Character count: 10786

Anticancer potential of holothurin A, holothurin B, and holothurin B3 from the sea cucumber *Holothuria scabra*

Cite as: AIP Conference Proceedings **2231**, 040084 (2020); https://doi.org/10.1063/5.0002552
Published Online: 22 April 2020

Teresa Liliana Wargasetia, Hana Ratnawati, and Nashi Widodo

Anticancer Potential of Holothurin A, Holothurin B, and Holothurin B3 from the Sea Cucumber *Holothuria scabra*

Teresa Liliana Wargasetia[1,a)], Hana Ratnawati[1,b)], and Nashi Widodo[2,c)]

[1]*Faculty of Medicine, Universitas Kristen Maranatha (Maranatha Christian University)*
Jl. Prof. Drg. Suria Sumantri MPH No. 65 Bandung 40164 Indonesia
[2]*Biology Department, Faculty of Mathematics and Natural Sciences, The University of Brawijaya*
Jl. Veteran Malang 65145 Indonesia

[a)]Corresponding author: Teresa.lw@med.maranatha.edu
[b)]hana.ratnawati@gmail.com
[c)]widodo@ub.ac.id

Abstract. Sea cucumbers have a wide distribution and high abundance in Indonesia. Previous research has shown that sea cucumbers have an anticancer function and have toxicity to various types of cancer cells. Furthermore, we identified the anticancer compounds of the sea cucumber *Holothuria scabra* collected from the South Malang sea. *H. scabra* was extracted using methanol, and then the active compound content was analyzed using liquid chromatography-mass spectrometry (LC-MS). From the results of the analysis, it is known that the methanol extract of *H. scabra* contains three types of anticancer compounds, namely holothurin A, holothurin B, and holothurin B3. Based on in silico analysis, it is estimated that the holothurin compounds have target proteins, namely BCL2, HDAC1, and PTPN2, which play an essential role in the process of apoptosis, cell cycle, and suppressing tumor. The results of this study show that sea cucumber might have anticancer, although various *in vitro* and in *vivo tests* are needed to prove the anticancer mechanism.

INTRODUCTION

Sea cucumbers (or holothurians) are soft-bodied echinoderms, shaped like cucumber with leathery skin and elongated body, habitually live on the lowest level of deep seas [1,2]. Some of them are consumed as nutritious food in some parts of Asia and have been long utilized in Asiatic folk medicine [1,3]. Sea cucumbers contain many valuable compounds for human's health such as vitamins (A, B1, B2, and B3), minerals (calcium, magnesium, zinc, and iron), chondroitin sulfates, fucoidan, glycosaminoglycans, glycoproteins, glycosphingolipids, lectins, non-glycosaminoglycan sulfated glycans, non-sulfated triterpene glycosides (variegatusides), peptides, sphingoid bases, triterpene glycosides (cucumariosides, Ds-echinoside, frondoside A, saponins, sti-choposides), and sterols [2,4].

Many studies have reported anticancer activity of bioactive compounds from sea cucumber through several molecular mechanisms in cancer cells such as cytotoxicity activity, induction of apoptosis, cell cycle arrest, reduction of tumor growth, antimetastatic, anti-angiogenic, and inhibition of drug resistance [5]. Cancer researchers have explored the potential use of substances derived from sea cucumbers as anticancer, which are A1 (HA1) and 24-dehydroechinoside A (DHEA), colochiroside A, cucumarioside A2-2, ds-echinoside A, echinoside A, frondanol A5, frondoside A, glycosides 1 and 2, holothurin A, holothurin B, intercedensides A (1), B (2), and C (3), philinopside A, philinopside E, saponin, scabraside D, sphingoid bases, stichoposide C, and stichoposide D [4,5].

More than 350 species of sea cucumbers were collected and identified from Indonesia ocean, and even new species are still being discovered in eastern Indonesia [6]. Indonesia is the major exporter of sea cucumbers to supply the oriental market of sea cucumbers worldwide [7]. Based on that fact, the authors want to explore Indonesia's natural wealth by studying bioactive compounds from sea cucumber Holothuria scabra and their anticancer mechanism in cells.

International Conference on Life Sciences and Technology (ICoLiST)
AIP Conf. Proc. 2231, 040084-1–040084-5; https://doi.org/10.1063/5.0002552
Published by AIP Publishing. 978-0-7354-1990-2/$30.00

EXPERIMENTAL DETAILS

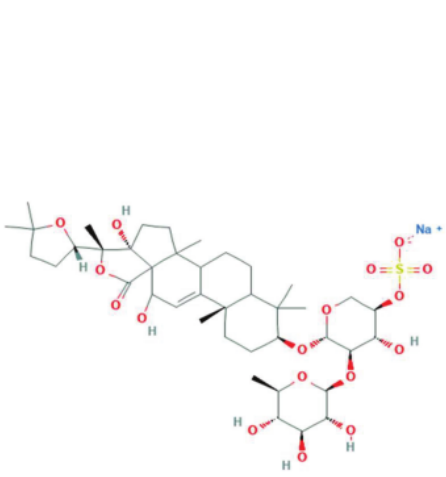
H. scabra were collected from South Malang sea, East Java, Indonesia. Sea cucumber active compound extraction was done by soaking sea cucumber slices in methanol overnight, then was filtered, and methanol evaporated with a rotary evaporator. Then, the active compound content from H. scabra extract was analyzed using liquid chromatography-mass spectrometry (LC-MS).

The active compounds were predicted based on mass and molecular formula from the LC-MS software. The target proteins of the active compounds were predicted by using Swiss Target Prediction (http://www.swisstargetprediction.ch). The compound's properties were examined by using Swiss ADME (http://www.swissadme.ch).

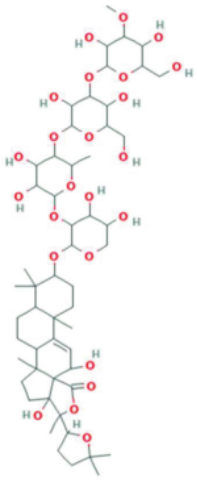
The functions of target proteins on cancer-related pathways were identified using The Biological General Repository for Interaction Datasets (BioGRID, https://thebiogrid.org). BioGRID is an open access repository consist of a database of protein, genetic, and chemical interactions in humans and significant model organisms [8]. Protein-protein interaction networks were identified using a Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, https://string-db.org/) [9]. Mapping of protein in a particular pathway was conducted based on the biological process database on Kyoto Encyclopedia Gene and Genome (KEGG) [10].

RESULTS AND DISCUSSION

The analysis result of active compound content from H. scabra extracts using liquid chromatography-mass spectrometry (LC-MS) is shown in Tabel 1. The analysis result shown that the extract has three main components, namely holothurin A, holothurin B, and holothurin B3. The structures of the holothurians were retrieved from Pubchem, and their drug-likeness properties, which are predicted using Swiss ADME indicated that the compounds have potential as drug profiles (Fig. 1). Both compounds, holothurin A and holothurin B have good solubility, can not penetrate the blood-brain barrier, do not inhibit cytochrome, which is thought to be potential and safe drugs.



(A)



(B)

Molecule	TPSA	MLOGP	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
Holothurin A	268.64	-0.39	No	No	No	No	No	No	-11.37
Holothurin B	361.36	-4.33	No	No	No	No	No	No	-14.42

(C)

FIGURE 1. The structure and characteristics of holothurin A (a) and holothurin B (b) show that both compounds have good solubility, can not penetrate the blood-brain barrier, do not inhibit cytochrome (c)

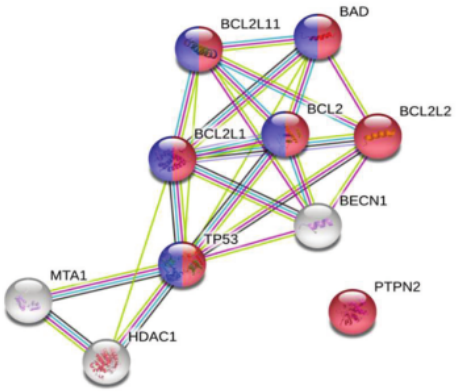
Holothurin is the first anticancer compound of sea cucumber glycoside that was studied by Nigrelli from Actinopyga agassizi [11]. Holothurin, A isolated from the sea cucumber Holothuria fuscocinerea, showed in vitro cytotoxicity against human promyelocytic leukemia cell line (HL-60) and human hepatoma cell line (BEL-7402) [12]. A study by Wang et al. found holothurin A and holothurin B (from Holothuria scabra) cytotoxic activities towards human cervical cancer cell line (HeLa), human hepatoma cell line (HepG2), and human leukemia cell line (K562) which were measured using MTT assay [13]. Yu *et al.* explored bioactive sulfated saponins from Holothuria moebii and revealed that holothurin A and holothurin B had potent dose-dependent inhibiting activity on the proliferation of rat glioma cell line (C6), and human glioma cell lines (U87-MG, U251, and SHG-44)[14].

TABLE 1. Important active compounds of *H. scabra* methanolic extract

No	Name	Amount (%)	Similarity (%)
1	Holothurin A	6.01	92
2	Holothurin B	5.41	92
3	Holothurin B3	6.07	92

In silico analysis gave information about the structure and characteristics of holothurin A and holothurin B that might have targeted some protein. Antiapoptosis protein BCL2, BCL2L1, and BCL2L2 are targets of holothurin A, whereas holothurin B might interact with HDAC1 and PTPN2. Further analysis using BioGRID and STRING revealed the targeted proteins develop a network that may have a role in the pathomechanism of cancer (Fig. 2). This analysis is essential to revealed functions of proteins that interact with holothurin A and B. Mapping of the BCL2 protein, which is the target of holothurin in the apoptotic pathway, is shown in Fig.3.

Compound–protein interactions are crucial to the discovery of new drugs by screening a candidate compound. The H. scabra seems two active compounds, holothurin A and holothurin B, that might have activity as anticancer by targeting BCL2, HDAC1, and PTPN2. The BCL2 inhibits apoptosis by increasing the time-to-death and inherent cell-to-cell variations in the mitochondrial pathway of cell death.[15] HDAC1 has functions in cell cycle regulation and hematopoiesis.[16] Tyrosine-protein phosphatase non-receptor type 2 (PTPN2) is a member of the protein tyrosine phosphatase (PTP) family. The PTP family regulates a variety of cellular processes, including cell growth, differentiation, mitotic cycle, and oncogenic transformation. PTPN2 modulates pancreatic β-cell apoptosis, so that becomes a candidate gene for type 1 diabetes. A study by Kleppe et al. revealed that PTPN2 has a role as a tumor suppressor repressing the proliferation of T cells in T-cell malignancies.[17] Taken together, the study suggested that the H. scabra may contain anticancer substances that warrant for candidates of cancer agent sources.



No	Compound	Predicted of Gene Target		
1	Holothurin A	BCL2	BCL2L1	BCL2L2
2	Holothurin B	PTPN2	HDAC1	

FIGURE 2. Holothurin A and holothurin B are predicted to have target proteins that play a role in the regulation of intrinsic apoptotic signaling pathway (red) and apoptosis pathway (blue)

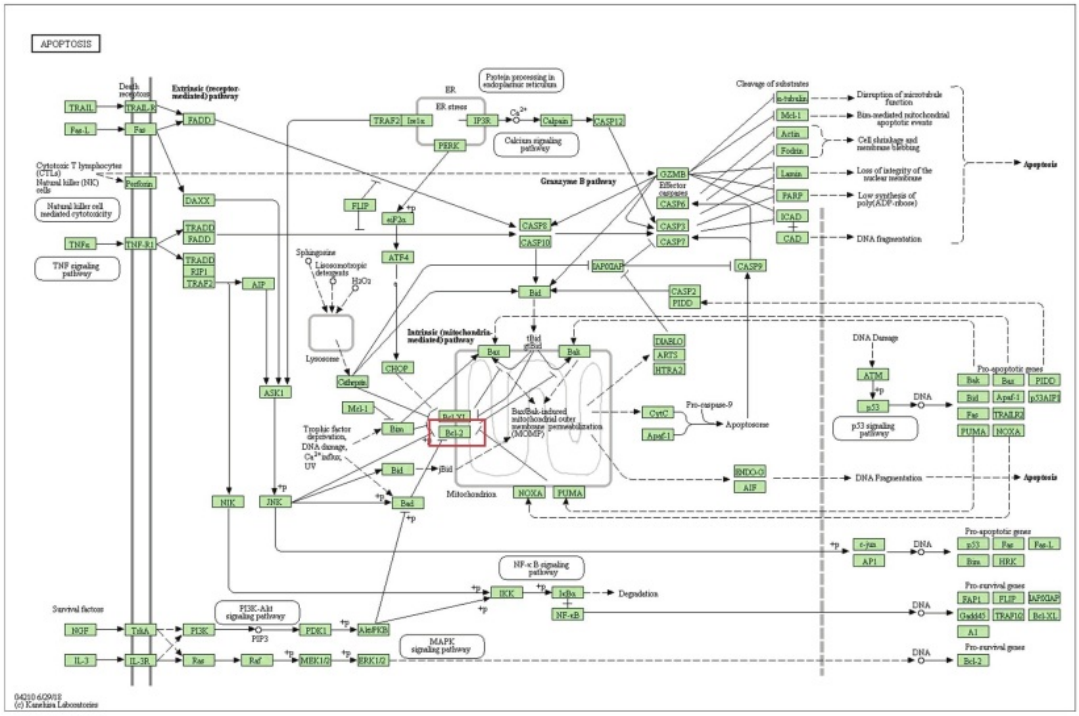


FIGURE 3. The position of the BCL2 protein, which is the target of holothurin in the apoptotic pathway. The pathway was adopted from KEGG Database.

SUMMARY

The methanol extract of H. scabra isolated from Malang coastal contains three types of anticancer compounds, namely holothurin A, holothurin B, and holothurin B3. The holothurin compounds might have proteins target, namely BCL2, HDAC1, and PTPN2, which play an essential role in the process of apoptosis, cell cycle, and suppressing tumor. The results of this study show that sea cucumbers might have the potential for further research for developing anticancer.

ACKNOWLEDGMENTS

The work was supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia with Grant Number: 226/SP2H/LT/DRPM/2019.

REFERENCES

1. N.X. Cuong, L.T. Vien, L. Hoang, T.T.H. Hanh, D.T. Thao, N. Van Thanh, N.H. Nam, D.C. Thung, P. Van Kiem, and C. Van Minh, Bioorganic and Medicinal Chemistry Letters **27**, 2939 (2017).
2. S. Bordbar, F. Anwar, and N. Saari, Marine Drugs **9**, 1761 (2011).
3. M. Shahinozzaman, T. Ishii, R. Takano, M.A. Halim, M.A. Hossain, and S. Tawata, Scientia Pharmaceutica **86**, 1 (2018).
4. T.L. Wargasetia, S. Permana, and Widodo, Current Pharmacology Reports (2018).
5. T.L. Wargasetia and Widodo, Investigational New Drugs **35**, 820 (2017).
6. H. Madduppa, A.A. Taurusman, B. Subhan, N.P. Anggraini, R. Fadillah, and K. Tarman, Biodiversitas **18**, 893 (2017).
7. V. Toral-Granda, L. A, and M. Vasconcellos, *Sea Cucumbers: A Global Review of Fisheries and Trade* (2008).

8. A. Chatr-Aryamontri, R. Oughtred, L. Boucher, J. Rust, C. Chang, N.K. Kolas, L. O'Donnell, S. Oster, C. Theesfeld, A. Sellam, C. Stark, B.J. Breitkreutz, K. Dolinski, and M. Tyers, Nucleic Acids Research **45**, D369 (2017).
9. D. Szklarczyk, A.L. Gable, D. Lyon, A. Junge, S. Wyder, J. Huerta-Cepas, M. Simonovic, N.T. Doncheva, J.H. Morris, P. Bork, L.J. Jensen, and C. Von Mering, Nucleic Acids Research **47**, D607 (2019).
10. M. Kanehisa and S. Goto, Nucleic Acids Research **28**, 27 (2000).
11. D. Aminin, E. Menchinskaya, E. Pisliagin, A. Silchenko, S. Avilov, and V. Kalinin, Marine Drugs **13**, 1202 (2015).
12. S.Y. Zhang, Y.H. Yi, and H.F. Tang, Journal of Natural Products **69**, 1492 (2006).
13. J. Wang, H. Han, X. Chen, Y. Yi, and H. Sun, Marine Drugs **12**, 4274 (2014).
14. S. Yu, X. Ye, H. Huang, R. Peng, Z. Su, X.Y. Lian, and Z. Zhang, Planta Medica **81**, 152 (2015).
15. J. Skommer, T. Brittain, and S. Raychaudhuri, Apoptosis **15**, 1223 (2010).
16. R.H. Wilting, E. Yanover, M.R. Heideman, H. Jacobs, J. Horner, J. Van Der Torre, R. a. Depinho, and J.H. Dannenberg, EMBO Journal **29**, 2586 (2010).
17. M. Kleppe, T. Tousseyn, E. Geissinger, Z.K. Atak, S. Aerts, A. Rosenwald, I. Wlodarska, and J. Cools, Haematologica **96**, 1723 (2011).

Holothurin

ORIGINALITY REPORT

7%	0%	5%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

6%

★ Teresa Liliana Wargasetia, Widodo. "Mechanisms of cancer cell killing by sea cucumber-derived compounds", Investigational New Drugs, 2017

Publication

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	On		