

# Phytochemistry profile and *in vitro* cytotoxicity of seaweed macroalgae *Sargassum polycystum* against colon HCT-116 and lung A-549 cancer cells

Ade Arsianti<sup>1,2</sup>, Fadilah Fadilah<sup>1,2</sup>, Anton Bahtiar<sup>3</sup>, Micheylla Kusumaning Dewi<sup>4</sup>, Zoya Marie Adyasa<sup>4</sup>, Daniel Martin Simadibrata<sup>4</sup>, Daniel Amartya<sup>4</sup>

<sup>1</sup>Department of Medical Chemistry, Faculty of Medicine, University of Indonesia, Depok, Indonesia, <sup>2</sup>Drug Development Research Cluster, Indonesia Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jalan Salemba Raya 6, PO Box 1358, Jakarta 10430, Indonesia, <sup>3</sup>Department of Pharmacology, Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia, <sup>4</sup>Medical Student, Faculty of Medicine, Universitas Indonesia, Jalan Salemba Raya 6, PO Box 1358, Jakarta, Indonesia

## Abstract

**Aim:** This research is aimed to develop marine resources which are focused on the phytochemistry profile and exploration of seaweed macroalgae *Sargassum polycystum* as a potential anti-colorectal and anti-lung cancer agents. **Materials and Methods:** Seaweed macroalgae *S. polycystum* collected from Lengkuas Beach, Tanjung Pandan, Province of Bangka-Belitung, South Sumatera, Indonesia, were extracted in organic solvent of *n*-hexane (Hex), ethyl acetate (EA), chloroform (CHCl<sub>3</sub>), and ethanol (ET), respectively. The concentrated extract of Hex, EA, CHCl<sub>3</sub>, and ET was then analyzed by thin-layer chromatography. Phytochemistry test of the extracts was conducted to identify the secondary metabolites containing in the seaweed macroalgae. Furthermore, cytotoxic activity of Hex, EA, CHCl<sub>3</sub>, and ET extracts of *S. polycystum* was evaluated against colon HCT-116 and lung-A549 cancer cells by MTT cell proliferation assay. **Results:** Phytochemistry profile of the concentrated extracts of *S. polycystum* showed the positive result for secondary metabolites of steroid, tannin, alkaloid, triterpenoid, and glycoside. Concentrated extracts of *Sargassum* sp. exhibited cytotoxicity against colon HCT-116 and lung-A549 cells with median inhibitory concentration ranging from 21.3 µg/mL to 33.4 µg/mL. **Conclusion:** These results are suggesting that seaweed macroalgae *S. polycystum* as a potent candidate for the new anti-colorectal and anti-lung cancer agents.

**Key words:** Colon HCT-116 cells, *in vitro* cytotoxicity, lung A-549, phytochemistry, *Sargassum polycystum*

## INTRODUCTION

Cancer is a major cause of morbidity and mortality worldwide. About 14.2 million of new cancer cases arise worldwide in 2012. Cancer was responsible for about 8.8 million deaths in 2015, affecting 1 of 6 deaths overall. Almost 70% of deaths due to cancer happen in low-to-middle-income countries. The incidence of cancer is expected to increase up to 23.6 million by 2030.<sup>[1]</sup> In Indonesia, lung cancer is one of the cancers that have high mortality rate, whereas colorectal cancer is the second highest incidence of cancer suffered by men with 1.8 cases per 100,000 population in Indonesia.<sup>[2]</sup> This fact indicating that Indonesia has quite high incidence rate for colorectal and lung cancers.

Numerous efforts such as surgery, radiotherapy, chemotherapy, or a combination of these methods have been done with the aim to inhibit cancer growth and to cure cancer. However, studies revealed that these methods have various significant side effects and not all therapies are available in tertiary level facilities.<sup>[3]</sup> These facts encourage us to explore and develop new anticancer drug which is more practical and is capable

### Address for correspondence:

Ade Arsianti, Department of Medical Chemistry, Natural Product Chemistry Laboratory, Faculty of Medicine, University of Indonesia, Depok, Indonesia.  
E-mail: arsi\_ade2002@yahoo.com

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of killing cancerous cells without having to give serious side effects to patients, thus increasing their quality of life.

The previous studies reveal that seaweed macroalgae extracts might have the capability in inhibiting growth and treat cancer. It might even beat chemotherapy in its effectiveness of beating cancer.<sup>[4]</sup> The macroalgae synthesize many compounds such as xanthophyll's terpenoids, carotenoids, vitamins, polyunsaturated fatty acids, saturated fatty acids, amino acids, acetogenins, and antioxidants such as alkaloids, polyphenols, halogenated compounds, and polysaccharides such as agar, proteoglycans, carrageenan, alginate, rhamnan sulfate, galactosyl glycerol, laminaran, and fucoidan. Those compounds have been used for many years in pharmaceutical industry to treat a variety of diseases such as AIDS, inflammation, arthritis, infections, and cancer.<sup>[5]</sup>

In our recent studies on seaweeds macroalgae as an anticancer agent, we have investigated phytochemical analysis and cytotoxic activity of seaweeds macroalgae *Eucheuma cottonii*, *Ulva lactuca*, and *Gracilaria verrucosa* against breast MCF-7, colon HCT-116, and cervical HeLa.<sup>[6-9]</sup>

In this work, we focused on *Sargassum polycystum* [Figure 1], one of the brown seaweed macroalgae species that commonly found in Indonesia, and can be developed as anticancer agent. Khanavi *et al.* conducted a study to investigate the cytotoxic activity of *Sargassum swartzii* extract against colorectal adenocarcinoma cells (Caco-2) and reported that n-hexane (Hex) extract of *S. swartzii* which contains non-polar structure of cytotoxic compounds, showed cytotoxicity against Caco-2 cells with median inhibitory concentration (IC<sub>50</sub>) value of 19.38 µg/mL.<sup>[10]</sup> Kim *et al.*, in 2010, revealed non-polar dimer glycoside and polysaccharide fucoidan containing in brown seaweed of *Fucus vesiculosus* demonstrated cytotoxicity on colon HCT-116 cells.<sup>[11]</sup> Another study conducted by Lee *et al.*, in 2013, revealed that polar extract of *Sargassum oligocystum* inhibits the proliferation of leukemia cancer cell, while heterofucan isolated from *Sargassum filipendula* showed the effect of antiproliferation against cervical and prostate cancer cells.<sup>[12]</sup> On the basis of promising cytotoxic activity of *Sargassum* family against a variety of cancer cells has encouraged us to study further the potential cytotoxicity of brown seaweed macroalgae species of *S. polycystum*. This research aimed to investigate the phytochemistry profile of brown macroalgae *S. polycystum*, as well as to evaluate its *in vitro* cytotoxicity against colon HCT-116 and lung A-549 cells. This work is an experimental study to evaluate the effects of *S. polycystum* extract on colon HCT-116 and lung A-549 cancer cells, which will be conducted in several steps. The first step is the collecting and identification of sample *S. polycystum*, followed by maceration and extraction of *S. polycystum* with four different polarity of organic solvent, which are Hex (non-polar), chloroform (CHCl<sub>3</sub>) (non-polar), ethyl acetate (EA) (semipolar), and ethanol (ET) (polar) in the second step. The third step is to analyze the hexane, CHCl<sub>3</sub>, EA,

and ET extracts of *S. polycystum* by phytochemistry test and thin-layer chromatography (TLC). The fourth step is *in vitro* cytotoxicity evaluation of the four extracts of *S. polycystum* against colon HCT-116 and lung A-549 by MTT assay. *S. polycystum* and its classification is displayed in Figure 1.<sup>[13]</sup>

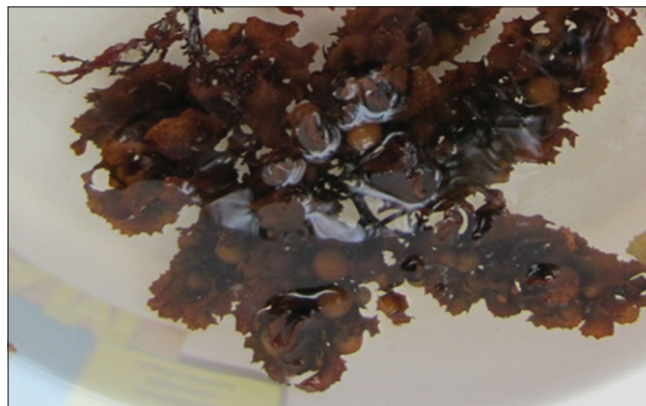
## MATERIALS AND METHODS

### Chemical and Seaweed Materials

All maceration and phytochemistry test were conducted in common oven-dried glassware. Hex, CHCl<sub>3</sub>, EA, ET, and all chemical reagents for phytochemistry test were purchased from Brataco, Indonesia chemical distributor. Cisplatin was purchased from Sigma-Aldrich Chemical Company. TLC of the extracts was analyzed by Merck & Co. precoated silica gel 60 F254 plates. Compounds were visualized by an ultraviolet lamp at the wavelength of 254 nm and 366 nm. Seaweed macroalgae of *S. polycystum* are collected from Lengkuas beach, Province of Bangka Belitung, South Sumatera, Indonesia, on the rainy season of February 2018. The seaweed *S. polycystum* was identified and authenticated at Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences in Cibinong, Bogor, Indonesia. Seaweed material was then dried and grinded to produce powder

### Maceration and Extraction of *S. polycystum*

About 250 g of dry powder sample of *S. polycystum* will be macerated in separate four organic solvents, namely Hex, CHCl<sub>3</sub>, EA, and ET, for 7 days inside a sealed vase container while being stirred at sometimes, 2–3 times a day. Maceration will be done 3 times to get as many extracts as possible. The result of maceration will be filtered and concentrated with rotary evaporator to get Hex extract, CHCl<sub>3</sub> extract, EA extract, and ET extract, respectively. The given extracts will then be analyzed with TLC to a number of phytochemical compounds



**Figure 1:** *Sargassumpolycystum*. The classification of *Sargassum polycystum*: Kingdom - Chromista, Division - Phaeophyta, Class - Phaeophyceae, Order - Fucales, Family - Sargassaceae, Genus - *Sargassum*, Species - *Sargassum polycystum*

containing in each extract. Subsequently, phytochemistry test is done to identify the content of secondary metabolites in *Sargassum polycystum* extract. *In vitro* cytotoxic activity of the four extracts of *S. polycystum* against colon HCT-116 and lung A-549 cancer HeLa cells will be tested using MTT cell proliferation assay.

### Phytochemistry Test

Classes of organic compounds inside Hex,  $\text{CHCl}_3$ , EA, and ET extracts of *S. polycystum* can be identified through phytochemistry test which is done with these following steps.<sup>[14,15]</sup>

Saponin screening is done with vertical shuffle of 10 mL of solution in a test tube for 10 s and then left it still for 10 s. If there is a presence of stabile foam of around 1–10 cm in height for <10 min which stays after the addition of 1 drop of HCl 2N, this means that saponin is present in the solution. Flavonoid screening is done with evaporating 1 mL of solution until it is dry, and the solution left is drenched with acetone and a pinch of smooth powder boric acid and oxalate acid is added, heated carefully with water bath (avoid overheating). The remaining solution is mix with 10 mL of ether. Under 366 nm of UV light, intensive yellow fluorescence suggests the presence of flavonoid compound. Triterpenoid and steroid screening can be identified through Liebermann–Burchard reaction where about 2 mL of solution will be evaporated through porcelain cup and the residue will be diluted into 0.5 mL of  $\text{CHCl}_3$ , and added with 0.5 mL of acetic acid anhydrate. An addition of 2 mL of concentrated sulfuric acid is put into the wall of the tube. Brownish or violet ring on the border of the solution suggests the presence of triterpenoid while blue-greenish ring suggests the presence of steroid. Alkaloid screening is done through evaporating 2 mL of the solution on a porcelain cup. The residue formed after the evaporating process will be diluted with 5 mL of HCl 2 N. The mixture of the solution is divided into three reaction tubes. The first tube will be added with HCl 2 N and this functions as the control. The second tube will be added with three drops of Dragendorff reagent and the third tube will be added with three drops of Mayer reagent. The presence of orange precipitate in the second tube or yellow precipitate in the third tube suggests the presence of alkaloid. Tannin screening is done with reacting 1 mL of solution with iron (III) chloride 10% solution. Dark blue or black-greenish color suggests the presence of tannin. Glycoside screening is done by evaporating 0.1 mL of solution on water bath. The remaining solution is diluted into 5 mL of acetic acid anhydrate and 10 drops of concentrated sulfuric acid will be added. Blue or green product suggests the presence of glycoside (Liebermann–Burchard reaction).

### TLC

TLC is a qualitative analysis to identify the amount of chemical compounds containing in the extract sample, which

is expressed by retention factor (Rf) values. TLC analysis of the extract was begun by preparing the precoated silica gel 60 F254 plates. The extract sample was then applied on the plates by capillary pipe. The plate and mixture of  $\text{CHCl}_3:\text{CH}_3\text{OH} = 3:1$  as a mobile phase was added into the TLC chamber. The mobile phase will diffuse and move along the plate. After this process was complete, the plate was placed under ultraviolet lamp at wavelength of 254 nm and 366 nm to visualize the spots of chemical component and determine the Rf value of each spot.<sup>[16,17]</sup>

### *In Vitro* Cytotoxicity Evaluation by MTT Assay

Cancer cells of colon HCT-116 and lung A-549 taken from the Department of Pathology Anatomy, Faculty of Medicine, University of Indonesia, will be cultured into RPMI 1640 (Gibco, USA) and are supplemented with 10% of fetal bovine serum (Gibco, USA). The supplemented cultured cells will later be incubated in temperature of 37°C and 4%  $\text{CO}_2$  in a humidified atmosphere. The antiproliferating effects of Hex,  $\text{CHCl}_3$ , EA, and ET extracts of *S. polycystum* toward cervical cancer HeLa cells are determined by MTT assay. The sample is diluted until the concentrations reach 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, and 0.4  $\mu\text{g}/\text{mL}$ , and then, it is added to target cells and then it will be incubated for 48 h. An addition of 20  $\mu\text{L}$  of 5 mg/mL solution of MTT phosphate-buffered saline will be added into the target cells. The mixture inside the plates is incubated for 4 h; then, it will be centrifuged with separate medium. About 200  $\mu\text{L}$  of dimethyl sulfoxide is added into every well so that it will dissolve a blue-purple sediment. Absorbance is measured in 590 nm on a microplate reader (Model 550, Bio-Rad, USA). The inhibition rate is calculated using this formula:

$$\% \text{ Inhibition} = 1 - \left( \frac{\text{Absorbance of treatment group}}{\text{Absorbance of control group}} \right) \times 100$$

Where as the  $\text{IC}_{50}$  is calculated using Microsoft Excel 2013 by plotting concentration of extracts in X-axis and percentage of inhibition in Y-axis, from which a linear regression equation was then obtained.

One-way ANOVA test is used to determine statistically significant difference between extracts of *S. polycystum* in their cytotoxicity effect on colon HCT-116 and lung A-549 cells. First, the variance of data of the four extracts will be evaluated. Then, *P*-value will decide if the results show a significant difference or not.  $P < 0.05$ , it shows a significant difference and vice versa.

## RESULTS

Phytochemical analysis was conducted to determine phytochemical secondary metabolites contained in the



**Table 1:** Phytochemical analysis of *S. polycystum* extracts

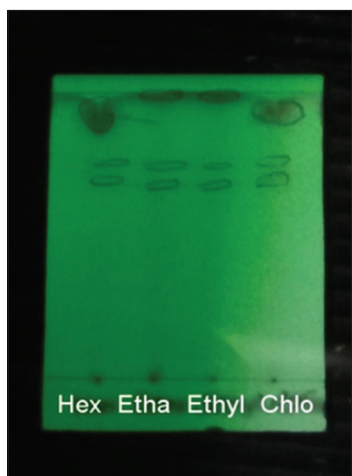
Metabolite	<i>S. polycystum</i>			
	n-Hexane	Chloroform	Ethyl acetate	Ethanol
Saponin	-	-	-	-
Flavonoid	-	-	-	-
Triterpenoid	+	+	+	+
Steroid	+	+	-	-
Alkaloid	-	-	+	+
Tannin	+	-	+	-
Glycoside	+	+	+	+

*S. polycystum*: *Sargassum polycystum*

**Table 2:** TLC results of *S. polycystum* extracts

Extract	Rf		
	1	2	3
n-Hexana (Hex)	0.91	0.73	0.67
Ethyl acetate (Ethyl)	0.32	0.5	0.735
Chloroform (Chlo)	0.91	0.73	0.67
Ethanol (Eth)			0.735

*S. polycystum*: *Sargassum polycystum*, TLC: Thin-layer chromatography



**Figure 2:** Thin-layer chromatography analysis of *Sargassum polycystum*

concentrated extract of Hex, CHCl<sub>3</sub>, EA, and ET of *S. polycystum*. In this research, phytochemistry test was carried out using procedure provided by Ciulei (1982) and Harborne (1997).<sup>[14,15]</sup> Phytochemistry test for concentrated extract of Hex, EA, and ET of *S. polycystum* is summarized in Table 1. TLC analysis of concentrated Hex (Hex), EA (Ethyl), ET (Eth), and CHCl<sub>3</sub> (Chlo) extract of *S. polycystum* is displayed in Figure 2. TLC analysis was applied on silica as stationary phase, using the combination of CHCl<sub>3</sub> and methanol (3:1) as mobile phase and UV lamp with  $\lambda$  255 and 366 nm as a visualized spot. TLC results for *S. polycystum* extracts are displayed in Table 2. Cytotoxicity of cisplatin (a positive control) and four extracts of *S. polycystum* were

evaluated against colon HCT-116 and lung A-549 cells. The results are summarized in Table 3.

## DISCUSSION

### Phytochemistry Profile of *S. polycystum*

As shown in Table 1, non-polar extract of Hex of *S. polycystum* showed positive result for metabolites of triterpenoid, steroid, tannin, and glycoside. Non-polar extract of CHCl<sub>3</sub> showed positive result for metabolites of triterpenoid, steroid, and glycoside. Semipolar extract of EA showed positive result for triterpenoid, alkaloid, tannin, and glycoside, whereas polar extract of ET showed positive result for metabolites of triterpenoid, alkaloid, and glycoside. These results supported by the previous studies on *Sargassum* sp. conducted by Mehdinezhad *et al.* that showed the three species brown seaweeds of *Sargassum* sp., namely *Sargassum angustifolium*, *S. oligocystum*, and *Sargassum boveanum* derived from Bushehr Province of Iran contain secondary metabolites of tannins, steroids, and triterpenoids.<sup>[18]</sup> Another study conducted by Raghavendran *et al.* reported *S. polycystum* collected from Rameswaram beach, India, contains metabolites of triterpenoids and glycosides,<sup>[19]</sup> whereas the study conducted in Malaysia by Daud *et al.* reported *S. polycystum* contains alkaloids, flavonoids, tannins, and steroids.<sup>[20]</sup>

### TLC of *S. polycystum*

TLC analysis in Table 2 showed that three spots of phytochemical compound with Rf are 0.91, 0.73, and 0.67 for Hex and CHCl<sub>3</sub> extract, respectively, whereas EA and ET extract have also three spots with Rf are 0.32, 0.5, and 0.735, respectively. TLC analysis showed that four concentrated extracts of *S. polycystum* have two similar phytochemical compounds in Rf of 0.73 and 0.67.

### Cytotoxicity of *S. polycystum*

Cytotoxic activity is represented by IC<sub>50</sub> value ( $\mu$ g/mL). IC<sub>50</sub> value <100 is considered as an active extract with

**Table 3:** Cytotoxic activity of cisplatin and *Sargassum polycystum* extracts

Extract	IC <sub>50</sub> (µg/mL)**			
	Colon HCT-116	Sig.**	Lung A-549	Sig.**
Cisplatin	27.6±1.1		32.0±1.5	
n-Hexane	31.2±1.3		21.3±1.6	
Ethyl acetate	26.0±1.2	0.027	26.1±1.3	0.031
Chloroform	33.4±1.5		28.4±1.5	
Ethanol	28.6±2.4		32.7±1.2	

\*IC<sub>50</sub> is the 50% half maximal inhibitory activity in µg/mL, expressed in mean value (n=3) ±SD; \*\*Sig.: Statistical significance between extracts of *Sargassum polycystum*, expressed in *P* value. IC<sub>50</sub>: Median inhibitory concentration, SD: Standard deviation

cytotoxic activity.<sup>[21]</sup> As shown in Table 3, all tested extracts have IC<sub>50</sub> value lower than 100 µg/mL that are assigned as active extracts. Compared to cisplatin and other extracts, EA extract of *S. polycystum* showed the strongest cytotoxicity against colon HCT-116 cells with IC<sub>50</sub> value of 26.0 µg/mL, whereas the strongest cytotoxicity against lung A-549 cells has shown by Hex extract of *S. polycystum* with IC<sub>50</sub> value of 21.3 µg/mL. One-way ANOVA test shows the *P* value between *S. polycystum* extracts on colon HCT-116 is 0.027, whereas the *P* value between *S. polycystum* extracts on lung A-549 cells is 0.031. Those *P*-values which are <0.05 indicating that there is statistically significant difference between extracts of *S. polycystum* in their cytotoxicity effect on colon HCT-116 and lung A-549 cells. The cytotoxicity of natural product is affected by the presence of anticancer metabolites. In this study, Hex and EA extracts of *S. polycystum* that contain metabolites of triterpenoid, steroid, glycoside, and tannin were more effective against colon HCT-116 and lung A-549, respectively. This result is comparable to those of another cytotoxic study of *S. swartzii* conducted by Khanavi *et al.* In that study, the hexane fraction of *S. swartzii* that contain a non-polar structure of cytotoxic compounds showed a potent cytotoxic effect on colorectal Caco-2 cells with mechanism cytotoxicity to increase the percentage of apoptotic cells among Caco-2 cells.<sup>[10]</sup> Cytotoxic activity on leukemia P-388 cells has also been noted with seaweed macroalgae *Laurencia mariannensis* that contain metabolite of triterpenoid.<sup>[22]</sup> Kim *et al.* (2010) reported that non-polar compound such as dimer of glycoside and fucoidan polysaccharide responsible for anticancer activity of brown seaweed *F. vesiculosus* against colon HCT-116 cells.<sup>[11]</sup> In addition, a study conducted by Sakagami *et al.* reported that tannin demonstrated the cytotoxicity against squamous cell carcinoma with possible mechanism to induce apoptotic cell death characterized by DNA fragmentation. This result suggesting that tannin is a potential cytotoxic metabolite.<sup>[23]</sup> Thus, the cytotoxicity of Hex and EA extracts of *S. polycystum* in this study might be attributed to the presence of cytotoxic metabolites of triterpenoid, glycoside, and tannin. The apoptosis-inducing properties for the cytotoxicity mechanism of the metabolites containing in Hex and EA extracts of *S. polycystum* remain to be considered in our future studies.

## CONCLUSION

Seaweed *S. polycystum* that contains metabolites of triterpenoid, steroids, glycosides, alkaloid, and tannin showed that the strong cytotoxicity against colon HCT-116 cells and lung A-549 with IC<sub>50</sub> value ranging from 21.3 to 33.4 µg/mL should be further developed as a candidate for new anti-colorectal and anti-lung cancer agents.

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