Role of Active Compounds of *Bohadschia argus* Inhibit Cancer Cell Survival

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Abstract

Sea cucumber is marine biota with a high economic value and also has potential for anti-cancer. The purpose of this study was to explore the mechanism of active compound of *Bohadschia argus* on regulating cancer cell survival. The *B. argus* samples were collected from the sea of Kamal Village, West Seram Maluku, then extracted by water. The constituents of water extract of *B. argus* were examined by LC-MS. The network among active compound and its protein target were determined by Cytoscape app. The result shows that *B. argus* has several active compounds, such as chondroitin sulfate, holothurin A, holothurin B, and scabraside that might play a role in cancer cell apoptosis, proliferation, and metastasis.

Keywords: Active compound**,** *Bohadschia argus*, LC-MS.

INTRODUCTION

Indonesia is archipelago country which surrounded by the ocean and has the potential natural resources, especially marine biota. One of the marine biotas that have high economic value is sea cucumber (Holothuriidae). Sea cucumber is an invertebrate animal which included into the phylum Echinodermata and has characters such as soft body, elongated body and rough skin [1].

The previous study has shown that some active compound of sea cucumber contains anti-cancer and anti-inflammatory properties include Monosulfated, Triterpenoid Glycoside, 12-Methyltetradecanoic, Frondoside A, Frondoside B, and Frondoside C [2]. Frondoside A is capable of the treatment for breast cancer [3]. *In vitro* study showed that frondoside A was able to fight cancer by reducing cell viability, inducing apoptosis (activated caspase-3) [4]. This study aimed to investigate the bioactive compounds in the crude extracts of sea cucumber *Bohadschia argus.*

MATERIAL AND METHOD

Bohadschia argus sample in this study was obtained from Kamal Village, West Seram, Mollucas Indonesia. The sample was washed with water to remove dirt and sand. Then the sample was stored on a cold state.

Bohadschia argus Extraction

Extraction of *B. argus* carried out by maceration method. *B. argus* was weighed 25 g and crushed. The sample was macerated into the hot water 80°C ± 500 mL for ± 2 hours. Maceration results were filtered using filter paper. After filtered, the extraction is then separated from water contain using *freezdry.*

Analysis Using LC-MS

The analysis of compound extract *B. argus* was performed by the LC-MS (Shimadzu LCMS - 8040 LC/MS), 1 µL sample was injected into the column 2 mm D x 150 mm 3 µm, Capillary voltage 3.0 kV, with column temperature 35°C and Flow rate 0,5 $mL.min^{-1}$. Desolvation gas flow 6 $L.hr^{-1}$, run time 120 minutes.

Network Construction

Network analysis was used to understanding the effect of the active compound in sea cucumber *B. argus.* The network analyzing active compound with protein was constructed using App Cytoscape 3.6.0.8 [20]. Six proteins related cancer cell was obtained by STRING network diseases then active compounds and protein interaction was established with STICH proteins/ compound network. In the network graphic, proteins and active compounds were presented as nodes, while compounds-proteins and proteins-proteins interaction were presented as edges.

RESULT AND DISCUSSION

Based on the results of LC-MS, the active compound of *B. argus* extract are shown in Table 1. Chondroitin sulfate is an active compound of *B. argus* extract that may act as anti-metastasis [5]. Another compound like Holothurin B and Holothurin A that included in the Triterpene glycoside [6]. Triterpene glycoside is the most abundant compound in sea cucumber [7]. It was

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secondary metabolite on sea cucumber which plays a role in cancer cells with inducing apoptosis by activating caspase, anti-proliferation and arrest cell cycle on S or G2/M [8].

Table 1. Sea cucumber *B. argus* active compound induce apoptosis and cell cycle arrest in breast cancer cell

Compound	Activity	Ref
Frondoside A	Induce Apoptosis	[9]
Holohurin A	Induce apoptosis, Anti-metastasis	[10]
Holohurin B	Induce Apoptosis	[13]
Echinoside A	Induce Apoptosis G0/G1 arrest	[11][12]
Cucumarioside	S phase Arrest	[13]
Chondoitin sulfate	Inhibit proliferation, Anti-Metastasis	[5]
Scabraside	Activated Caspase 3	[4]

Induction apoptosis is one of the most prominent markers of cytotoxic antitumor agents. Some of the natural compounds from sea cucumber induce apoptotic pathways to inhibit cancer progression. Frondoside A induces apoptotic cell through increased expression of P53, and induction CASP9, CASP3, CASP7 cell death in breast cancer cells [4]. Cucumarioside demonstrated anticancer effects through its ability to cause the arrest of the cell cycle during the S phase and was shown to induce apoptosis [13].

Analysis of *B. argus* Active Compound Target Network

Sea cucumber contains several active compounds that potentially for anti-cancer [8]. The interaction among active compound and its protein target have been constructed (Fig. 1). The active compounds interacted with proteins that involve in the apoptosis, metastasis, and proliferation.

The network constructed is shown that several compounds from sea cucumber targets are proteins which play a role in breast cancer like MMP9, CASP9, TP53, CASP3, CHSY1. Some of the active compounds have direct interaction with a protein related to breast cancer and other compounds have indirect interaction. Frondoside A has direct interaction with Matrix metallopeptidase 9 (MMP9), and inhibit the growth of cancer cell [4,9,15]. MMP9 have interaction with several proteins such as TP53, BCL2, NFKB1, and BAX. The compund is capabale of inducing apoptosis through various mechanisms, including the intracellular caspase, decreasing BCL2 and increasing CASP3 [16].

Chondroitin sulfate is one of the active compounds found in *B. argus* interact directly with several proteins like CHSY1, ARSB, and CSPG4. The compound is able to inhibit Chondroitin synthase-1 (CHSY1) expression that links to cell apoptosis and proliferation [17]. CHSY1 interact with CSPG4 responsible for regulating apoptosis and suppressing cell proliferation and metastasis [18]. Several compounds like Echinoside A whereas indirect interact with a protein that has a function for controlling cell cycle and apoptosis [12]. The active compounds in *B. argus* interacted with many proteins that involved in the cell cycle, metastasis, and apoptosis [19]. The ability of the active compounds in sea cucumber compounds to inhibit cancer cell can be modified to fit the genetic profile of cancer cells for the purpose of treatment.

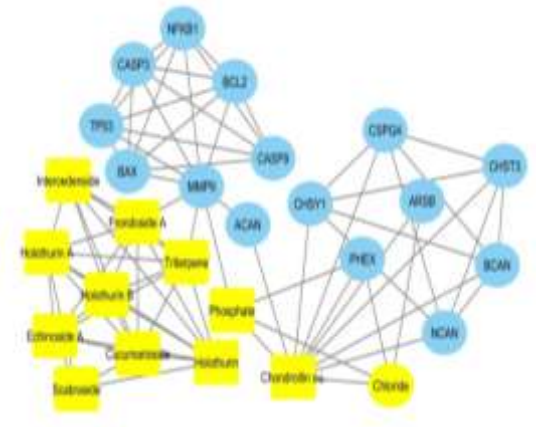

Figure 1. Active compound-protein related cell cycle arrest and apoptosis pathway in the breast cancer cell. The yellow rectangular represents active compound from *B. argus.* The blue circle represents proteins in breast cancer.

CONCLUSION

The *B. argus* contains active compound such as Frondoside A, chondroitin sulfate, echinoside A, holothurin A, holothurin B, and scabraside. The compound has interaction with some of the proteins like MMP9, CASP9, TP53, CASP3, BCL2, BAX, NFKB1, and CHSY1 that play a role on cancer cell apoptosis, cell cycle and metastasis. The mechanism for killing cancer cells using the active compounds contained in sea cucumber *B. argus* has been investigated. Future researchers may conduct a study that develops treatment using the active compounds in *B. argus.*

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