The reducibility of HeLa cell viability by Sargassum polycystum extracts

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Abstract. Cervical cancer is the second largest cause of death-related cancer in women. The efficacy of cancer drugs is still low. Bioactive of brown seaweed has been studied by in vitro and in vivo as anticancer. The aim of this study was to evaluate the cytotoxicity of Sargassum polycystum extracts on HeLa cell, to recognize bioactive on extract and estimate the interaction between the bioactive and target protein. S. polycystum was found from Talango Island waters and HeLa cell was obtained from Indonesian Science Institute. Sample was extracted by ethanol, ethyl acetate and hexane, concentrated and finally, extracts were assayed on HeLa cell. The viability of this cell was quantified on ELISA-Reader. The bioactive compounds of the extract were elucidated by GC-MS. The interaction between bioactive and target protein was evaluated by using in silico method. The result showed that the lowest viability of HeLa cell on n-hexane extracts treatment. The n-hexane extract of this seaweed contained benzenepropanoic acid. This compound reduced HeLa cell viability by reducing of thrombin concentration. In conclusion, the benzene propanoic acid of S. polycystum was the cytotoxic agent and it is potential agent for anti-cervical cancer.

1. Introduction

Cervical cancer is the second highest cause of death in women after heart disease. More than three hundred thousand women died from this cancer each year and mostly occurs by the persistent infection of human papillomavirus [1]. In the future, this disease is predicted to be the main causative of death in women if early detection and curative measures on this disease is not done. Nowadays, the efficacy of cancer drugs distributed and consumed by patients is still low and also has side and resistant effects. The World Health Organisation (WHO) predicts that more than 80% of the world population relies on plant-based medicine for healthcare. These substances are safer and more natural [2].

Brown seaweed is a natural marine resource that contains many secondary metabolites or bioactives and also shows capability as an anticancer ingredient. Fucoidan is the bioactive of this seaweed that is known to have anti-proliferation, antitumor and anticancer properties by inducing apoptosis and inhibiting invasion, metastasis, and angiogenesis of cancer cells. This substance has been known to be able to inhibit the growth of several cancers [3, 4, 5]. There are several active compounds of this seaweed that is also capable in inhibiting and destructing cancer cells. These substances kill cancer cells by activating apoptosis, inhibiting the binding between the oncoprotein, tumor suppressor protein, and function of HPV-16-harboring cervical cancer cells [6].
Sargassum sp. has been grouped as brown seaweed and this alga is also found in Indonesia waters. One species is *S. polycystum* that is obtained and grows abundantly in the waters of Talango Island, District of Sumenep, East Java. Brown seaweed has been known for their anti-cancer potency, however, the exploration and usage of *S. polycystum* as sources of bioactive compounds is very limited and its inquiry as an anti-cervix cancer ingredient has not yet been done. Therefore, the purpose of this study was to obtain the cytotoxic activity of brown seaweed (*S. polycystum*) extract on HeLa cells, find the identity of their bioactives and their interaction with the target protein.

2. Material and methods

2.1. Material

*S. polycystum* was obtained from the waters of Talango Island, Sumenep District, East Java in September 2013. HeLa cells were obtained from the Research Center of Biotechnology, Indonesian Science Institute, Cibinong. Hexane, ethyl acetate, and ethanol were obtained from Merck. 3-(4, 5-dimethylthiazolil-2)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich.

2.2. Methods

*S. polycystum* was washed and dried under the sun until dry. The dried sample was crushed and then sieved to get powder matter. This material was macerated by n-hexane, ethyl acetate and ethanol (1:3; b:v) at 4°C overnight. Afterwards it was filtered by Whatman paper to obtain a supernatant. This was concentrated by a rotary evaporator and was collected in a tight bottle and saved until used for assaying.

2.2.1. Cell Viability

This assay used 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide (MTT) to observe the viability of HeLa cells [7]. A hundred μL of RPMI 1640 was poured and mixed with HeLa cells in 96 microwells to obtain 3 x 10⁴ cell/mL of HeLa cell suspension. Each well was poured with 100 μL of HeLa cells. One well filled with HeLa cell was stated as the negative control. Six wells were mixed with 15 μL of doxorubicin as the positive control and the others were added with 125 μL of ethanol, ethyl acetate and hexane extract of *S. polycystum*, respectively. The viability of cells was observed by the ELISA reader. Absorbance value related positively to the color formation of formazan that shows metabolism activity of the cell.

2.2.2. Gas Chromatography Determination

This analysis used GC-17A Agilent 5890 series II Plus and GC-MS Agilent Technologies 5975C inert MSD with Triple-Axis Detector and the column HP 5-MS. One μL of sample was injected in the column port and carried by Helium with a flow rate 0.7 mL each minute. The injector temperature of GC was 295°C and the oven temperature was programmed starting from 70°C for 2 minutes and was increased by 10°C each minute until 295°C for 28 minutes. The identity of its mass spectra was confirmed by the Wiley Library.

2.2.3. In-silico

HITPICK was used to predict the protein target of *S. polycystum* bioactive and STITCH was exercised to determine the interaction between the *S. polycystum* bioactive and protein target.

2.3. Statistical analysis

Data of HeLa cell viability were analyzed by one way methods. The significant level was set at *P* < 0.05.

3. Results and Discussion

3.1. HeLa cell Viability

Figure 1 illustrates the percentage of HeLa cell viability exposed by the control and n-hexane, ethyl acetate and ethanol of *S. Polycystum* extract. The percentage of HeLa cell viability of n-hexane extract was the lowest among other treatments.
Figure 1. Percentage of HeLa cell viability was treated positive control and n-hexane, ethyl acetate and ethanol extract of S. polycystum.

The viability of cell is a method of measurement on the life or death of the cell. Cytotoxicity of the cell is generally indicated by decreasing cell proliferation, nucleic acid or protein synthesis and cell viability. The alteration on membrane permeability or disturbance on several metabolic pathways is the causality of cell death that is observed by cell viability. Therefore, cell viability can used as the cytotoxicity indicator of a substance [8]. The life cells are able to produce succinic dehydrogenase in their mitochondria. This enzyme will react with the MTT substance to form a formazan crystal that will turn to the color violet. The darkness of the color is indicated and positively correlated with cell life [9].

Figure 1 showed that the HeLa cell viability that was exposed to the n-hexane S. polycystum extract exhibited the lowest measurement. It means that the n-hexane extract of S. polycystum has the capability to kill HeLa cells. The methanol extract of brown algae (Dictyotaciliolata) extract showed the strongest capability to kill HeLa cells [10]. Meanwhile, the dichloromethane extract of brown alga (Cystoseiraabies-marina) acted as a HeLa cell anti-proliferation substance [11]. Although this experiment shows different results from the previous studies, it indicates that brown seaweed is a natural marine resource that contain many bioactive that can be developed and used to act as anticancer drugs [12]. The ability of brown alga extracts to eliminate HeLa cells is caused by their being able to change the morphology and biochemical of HeLa cell. These phenomena are characterized by apoptosis that is marked by loss of cell viability, chromatin condensation, phosphatidylserine externalization and phase accumulation of sub-G1 cycle [10].

3.2. Bioactive Identity
Figure 2 shows the spectra of S. polycystum n-hexane extract. Based on the Wiley Library, it exhibited that the identity of S. polycystum n-hexane extract is grouped in oncarboxylic and fatty acids, i.e.: hexadecanoic acid, benzenepropanoic acid, and arachidonic acid.

Figure 2. Spectra of S. polycystum n-hexane extract by gas chromatography mass spectra.
Figure 2 shows that bioactive compounds that were contained in the n-hexane extract of *S. polycystum* are grouped in the derivation of fatty and carboxylic acids. Many studies have reported that fatty acids were anticancer agents in a variety of human cancer cell lines derived from colonic, pancreatic, prostate, and breast cancers. The fatty acids compound is capable as an anticancer agent by attenuation growth and inducing apoptosis [13]. The mechanisms underlying the anti-tumor effects of omega3-FA are complex. The incorporation of omega3-FA in biological membranes alters the profile of lipid mediators generated during inflammatory reactions. Furthermore, omega3-FA acts as ligands of nuclear peroxisome proliferator-activated receptors that attenuate transcription of NF-kappaB-dependent genes. Thereby, the cyclooxygenase-2/prostaglandin E(2)-dependent production of pro-angiogenic vascular endothelial growth factor and levels of anti-apoptotic Bcl-2 and Bcl-X(L) are decreased. Eicosanoid-independent pro-apoptotic pathways include enhanced lipid peroxidation, modulation of mitochondrial calcium homeostasis and enhanced production of reactive oxygen species as well as activation of p53 [13].

3.3. *Target Protein Prediction and Interaction*

Based on HITPICK and STITCH software analysis, it is suggested that benzene propanoic acid is capable of reducing the viability of HeLa cells by the reduction of thrombin concentration. This protein is known as an anticoagulant, however if its concentration is high in the blood, it will induce tumor growth, angiogenesis, and metastasis. The depressing of thrombin concentration will inhibit several mechanisms of cancer cell viability, such as (1) tumor cell adhesion to platelet cells, endothelial cells, and sub-endothelial matrix protein, (2) enhancement of tumor cell growth, and (3) increasing of tumor cell seeding and spontaneous metastasis [14].

4. Conclusion

The benzene propanoic acid of the n-hexane extract of *S. polycystum* was the cytotoxic agent on HeLa cells by reducing the thrombin concentration mechanism.

5. References

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