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Cytotoxic Activity of Extract and Active Fraction of *Turbinaria decurrens* Bory on Colon Cancer Cell Line HCT-116

Actividad Citotóxica del Extracto y la Fracción Activa de *Turbinaria decurrens* Bory en la Línea Celular de Cáncer de Colon HCT-116

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SUMMARY: Turbinaria deccurrens Bory contains bioactive compound that is beneficial for health. *Turbinaria deccurrens* Bory is one of many species of brown seaweed that grows in Indonesian marine life and has been known to have cytotoxic activity. The aim of this study is to determine fucoxantin content and the cytotoxic activity of extract and fraction *T. decurrens* on colon cancer cell lines. Cytotoxic assay of ethanolic extract, n-hexane, ethyl acetate and ethanolic fractions against HCT-116 by MTS assay using Cell Counting Kit-8 (CCK-8). Fucoxantin content in extract and fraction were analyzed using Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) analysis. Extract and fraction of *T. decurrens* contain fucoxanthin with the highest content of fucoxanthin was in ethyl acetate fraction. CCK-8 assay showed that extract, n-hexane and ethyl acetate fraction inhibited the growth of HCT-116. Brown seaweed Turbinaria decurrens was potential as an anticolon cancer agent.

KEY WORDS: Brown seaweed; Turbinaria decurrens; Colon cancer; HCT-116; Fucoxanthin.

INTRODUCTION

As a maritime country Indonesia has abundant biodiversity including seaweeds or algae. Seaweeds contain various compounds as primary and secondary metabolites. Primary metabolites such as polysaccharides including alginat, carrageenan and agar, have been commercial used in food, nutraceutical, cosmetic and agricultural industries. However secondary metabolites are still less used. Therefore the economic value of brown seaweed is lower than red or green seaweed. So the aim of this study was to find the bioactivity of extract and fraction of brown seaweed.

Six species of brown seaweed (*Sargassum ilicifolium, Sargassum binderi, Turbinaria decurrens, Turbinaria ornata, Padina australis* and *Hormophyta triquetra*) has been studied the cytotoxicity activity to T47D and HeLa cell line. Turbinaria decurrens showed the cell growth inhibition for both cell lines. *T. decurrens* also showed the antioxidant activity and contain high fucoxanthin (Nursid *et al*., 2013). Fucoxanthin is the main component of carotenoid non-provitamin A, with C42-H58O6 formula (Kim *et al*., 2012). Fucoxanthin showed cytotoxic effects by inducing apoptosis of prostate cancer cell line PC-3 (Kotake-Nara *et al*., 2001), leukemia HL-6 (Nakazawa *et al*., 2009), and 6 colorectal cell lines, Caco-2, WiDr, HCT116, DLD-1, SW620, Colo205, Caco-2 cell lines (Takahashi *et al*., 2015). Turbinaria decurrens Bory also contain fucoidan that has been studied showing neuroprotective (Meenakshi *et al*., 2016) and hepatoprotective effects (Meenakshi *et al*., 2014).

Cancer is one of the leading causes of morbidity and mortality worldwide, 8.8 million deaths in 2015, colorectal cancer causes 774,000 deaths (WHO, 2017). Colon cancer can be prevented by selecting the appropriate foods and the lifestyle. The most effective treatment is surgical removal, and patients whose tumour cannot be removed are treated using chemotherapy and radiotherapy. Unfortunately, the five-year survival rate for metastatic colon cancer is below 10 % (Chen & Huang, 2009). Therefore, this study used Turbinaria decurrens to study the activity to another cell line, that was colon cancer cell line HCT-116.

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MATERIAL AND METHOD

Chemicals. Ethanol, n-hexane, ethyl acetate, aquadest, methanol, acetonitrile (Merck), Counting Cell Kit-8 (Dojindo Laboratories, Japan), 5-Fluorouracil (5-FU) (Kalbe), fucoxanthin (Sigma).

Brown seaweed. Brown seaweed Turbinaria decurrens was collected from Binuangen Beach, Banten, Indonesia. The collected seaweeds were washed with tap water and distilled water to remove salt and other debris then packed with iced to keep cool during the transportation to the laboratory.

Extraction and fractination. Two kg wet weight Fresh seaweed Turbinaria decurrens Bory were extracted with 70 % ethanol. Filtrate were evaporated in evaporator with vacuum. Crude extract was dissolved by aquadest (100 ml) and fractionated by liquid-liquid fractionation by n-hexane and ethyl acetate (each 100 ml x 3) respectively and both evaporated in vacuum to get n-hexane and ethyl acetate fractions. Ethanol-aqueous residue was evaporated to get ethanol fraction.

Analysis of fucoxanthin in the extract and fraction. Fucoxanthin content of the extract was analyzed using High Performance Liquid Chromatography (HPLC). Fucoxanthin in extract expressed as mg per 1 gram ethanolic extract and in fraction expressed as mg per 1 gram fraction. HPLC condition used the method as described by Noviendri *et al*. (2011). HPLC analysis carried out using a Shimadzu system equipped with a pump (LC-20AT), UV/Vis detector (SPD-20A). Reversed-phase HPLC (RP-HPLC) analysis were carried out using a C18 RP column, 5 µm particle size, 250 mm x 4,6 mm (YMC Co., LTD). Fucoxanthin content was determined with methanol-acetonitrile (7:3, v/v) as the mobile phase at a flow rate 0.5 ml/min. The amount of fucoxanthin was quantified from the peak area using a standard curve with fucoxanthin standard (Sigma).

Cytotoxic assay. A Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Japan) were used to measure the cytotoxicity of ethanolic extract and n-hexane, ethyl acetate and ethanolic fraction of *T. decurrens* on human colon cancer cell HCT-116. 100 mL HCT-116 cell line (1000 cell/well) were grown in 96-well plates and preincubated for 24 h (temperature at 37 ºC, 5 % CO_2). After 24 h, treated with 100 mL extract and fraction with various concentrations (0-240 mg/mL). 5-fluorourasil (5-FU) was used as positive control and distributed with various concentrations 0-625 mg/mL (Davoodi *et al*., 2013) and fucoxanthin standard (Sigma) as marker substance of brown seaweeds with various concentrations 0 - 150 mg/mL. All samples then incubated for 48 h in incubator. The extent of cell growth was assessed using a CCK-8 assay, CCK-8 solution (10 mL) was added to each well, followed by incubation for 1 h at 37 ºC. The absorbance at 450 nm was determined by multiplate reader. The inhibition percentage of cell growth were calculated with formula: (A-D)-(B-C)/(A-D) x 100 %, where A = control cell absorbance, B = compounds absorbance, C = controls compound absorbance, and D = control media absorbance. The inhibition concentration 50 (IC50) value is defined as the concentration of compound which inhibited 50 % of the cell growth (Nursid *et al*.).

RESULTS AND DISCUSSION

Fucoxanthin content. Fucoxanthin is main component of non-provitamin A carotenoid and has free radical scavenging activity due to unusual allenic double bonds (C=C=C) that is believed to be responsible for its higher antioxidant activity (Agatonovic-Kustrin *et al*., 2016). Fucoxanthin has been investigated as antitumor and cancer-preventative function that indicate inducing G1 cell-cycle arrest and apoptosis in various cell lines (Satomi, 2017).

Ethanol solvent was chosen for extraction because it

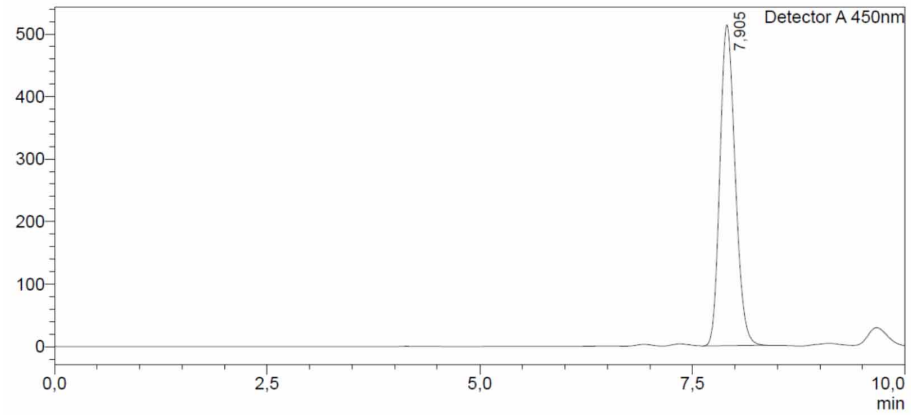

Fig. 1. Chromatograms of 25 ug/ml fucoxanthin standard.

Table I. Yield percentage and fucoxanthin content of *T. decurrens* extract and fractions

	Yield (%)	Fucoxanthin content (mg/g)
Extract	1.4	9.851
n-hexane fraction	19.3	9.869
Ethyl acetate fraction	21.13	10.148
Ethanol fraction	39.27	9.781

provided the best fucoxanthin extraction yield from Phaeodactylum tricornutum (15.71 mg/g freeze-dried sample weight (Kim *et al.*). Fucoxanthin content was analyzed using HPLC in ethanolic extract, n-hexane, ethyl acetate and ethanolic fraction of Turbinaria decurrens. Based on retention time of fucoxanthin standard, peak of fucoxanthin detected in 7.8 min. Peak in thats retention time also showed in extract and fraction (Fig. 2a-d).

The results showed that of fucoxanthin content in the extract and fraction as shown in Table I were analysis using chromatogram of fucoxanthin standard. Fucoxanthin standard has retention time 7.905 min as shown in Figure 1, the extract and all fractions have similar chromatogram at the RT around 7.9 min as shown by red arrow in Figure 2. From this result the fucoxanthin concentration of the ethanolic extract, ethyl acetate fraction, n-hexane fraction, ethanolic fraction are 9.851 mg/g, 10.148 mg/g, 9.869 mg/g and 9.781 mg/g respectively.

Cytotoxicity test. Cytotoxic activity of the extract and fractions of *T. decurrens* was evaluated using CCK-8 assay. After 48 h of treatment, the mortality of HCT-116 cell was shown by treatment of extract, n-hexane and ethyl acetate fraction, but didn't show inhibition activity from ethanolic fraction (Table II). IC50 values of extract and n-hexane, ethyl acetate fraction was 215 µg/ml, 1.512 µg/ml, 3.058 µg/ml, respectively.

Table II. Cytotoxicity against HCT-116 cell line.

	IC_{50} (µg/ml)
Extract	281,16
n-hexane fraction	16,38
Ethyl acetate fraction	11,24
Ethanol fraction	483,32
Fucoxanthin	12,37
5-FU	11.84

Cytotoxic activity from *T. decurrens* to another cancer cell line has been reported. Methanolic extract showed cytotoxic activity to T47D, HepG2 and C26 cells with IC50 value were 172, 360, and 330 µg/ml . After fractionation of the Turbinaria decurrens Bory extract, the cytotoxicity increased with IC50 of n-hexane, ethyl acetate and methanol fraction were 43.1, 51.9, 383.0 µg/ml (Nursid *et al.*). This research indicated that extract *T. decurrens* was potential as an anticancer agent, n-hexane and ethyl acetate fraction also can be considered for the development of anticancer agent.

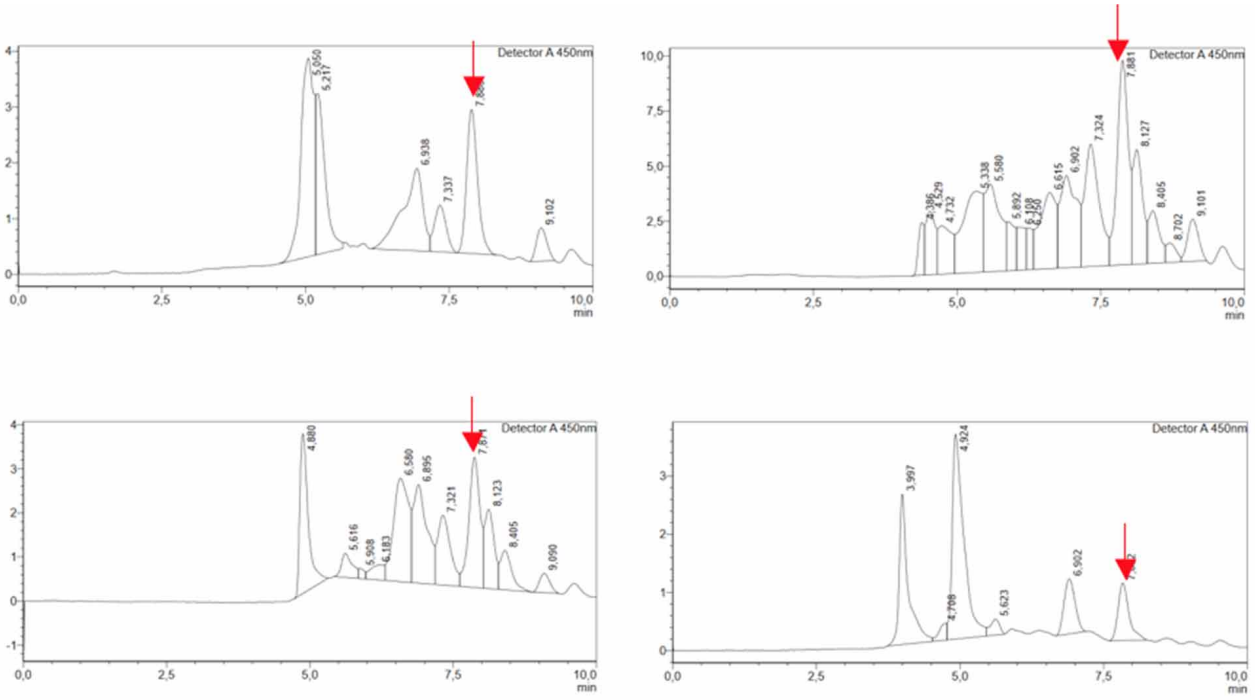

Fig. 2. Chromatograms of fucoxanthin in ethanolic extract (a), ethyl acetate (b), n-hexane (c), and ethanolic (d) fraction of *T. decurrens*. *red arrow indicated fucoxanthin

Cytotoxic activity of *T. decurrens* was compared with fucoxanthin standard and 5-fluorouracil as a positive control. Cytotoxic activity of extract was lower than fucoxanthin and 5-FU, while n-hexane and etyl acetate fraction seem higher than 5-FU but still lower than fucoxanthin.

Fucoxanthin showed inhibition values (IC50) of HCT-116 cell at 1.207 µg/ml, which means fucoxanthin is a potential anticancer agent and supported by antioxidant activity that has IC50 values at 1.0974 ppm (Zailanie *et al*., 2015). Antiproliferative effect of cancer cell line also has been evaluated by Asai *et al*. (2003) and IC50 values on the proliferation of PC-3 cells was 4,6 µM for fucoxanthin. IC50 -of 5-FU was 11.843 µg/ml, its value was not significantly different from Davoodi *et al*., that showed IC50 of 5-FU after 48 h treatment to HCT-116 was 10 µg/ml.

From cytotoxic test, it's seem not only fucoxanthin that affected the cytotoxic activity because it's not showed in ethanolic fraction although it has fucoxanthin. Cytotoxic activity in brown seaweed was not always related to fucoxanthin because brown seaweed has another component, such as steroid, poysaccharide and phenol. 3-keto-22-epi-28-nor-cathasteron and kolest-4-ene-3,6-dion has cytotoxic activity to HEPG-2 and HCT 116. Fucosterol also showed cytotoxic activity to HT-29, Caco-2 dan T47D (Hussain *et al*., 2016). Fucoidan, a polysaccharide, has cytotoxic activity to HCT-15 with IC50 value was 75 µg/mL (Somasundaram *et al*., 2016). Flavonoid that isolated from Turbinaria ornata showed antiproliferation to A549, PC-3, HCT-15 dan MG-63 cells. Bromophenol had IC50 value at 10 µg/mL to HT-1080 and HCT-8 cells (Kim, 2011).

CONCLUSION

Extract, n-hexane and ethyl acetat fraction of Turbinaria decurrens Bory had cytotoxic activity against HCT-116 cell line, and contain fucoxanthin in extract and fraction.

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RESUMEN: *Turbinaria deccurrens* Bory contiene compuestos bioactivos que son beneficiosos para la salud. *Turbinaria deccurrens* Bory es una de muchas especies de algas pardas que crecen en aguas marinas de Indonesia y se ha estudiado su actividad citotóxica. El objetivo de este estudio fue determinar el contenido de fucoxantina y la actividad citotóxica del extracto y la fracción de *T. decurrens* en líneas celulares de cáncer de colon. Se llevó a cabo un ensayo citotóxico de extracto etanólico, n-hexano, acetato de etilo y fracciones etanólicas contra HCT-116 mediante ensayo MTS utilizando Cell Counting Kit-8 (CCK-8). El contenido de fucoxantina en el extracto y la fracción se analizaron usando cromatografía líquida de alta resolución de fase reversa (RP-HPLC). El extracto y la fracción de *T. decurrens* contienen fucoxantina conmayor contenido de fucoxantina en la fracción de acetato de etilo. El ensayo CCK-8 mostró que la fracción de extracto, n-hexano y acetato de etilo inhibía el crecimiento de HCT-116. El alga marrón *Turbinaria decurrens* es un agente potencial contra el cáncer de colon.

PALABRAS CLAVE: Alga marrón; *Turbinaria decurrens*; Cáncer de colon; HCT-116; Fucoxantina.

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