

# Phytochemical Composition and Evaluation of Marine Algal *Sargassum polycystum* for Antioxidant Activity and *In Vitro* Cytotoxicity on Hela Cells

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## ABSTRACT

**Introduction:** *Sargassum polycystum* is one of marine algal which has a potent antioxidant anticancer activities. This research aims to investigate phytochemical composition, antioxidant activity, and *in vitro* cytotoxicity of marine algal *Sargassum polycystum* on cervical HeLa cancer.

**Methods:** *Sargassum polycystum* collected from Dompu beach, Lombok, Nusa Tenggara Barat Province, Indonesia, were extracted into organic solvent of *n*-hexane, ethylacetate, chloroform and ethanol, respectively. Subsequently, *Sargassum polycystum* extracts were applied for Thin Layer Chromatography (TLC) analysis, phytochemistry test, total phenolic and total flavonoid contents, as well as for antioxidant activity test by DPPH (2,2-diphenyl-1-picrylhydrazyl) method, and *in vitro* cytotoxicity evaluation on HeLa cells by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. **Results:** Phytochemical analysis of *S. polycystum* extracts are positive for metabolites of flavonoid, steroid, tannin and glycoside. TLC analysis revealed that *S. polycystum* extracts containing four phytochemical components. Ethylacetate extract of *S. polycystum* showed the highest total phenolic content, and exhibited greater antioxidant activity than ethanol extract. Total phenolic and total flavonoid content in ethylacetate extract are 548.61 µg/mL and 40.06 µg /mL, respectively. Ethylacetate extract of *S. polycystum* with IC<sub>50</sub> value of 298.3 µg/mL is assigned to have a weak antioxidant activity against DPPH free radical. The results indicate that antioxidant activity of ethylacetate extracts of *S. polycystum* is directly correlated with its total phenolic and flavonoid content. Moreover, *S. polycystum* extracts demonstrated a strong anticancer activity on cervical HeLa cells with IC<sub>50</sub> ranging from 38.3 µg/mL to 112.8 µg/mL. **Conclusion:** This work confirmed that *S. polycystum* are promising natural antioxidant and anti-cervical cancer agents.

**Key words:** *Sargassum polycystum*, phytochemistry, antioxidant, cytotoxicity, HeLa cells.

## INTRODUCTION

Cancer is one of the diseases that is life-threatening and health-threatening in the world as well as in Indonesia. According to data obtained from the Information Center on human papiloma virus (HPV) and Cancer in 2017 in Indonesia, breast cancer and cervical cancer rank the first and second most cancer incidents suffered by women respectively, with the number of cases 39.9 and 17.0 per 100,000 population.<sup>1</sup> The data showed that the prevalence of cervical cancer is quite high in Indonesia. Various attempts have been made to cure cancer which include surgical methods, chemotherapy, hormonal therapy, radiotherapy or combinations of these methods. However, studies conducted by Lage (2003) and Aziz (2006) revealed that some of the previously mentioned healing methods not only cause side effects to occur, but also show resistance to cancer cells, so that they are no longer effective to be applied.<sup>2,3</sup> One of the marine biota that has fairly high diversity and shows anticancer activity is marine macroalgae. Korean researchers reported that the cell wall of brown macroalgae *Fucus vesiculosus* contains sulphate polysaccharide compound, fucoidan, that shows

anticancer activity by inducing apoptosis in HCT-116 colorectal cancer cells.<sup>4</sup> In 2013, researchers from China revealed that extract of water macroalgae *Sargassum oligocystum* inhibited the leukemia cancer cells proliferation, whereas heterofucan compounds isolated from brown macroalgae *Sargassum filipendula* showed antiproliferative effects on cervical and prostate cancer cells.<sup>5</sup> Michanek (1979) and Noda *et al.* (1990) reported that *Sargassum sp.* is an anticancer macroalgae species that is mostly found in Indonesian marine.<sup>6,7</sup> The anticancer potential of macroalgae *Sargassum sp.*, has inspired us to conduct studies that aim to develop Indonesian marine natural products that focus on the exploration of macroalgae *Sargassum polycystum* which is widely available in the coastal areas of Indonesia, as novel potential anticancer. Recently in 2019, we have completed cytotoxicity evaluation of *S. polycystum* extracts originated from Lengkuas beach, Tanjung Pandan, Belitung, Indonesia, which showed a strong cytotoxicity against colon HCT-116 and lung-A549 cells.<sup>8</sup> In this current work, the specific objectives which will be achieved is to obtain *S. polycystum* extract using maceration and extraction techniques; to perform phytochemical analysis and thin layer chromatography (TLC) of *S. polycystum* extract; to

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determine total phenolic and total flavonoid contents of the extract; to evaluate its antioxidant activity and *in vitro* cytotoxicity against cervical HeLa cells.

## MATERIAL

### Marine Algal *Sargassum polycystum*

*Sargassum polycystum* were collected from Dompu beach, Nusa Tenggara Barat, Indonesia (Figure 1).

The morphology of *S. polycystum* is cylindrical talus with small thorns, holdfast forms a small disc and on its top, there is a stolon root which expands to all directions. Short stems with branches, have air bubbles (bladder) which are generally in groups, reaching 7 metres in length, with the brown color of the talus generally.<sup>9,10</sup> The habitat where the *S. polycystum* can grow is widely spread in Indonesia. In Kepulauan Seribu, another area in Indonesia, *Sargassum sp.* is called *oseng*, whilst in Banten this marine algal is known as *kembang karang* or coral flower. *Sargassum sp.* flourish well in the tropics with water temperatures of 25-30°C, salinity of 32-33.5‰, and sunlight intensity ranging from 6500 to 7500 lux. The environment where *Sargassum sp.* grows is usually in clear waters that have the basic substrate composition of a coral reef, dead coral, and volcanic rock. The southern coast of Java, the Sunda Strait, some of the islands in Batam and Bangka-Belitung province in Indonesia that have beaches with coral reef substrate, are ideal habitats for the marine algal *Sargassum sp.*<sup>11</sup>

### Cervical HeLa cancer cell lines

Cervical HeLa cancer cell is the cell culture collection of the Medical Chemistry Department, Faculty of Medicine, University of Indonesia.

## METHODS

### Extraction of Marine Algal *Sargassum polycystum*

Maceration and extraction technique of marine algal *S. polycystum* as follows, 250 gram of dried powder of marine algal *Sargassum polycystum* is macerated with each n-hexane, chloroform, ethylacetate, and ethanol solvents in a tightly closed glass vessel for 7 days, with occasional stirring. Maceration is done three times to extract as



**Figure 1:** *Sargassum polycystum*

Kingdom	: Chromista
Division	: Phaeophyta
Class	: Phaeophyceae
Order	: Fucales
Family	: Sargassaceae
Genus	: <i>Sargassum</i>
Species	: <i>Sargassum polycystum</i>

much substances as possible from algal powder. The mixture of the maceration process are then filtered, the filtrates are concentrated using a rotary evaporator, to produce crude extract of n-hexane, chloroform, ethylacetate and ethanol, respectively. Extracts obtained are then applied for phytochemical test, total phenolic and total flavonoid contents, as well as Thin-Layer Chromatography (TLC) analysis to determine how many chemical components contained in the extracts. Subsequently, antioxidant activity of *Sargassum polycystum* extracts were determined by DPPH method. Furthermore, the cytotoxic activity of the four extracts of *Sargassum polycystum* towards cervical HeLa cells will be tested *in vitro* by using the MTT cell proliferation assay.

### Phytochemistry test

Phytochemistry test is performed to identify the content of chemical secondary metabolites, such as saponin, flavonoid, triterpenoid, steroid, glycoside, tannin and alkaloid in the extract. Procedure of phytochemistry test was adopted from Harborne (1987)<sup>12</sup> and Ciulei (1982)<sup>13</sup>, and was conducted as described in our previous research.<sup>8</sup>

### Determination of total phenolic content

Total phenolic content in *S. Polycystum* extracts were determined by procedure provided by Banerjee *et al* (2008)<sup>14</sup>, with some modifications. A stock solution was prepared by dissolving folin ciocalteu phenol reagent in distilled water (1:10), amount of 100 mL aqueous solution of Na<sub>2</sub>CO<sub>3</sub> at the concentration of 2% (b/v) was also prepared. *S. polycystum* extract (10 mg) was dissolved in 75% of ethanol (10 mL), subsequently, triplicates of 0.5 mL diluted *S. polycystum* extract were added into the test tubes and mixed with 1 mL of Na<sub>2</sub>CO<sub>3</sub> solution. The mixture in the test tubes were then added 0.5 mL folin ciocalteu stock solution, the mixture was then incubated in a dark chamber. After incubation in 30 minutes, the mixture was analyzed by visible spectrophotometer at wavelength of 765 nm. Blank solution was prepared with the same procedure mentioned previously, but without addition of *S. polycystum* extract. Whereas standard solutions were prepared by diluting 1000 µg/mL of gallic acid with 75% (v/v) of ethanol until reached one-set variety concentration of gallic acid standard solution at 0, 6.25, 12.5, 25, 50, and 100 µg/mL. The absorbance of gallic acid standard solutions were measured with UV-Visible Spectrophotometer at 765 nm, and plotted The absorbance versus concentration for linear regression. The absorbance of triplicate extract samples was averaged, the average of absorbance value was inputted and interpolated into the linear regression equation of standard solution gallic acid to obtain total phenolic content of extract sample in µg/mL.

### Determination of total flavonoid content

Determination procedure of flavonoid content was modified from Do *et al.* (2014).<sup>15</sup> *S. polycystum* extract (10 mg) was dissolved in 75% of ethanol (10 mL), subsequently, 600 µL of the extract (triplicates) were placed into the test tubes and were added 20% of AlCl<sub>3</sub> (30 µL) and 2M CH<sub>3</sub>COOK (30 µL). The mixture was then added distilled water until reach volume 1.5 mL and homogenized by vortex mixer. The blank solution was prepared by the same procedure, but without addition of the extract. All mixture solutions in test tubes were incubated in the dark chamber at room temperature. After incubation for 30 minutes, the absorbance values of mixture solutions were measured UV-Vis spectrophotometer at 440 nm.

Standard solutions at the variety concentration of 0, 6.25, 12.5, 25, 50, and 100 µg/mL were prepared by diluting 1000 µg/mL of quercetin with 75% (v/v) of ethanol. The absorbance of quercetin standard solutions were measured with UV-Visible Spectrophotometer at 440 nm. Linear regression graph was generated by plotting the absorbance versus concentration for linear regression. The absorbance of triplicate extract samples was averaged, the average of absorbance value was inputted and

interpolated into the linear regression equation of standard solution to obtain total flavonoid content of extract sample in µg/mL.

To determine total phenolic content in the extract, the absorbance of triplicate extract samples was averaged, the average of absorbance value was inputted and interpolated into the linear regression equation of standard solutions of quercetin.

### Thin layer chromatography (TLC) analysis

Thin layer chromatography (TLC) is a useful method to identify and separate chemical substances in the crude extract based on its polarity and consisting of a mobile phase and the stationary phase. The stationary phase is the TLC plate combined with adsorbent material, such as silica gel. In this work, silica gel plates was used as a stationary phase, whereas the mixture of chloroform (CHCl<sub>3</sub>) and methanol (CH<sub>3</sub>OH) was used as a mobile phase. The sample extract was dropped on the silica gel plate using capillary tube. Then, the silica gel plate was placed into the isolated chamber. The chemical components containing in the extract will move in different rates depending on the polarity and will appear as spot on silica gel plate. UV light at the wavelength of 254 nm and 366 nm is used as visualized spot.

Retention factor (Rf) of each spot of chemical compound can be calculated by the formula:

Rf (Retention factor) = distance traveled by sample/ distance traveled by mobile phase

### Determination of antioxidant activity by DPPH method

DPPH radical scavenging method used in this work was adopted from Rajurkar and Hande (2011)<sup>16</sup>. DPPH fresh solution in concentration of 100 ppm was prepared by dissolving 5 mg of DPPH with 75% of ethanol (50 mL) in dark condition by storing it in amber glass bottle covered in aluminium foil at room temperature. *S. polycystum* extract and ascorbic acid (a positive control) in concentration of 500 ppm was prepared by dissolving 5 mg of *S. polycystum* extract and 5 mg ascorbic acid with 75% of ethanol (10 mL), respectively. Subsequently, 500 ppm of *S. polycystum* extract was diluted with 75% of ethanol to give extract sample solution of *S. polycystum* in concentration of 6.25, 12.5, 25, 50, and 100 ppm (triplicates). Whereas serial ascorbic acid solution in concentration of 3.125, 6.25, 12.5, 25 and 50 ppm were prepared by diluting 500 ppm of ascorbic acid solution with 75% of ethanol. Subsequently, 1.5 mL of each concentration of extract sample solutions and 1.5 mL of each concentration of ascorbic acid solutions were placed into the test tubes and were added 1.5 mL of DPPH solution, respectively. The blank solution was prepared by the same procedure, but without addition of the extract solution or ascorbic acid solution. All of the mixtures in test tubes were incubated for 30 minutes in dark isolated system at room temperature. After incubation in 30 minutes, the mixtures were then transferred into cuvettes and measured the absorbance with UV-Vis spectrophotometer at 517 nm.

DPPH radical scavenging activity of the sample (%inhibition on DPPH) is calculated using

$$DPPH \%_{inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100\%$$

A<sub>control</sub> is an absorbance of control DPPH solution (100 ppm) and A<sub>extract</sub> is the absorbance of samples, either ascorbic acid solutions or *S. polycystum* extract solutions). The absorbance value is an average from triplicates which subtracted with blank absorbance. A linear regression equation: y= ax + b, is obtained by plotting DPPH % inhibition of sample with the concentration. The IC<sub>50</sub> value of the sample (either extract of *S. polycystum* and ascorbic acid) were generated by substitute the coefficient y with value of 50 to give the coefficient x value, which equal to IC<sub>50</sub> value

### *In vitro* Cytotoxicity Evaluation by MTT assay

Cervical HeLa cancer cells were seeded in RPMI 1640 (Gibco, USA) culture medium, which has been supplemented with 10% fetal bovine serum (Gibco, USA). Then, it is incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. The cell viability is determined by 0.1% trypan blue method. Each extract sample of *S. polycystum* were diluted until reach the serial final concentration of 51.2; 25.6; 12.8; 6.4; 3.2; 1.6; 0.8 and 0.4 µg/ml. Subsequently, diluted extract samples were added separately to the target cells and incubated for 48 hours. Amount of 100 µl of 5 mg/mL of MTT phosphate-buffered saline (PBS) was then added into the target cells in the well plate, and the mixture in the well plate was reincubated for 4 hours. The mixture was then centrifuged, the medium is separated. About 100 µl DMSO is added to each well to dissolve the blue purple-colored sediments. The absorbance is measured at 590 nm on a microplate reader (Model 550, Bio-Rad, USA) at the wavelength of 590 nm. The percentage of inhibition was calculated using the formula:

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

Cytotoxicity of the samples of *S. polycystum* extracts on HeLa cells are expressed by median inhibitory concentration (IC<sub>50</sub>) value.

## RESULTS AND DISCUSSION

### Phytochemistry of *Sargassum polycystum*

Phytochemistry test for extract of n-hexane, ethyl acetate, Chloroform and ethanol of *S. polycystum* is summarized in Table 1. As shown, non-polar extract of n-hexane and chloroform of *S. polycystum* showed positive result for metabolites of flavonoid, steroid, tannin, and glycoside. Semi polar extract of ethyl acetate and polar extract of ethanol showed positive result for flavonoid, steroid, and glycoside. This phytochemistry results supported by Mehdinezhad *et al.* (2016) that reported the three species of marine algal *Sargassum sp.* i.e. *S. angustifolium*, *S. boveanum*, and *S. oligocystum*, originated from Province of Bushehr, Iran containing phytochemical metabolite of tannin, triterpenoid, and steroid.<sup>17</sup> Raghavendran *et al.* (2005) found that marine algal *Sargassum polycystum* from Rameswaram coast, India, contains triterpenoid and glycoside.<sup>18</sup> Furthermore, phytochemical studies by Malaysian researcher, Daud *et al.* (2015) revealed that *S. polycystum* showed the positive results for metabolites of alkaloid, flavonoid, tannin, and steroid.<sup>19</sup>

### Total phenolic and total flavonoid contents

Total phenolic content was determined from linear regression equation of the calibration curves of gallic acid (Figure 2a), by substituting Y value of the linear regression equation of gallic acid (Y = 0.0011x + 0.1833, R<sup>2</sup> = 0.9833), with absorbance value of the extract to obtain x value which is equal with the total phenolic content of the extract sample in µg/mL. Whereas, total flavonoid content was determined by substituting Y value of the linear regression equation of quercetin in Figure 2b (Y = 0.0152x + 0.0663, R<sup>2</sup> = 0.9993) with absorbance value of the extract to obtain x value which is equal with the total flavonoid content of the extract sample in µg/mL. Total phenolic and total flavonoid contents of

**Table 1: Phytochemical analysis of *Eucheuma cottonii*.**

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



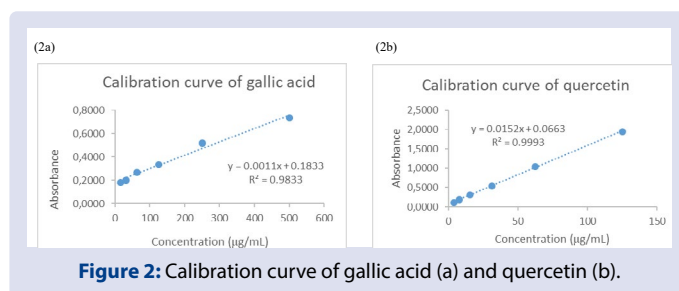


Figure 2: Calibration curve of gallic acid (a) and quercetin (b).

*Sargassum polycystum* extracts were summarized in Table 2. As shown, ethylacetate extract has the highest phenolic content (548.61 mg/mL), followed by chloroform extract (380.21 µg/mL). The highest flavonoid content (49.06 µg/mL) containing in chloroform extract, followed by ethylacetate extract (40.06 µg/mL), whereas the n-hexane and ethanolic extracts of *Sargassum polycystum* contained less amount of phenolic and flavonoid compounds.

### Thin layer chromatography (TLC) analysis of *Sargassum polycystum* extracts

TLC analysis of *Sargassum sp.* using silica plate stationary phase, and a mixture of hexane and ethylacetate in a ratio of 1:1 as a mobile phase, UV lamp at 254 nm and 366 nm as a visualized spot (Figure 3). Retention factor (Rf) of *S. polycystum* extracts is displayed in Table 3. Hexane and ethylacetate extracts of *S. polycystum* have three spots, each at the Rf of 0.69, 0.86 and 0.97 in hexane extracts, and at the Rf of 0.17, 0.86 and 0.97 in ethylacetate extract. Chloroform and ethanol extracts of *S. polycystum* have four spots respectively, at the Rf of 0.17, 0.72, 0.90 and 0.97 in chloroform extract, and at the Rf of 0.14, 0.72, 0.83 and 0.97 in ethanol extract. These results indicate that hexane and ethylacetate extracts contain three chemical components. Likewise, chloroform and ethanol extracts contain four chemical components. TLC results in Table 3 also showed that all these four extracts contain the same chemical compound at the Rf of 0.97. Hexane and ethylacetate extracts of *S. polycystum* has the same spots at the Rf of 0.86 and 0.97, indicating that both of extracts contain the same two chemical compounds. Moreover, ethylacetate and chloroform extracts of *S. polycystum* contain the same two chemical compounds at the Rf of 0.17 and 0.97, while the chloroform and ethanol extracts of *S. polycystum* contain the same two chemical compounds at the Rf of 0.72 and 0.97.

### Antioxidant Activity of *Sargassum polycystum*

Antioxidant activity of ethylacetate and ethanol extracts of *S. polycystum* were evaluated as DPPH free radical scavenger, and depicted by IC<sub>50</sub> value (Table 4). According to Marjoni et al. (2017), the IC<sub>50</sub> value of antioxidant activity can be categorized into four levels: very strong, strong, moderate, and weak. IC<sub>50</sub> value below 50 µg/mL is classified to have very strong antioxidant activity, IC<sub>50</sub> value ranging from 50 to 100 µg/mL is classified to have strong antioxidant activity, IC<sub>50</sub> value ranging from 100 to 250 µg/mL is classified to have moderate antioxidant activity, IC<sub>50</sub> value ranging from 250 to 500 µg/mL is classified to have weak antioxidant activity, and IC<sub>50</sub> value over than 500 µg/mL is classified to have no antioxidant activity.<sup>20</sup> As shown in Table 4, ascorbic acid (positive control) has the strongest antioxidant activity on DPPH free radical with IC<sub>50</sub> of 6.47 µg/mL. IC<sub>50</sub> value of ethanol extract of *S. polycystum* with IC<sub>50</sub> value of 624.76 µg/mL, is assigned to have no antioxidant activity, whereas ethylacetate extract of *S. polycystum* with IC<sub>50</sub> value of 298.32 µg/mL is assigned to have a weak antioxidant activity. Considering that ethylacetate extract of *S. polycystum* possess high total phenolic content (548.61 µg/mL) and high total flavonoid content (40.06 µg/mL), this result indicating that antioxidant activity of ethylacetate extracts of *S. polycystum* is directly correlated with its total phenolic and flavonoid contents.

### *In Vitro* cytotoxicity of *Sargassum polycystum* Against HeLa cells

*In vitro* cytotoxicity of *S. polycystum* extracts and cisplatin as a positive control were determined by the MTT assay, and is expressed by the IC<sub>50</sub> value. The smaller IC<sub>50</sub> value, the stronger cytotoxicity. Table 5 summarizes cytotoxicity of cisplatin and four extracts of *S. polycystum* against HeLa cells. Atjanasuppat et al. (2009) classified anticancer activity of the extract into four groups based on IC<sub>50</sub> value, as follows : IC<sub>50</sub> value below 20 µg/mL is classified as an active anticancer agent, the IC<sub>50</sub> value between 20-100 µg/mL is classified as a moderate active anticancer agent, IC<sub>50</sub> value between 100-1000 µg/mL is classified as a weak anticancer agent, and IC<sub>50</sub> value over 1000 µg/mL is classified as inactive agent.<sup>21</sup> As shown in Table 5, As cisplatin has a very strong cytotoxicity against HeLa cervical cancer cells with IC<sub>50</sub> value of 14.5

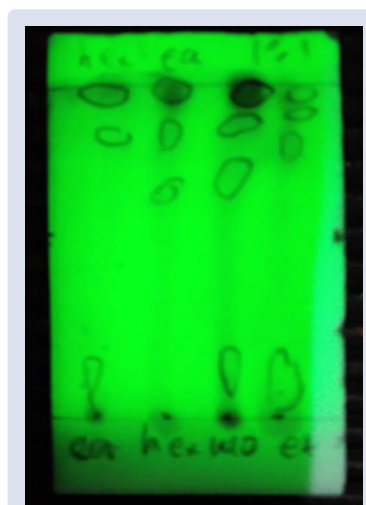


Figure 3: TLC analysis of *Sargassum polycystum*.

Ea: ethylacetate extract; hex: n-hexane extracts; klo: chloroform extract; Et: ethanol extract

Table 2: Total phenolic and total flavonoid contents of *Sargassum polycystum* extracts.

Extract of <i>S. polycystum</i>	Total Phenolic (mg/mL)	Total Flavonoid (mg/mL)
n-Hexane	233.91	28.56
Ethyl acetate	548.61	40.06
Chloroform	340.21	49.06
Ethanol	123.21	14.45

Table 3: Retention factor (Rf) of *Sargassum polycystum* extracts.

Extract	Rf value			
	1	2	3	4
n-Hexane	0.69	0.86	0.97	-
Ethylacetate	0.17	0.86	0.97	-
Chloroform	0.17	0.72	0.90	0.97
Ethanol	0.14	0.83	0.90	0.97

\*Rf = Retention factor

Table 4: Antioxidant activity of *Sargassum polycystum* extracts against DPPH free radical.

Tested Sample	IC <sub>50</sub> (µg/mL)
Ascorbic acid (positive control)	6.47
Ethylacetate extract of <i>S. polycystum</i>	298.32
Ethanol extract of <i>S. polycystum</i>	624.76

**Table 5: Cytotoxicity of *Sargassum polycystum* extracts against HeLa cells.**

Tested sample	IC <sub>50</sub> (µg/mL)
Cisplatin (positive control)	14.5
n-hexane	60.9
Ethyl acetate	112.0
Chloroform	38.3
Ethanol	112.8

\*IC<sub>50</sub> is the 50% half maximal inhibitory activity in µg/mL

µg/mL. N-hexane and chloroform extracts of *Sargassum polycystum* with IC<sub>50</sub> values of 60.9 µg/mL and 38.3 µg/mL, respectively, were classified to have a moderate cytotoxicity against HeLa cells. Whereas, ethylacetate and ethanol extracts of *Sargassum polycystum* were classified to have a weak cytotoxicity on HeLa cells with IC<sub>50</sub> values of 112.0 µg/mL and 112.8 µg/mL, respectively. Compared to other extracts, chloroform extract which has the smallest IC<sub>50</sub> value (38.3 µg/mL) and the highest total flavonoid content (49.06 µg/mL) exhibited the strongest cytotoxicity against HeLa cervical cancer cells. This result suggesting that chloroform extracts that contains high concentration of flavonoid, as well as containing metabolites of steroid, glycoside, and tannin is more effective as anticancer agent against cervical HeLa cells. Flavonoids are well known showing effectivity and interaction with several pathways to inhibit diseases, particularly as an antioxidant and anticancer agents. Flavonoids involve in the mechanism to treat cancers, such as to induce apoptosis, as carcinogenic enzyme and metabolism inhibitors, as well as to induce differentiation.<sup>22,23</sup> Steroid is also known to have ability in inhibiting the progression of cancer, whereas tannin showed antibiotic activity to prevent oral infection caused by dental biofilm.<sup>24</sup> Another study by Sakagami *et al.* (2000) revealed that tannins possessed cytotoxic effect on squamous cells carcinoma by inducing apoptosis.<sup>25</sup> Thus, the cytotoxicity of chloroform extract of *S. polycystum* this study might be due to the presence of flavonoid, steroid, glycoside, and tannin.

## CONCLUSION

Chloroform extract of *Sargassum polycystum* demonstrated the strongest cytotoxicity against cervical HeLa cells (IC<sub>50</sub> value of 38.3 µg/mL), which is potential to be developed as a candidate for new anti-cervical cancer agents. Whereas ethylacetate extract of *Sargassum polycystum* with IC<sub>50</sub> value of 298.32 µg/mL on DPPH free radical, is potential to be developed as a natural antioxidant.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

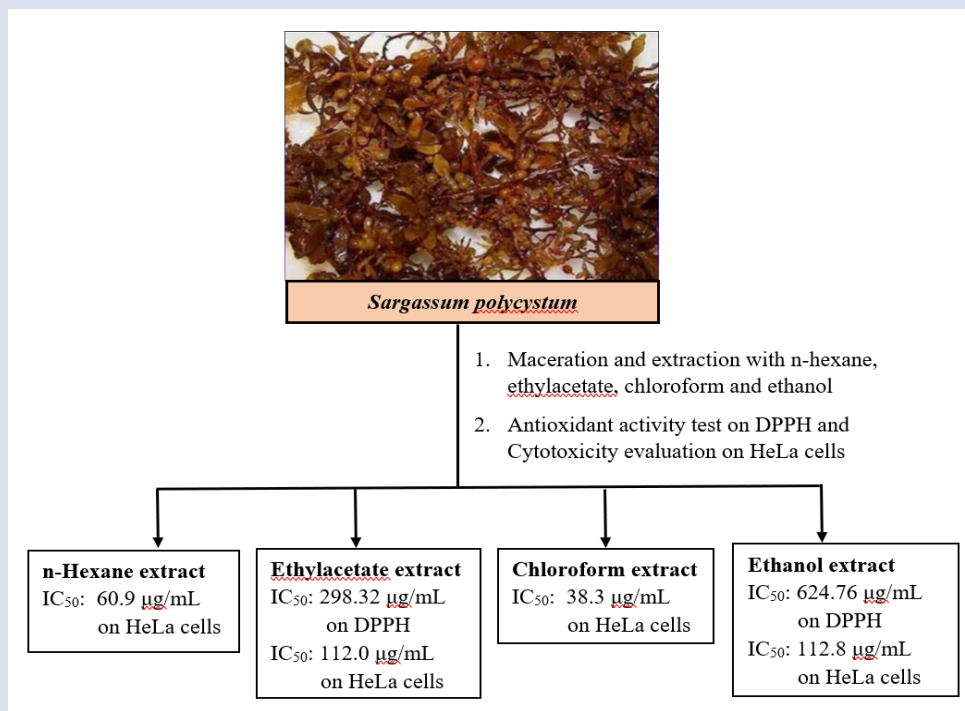
## ABBREVIATIONS

**TLC:** Thin layer chromatography; **DPPH:** (2,2-diphenyl-1-picrylhydrazyl) **MTT:** (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); **IC<sub>50</sub>:** median Inhibitory Concentration; **µg/mL:** microgram/milliliter; **HeLa:** Henrietta Lacks; **g:** gram; **mL:** milliliter; **N:** Normality; **UV-Vis:** Ultra violet-Visible; **RPMI:** Rosewell Park Memorial Institute; **h:** hour; **PBS:** Phosphate-Buffered Saline; **°C:** degree Celsius; **µL:** microliter; **CO<sub>2</sub>:** Carbon dioxide; **Rf:** Retention factor; **USA:** United States of America.

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## GRAPHICAL ABSTRACT



## SUMMARY

Marine algal *Sargassum polycystum* collected from Dompu beach, Nusa Tenggara Barat, Indonesia showed potent antioxidant anti-cervical cancer activities. Phytochemical analysis of *S. polycystum* extracts are positive for metabolites of flavonoid, steroid, tannin and glycoside. TLC analysis revealed that *S. polycystum* extracts containing four phytochemical components. Ethylacetate extract of *S. polycystum* with IC<sub>50</sub> value of 298.3 µg/mL exhibited a weak antioxidant activity against DPPH free radical. Moreover, *S. polycystum* extracts demonstrated a strong cytotoxicity against cervical HeLa cells with IC<sub>50</sub> ranging from 38.3 µg/mL to 112.8 µg/mL.

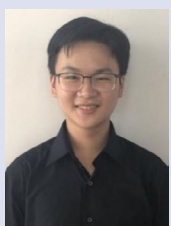
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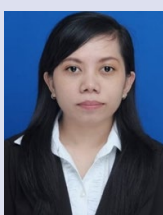
**Vincent Kharisma Wangsaputra:** Third-year medical student in Faculty of Medicine, Universitas Indonesia. Despite technological advancement in treatment and sophisticated therapeutic strategies, neoplasm still remains as the focus of medical problem causing mortality and morbidity. Many researches have been conducted in order to figure out substances that have high potential of anticancer or cytotoxic activity, especially in combating cell resistance. My personal interest of research includes the utilization and incorporation of Indonesian natural compound to be scientifically applied in a way that it can yield efficient antitumorogenic properties towards the cancer cells.



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