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Research Article

# PHYTOCHEMICAL COMPOSITION AND ANTICANCER ACTIVITY OF SEAWEEDS *ULVA LACTUCA* AND *EUCHEUMA COTTONII* AGAINST BREAST MCF-7 AND COLON HCT-116 CELLS

**ADE ARSIANTI[1]*, FADILAH[1], FATMAWATY[1], LIES K WIBISONO[1], KUSMARDI[2], NORMA NUR AZIZAH[1], RISTA PUTRIANINGSIH[1], TUTIK MURNIASIH[3], ABDULLAH RASYID[3], RATIH PANGESTUTI[3]**

**[1]Department of Medical Chemistry, Faculty of Medicine, University of Indonesia, Jalan, Salemba Raya 6 Jakarta 10430, Indonesia. [2]Department of Anatomical Pathology, Faculty of Medicine, University of Indonesia, Jalan, Salemba Raya 6 Jakarta 10430, Indonesia. [3]Marine Natural Product Division, Research Center for Oceanography, Indonesia Institute of Science, Jl. Pasir Putih I, Ancol Timur, Jakarta Utara, Indonesia. Email: arsi_ade2002@yahoo.com**

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## ABSTRACT

**Objective:** This study is aimed to develop marine resources, which is focused on the determination of phytochemical composition and exploration of seaweeds *Ulva lactuca* and *Eucheuma cottonii*, as a potential antibreast cancer and anticolorectal cancer agents.

**Methods:** Seaweeds *U. lactuca* collected from Parangtritis beach, Yogyakarta, Central Java, Indonesia, whereas *E. cottonii* collected from Salemo Island, South Sulawesi, Indonesia. Seaweeds *U. lactuca* and *E. cottonii* were macerated in organic solvents, *n*-hexane, chloroform, ethyl acetate, and ethanol, respectively. After maceration for 3 days, the mixture was filtered; the filtrate was concentrated by rotary evaporator. The concentrated extract of n-hexane, ethyl acetate, ethanol, and chloroform was then analyzed by thin layer chromatography. Phytochemical test of the concentrated extract was conducted to identify the metabolites containing in the seaweeds. Furthermore, the cytotoxic activity of the n-hexane, ethyl acetate, ethanol, and chloroform extract of *U. lactuca* and *E. cotonii* were evaluated as a growth inhibitor of breast MCF-7 and colorectal HCT-116 cancer cells by MTT cell proliferation assay.

**Results:** Phytochemical test for the concentrated extracts of *U. Lactuca* showed the positive result for metabolites of steroids, glycosides, flavonoids, and tannins, whereas the concentrated extracts of *E. cottonii* showed the positive result for metabolites of steroids, glycosides, and flavonoid. Both concentrated extracts of *U. lactuca* and *E. cotonii* exhibited anticancer activity against breast MCF-7 and colorectal HCT-116 cells with $IC_{50}$ ranging from 21 μg/mL to 99 μg/mL.

**Conclusion:** Our results clearly demonstrate seaweeds *U. Lactuca* and *E. cotonii* as promising candidates for the new antibreast and anticolorectal cancer agents.

**Keywords:** Phytochemistry, *Ulva lactuca*, *Eucheuma cottonii*, Anticancer, Breast MCF-7, Colorectal HCT-116.

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## INTRODUCTION

Cancer is a life-threatening disease. In the world, cancer is the second leading cause of death after cardiac disease. Cancer incidence increased from 12.7 million cases in 2008 to 14.1 million cases in 2012, with the number of died people up from 7.6 million people in 2008 to 8.2 million people in 2012. It is estimated that around 26 million people will suffer from cancer and 17 million people will die because of cancer in 2030 [1].

Based on the research data, in Indonesia, breast cancer is the most cancer suffered by women, with 28.7 cases per 100,000 population, whereas colorectal cancer is the second highest incidence of cancer suffered by men with 1.8 cases/100,000 population [2]. These data show the prevalence of breast cancer and colorectal cancer are quite high in Indonesia. Various attempts have been made to cure cancer, including using surgery, chemotherapy, hormonal therapy, radiotherapy, or a combination of these methods, which is effective to inhibit the growth of cancer cells. However, a recent study revealed that some of these methods have side effects and also showed resistance to the cancer cells [3-5]. This fact prompted us to search and develop new anticancer drugs which are more effective, safer, and potentially to increase the survival rate of cancer patients.

In 2012, we succeeded in synthesizing 18-membered analog of antimycin A which showed anticancer activity against HeLa cells, breast MDA-MB-231 cells, and prostate PC-3 cells [6]. In 2014, we conducted in silico molecular docking study on antimycin A analogs as inhibitors of antiapoptotic Bcl-2 of breast cancer [7]. Recently, in 2015, we have reported the synthesis of novel open-chain analogs of antimycin A which showed a strong inhibitory activity against colorectal HCT-116 cancer cells. As a part of our contribution to discover new anticancer drug, in this research, we explore Indonesia marine resources as potential anticancer agents [8].

Indonesia is a maritime country which has sea area of 2.7 million $km^2$ or it covers around 70% of the area of the Republic of Indonesia, whereas the land area is approximately 1.9 million $km^2$. President of the Republic of Indonesia, Mr. Joko Widodo, in the Summit East Asian Nations which took place in Myanmar on November 2014, described that Indonesia is one of the world's maritime axis based on the natural resources, culture, science, and geography. In his speech, the President asserted that Indonesia as a country of the maritime axis will maintain and manage marine resources [9]. Although it has been known that diversity in marine organism is a gold mine and the source of drugs to treat diseases, such as cancer, malaria, inflammation, and diseases caused by viral infections [10,11], the diversity of the marine resources in Indonesia as material for drug have not been explored optimally.

Seaweeds macroalgae are one of marine resources which have anticancer activity. Researchers from Korea reported that the brown macroalgae of *Fucus vesiculosus* contain a polysaccharide fucoidan, which showed anticancer activity by inducing apoptosis of colorectal cancer HCT-116 cells [12]. In 2013, researchers from China revealed that polar extract of the red macroalgae *Sargassum oligocystum* inhibit the proliferation of leukemia cancer cells, whereas heterofucan isolated from brown macroalgae of *Sargassum filipendula* showed the effect of antiproliferation against cervical and prostate cancer cells [13]. Michanek (1979) and Noda *et al*. (1990) reported the genus of seaweeds *Sargassum* sp., *Eucheuma* sp., and *Ulva* sp., which commonly found in the marine area of Indonesia, showed a potent anticancer activity [14,15]. Anticancer activity of seaweeds *Eucheuma* sp. and *Ulva* sp. has inspired us to conduct research that is aimed to develop marine resources of Indonesia which is focused on the exploration of seaweeds *Eucheuma cottonii* and *Ulva lactuca* as new anticancer agents.

*U. lactuca* and *E. cottonii* are widely spread in Indonesia oceans. *U. lactuca* has a shape like leaf and stands with a thin green thallus. It living in colonies with attached to the substrate with the aid of Holdfast. *E. cottonii* is a species of red seaweed producing carrageenan and has thallus, slippery surface, and cartilaginous. *E. cottonii* has varied color, namely, green, yellow, gray, or red. Classification and physical characteristics of *U. lactuca* and *E. cottonii* display in Fig. 1a and b [16,17].

Indonesian people utilize *U. lactuca* and *E. cottonii* in various fields such as biogas, medical, industrial, and food supplies. *U. lactuca* known to have active compounds as antioxidant, antibiotic, and anticancer. They are also used to cure lung disease (tuberculosis) and rheumatic diseases [18-20]. Metabolites of *U. lactuca* are an alkaloid compound, melatonin which can be found in green algae. Phytomelatonin is an active substance of melatonin containing in the plant which can act as inhibitors and anticancer activity. Whereas *E. cottonii* has been used as food and beverages, manufacture of gelatine. *E. cottonii,* which contains carrageenan, has been used to decrease blood glucose levels as well as to treat cancer [21].

In this research, seaweeds *U. lactuca* collected from Parangtritis Beach, Yogyakarta, Central Java, whereas *E. cottonii* collected from Salemo Island, South Sulawesi, Indonesia. Dried seaweeds of *U. lactuca* and *E. cottonii* were macerate in various organic solvents: n-hexane, chloroform, ethyl acetate, and ethanol, respectively. After maceration for 3 days, the mixture was filtered; the filtrate was concentrated by rotary evaporator. The concentrated extract of n-hexane, ethyl acetate, ethanol, and chloroform was then analyzed by thin layer chromatography. Phytochemical test of the concentrated extract was conducted to identify the metabolites containing in the seaweeds. Furthermore, the cytotoxic activity of then-hexane, ethy lacetate, ethanol, and chloroform extract of *U. lactuca* and *E. cottonii* were evaluated as a growth inhibitor of breast MCF-7 and colorectal HCT-116 cancer cells by MTT cell proliferation assay.

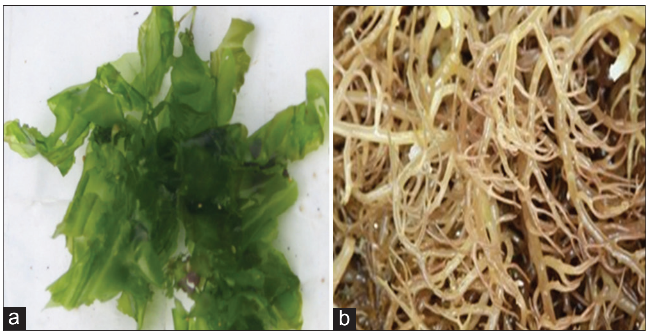


**Fig. 1: (a) *Ulva lactuca*. Kingdom: Plantae; Divisio: Chlorophyta; Class: Ulvophyceae; Ordo: Ulvales; Familly: Ulvaceae; Genus: Ulva; Species: *Ulva lactuca* (b) *Eucheuma cottonii*. Kingdom: Plantae; Divisio: Rhodophyta; Class: Rhodophyceae; Ordo: Gigartinales; Familly: Solieracea; Genus: Eucheuma; Species: Eucheuma cottonii**

## METHODS

### Extraction and fractionation samples of seaweeds

A total of 300 g of dry powder seaweeds of two species of macroalgae, i.e., *U. lactuca* and *E. cottonii* are macerated (soaked) with ethyl acetate solvent in a sealed glass vessel for 3 days, with occasional stirring. Maceration is done three times to extract as much as possible of existing substances in sample of macroalgae. The mixture was then filtered; the filtrate was concentrated using rotary evaporator. Concentrated extracts of each seaweed were further fractionated by column chromatography on silica gel G60 (230-400 mesh ASTM) consecutively using non-polar solvent of n-hexane, semi-polar solvent of ethyl acetate, and polar solvent of ethanol, to produce n-hexane fraction, ethyl acetate fraction, and ethanol fraction. Fractions were then analyzed by thin layer chromatography to determine the number of components or compound contained in the fraction. Furthermore, the fractions were subjected to phytochemistry test to identify the metabolites content in the fractions. *In vitro,* cytotoxic activity of the extracts was evaluated as a growth inhibitor of breast MCF-7 and colorectal HCT-116 cancer cells by MTT cell proliferation assay.

### Phytochemistry test

Phytochemical test was conducted to determine metabolites contained in the concentrated extract of n-hexane, chloroform, ethyl acetate, and ethanol fractions of macroalgae *U. lactuca* and *E. cottonii*. Phytochemistry test was carried out with the following procedures [22].

#### *Saponin test*

Saponin screening is carried out by placed of 10 mL of test solution in a test tube; then, it is shaken vertically for 10 seconds and then left to stand for 10 seconds. 1-10 cm tall foam formation that is stable for not <10 minutes indicate the presence of saponin. For the confirmation, after the addition of 1 drop of 2 N HCl, the foam does not disappear.

#### *Flavonoids test*

Flavonoid screening is done by placed of 1 mL of the test solution was evaporated to dryness, the remaining moistened with acetone, and then added a fine powder fine powder of boric acid and oxalic acid, carefully heated over a water bath and avoid overheating. Residual obtained is mixed with 10 mL of ether. Observed with the ultraviolet 366 nm; yellow fluorescence in solution showed flavonoids.

#### *Triterpenoid and steroids test*

The triterpenoids and steroids test are conducted using Liebermann-Burchard reaction. 2 mL of test solution was evaporated in a porcelain cup. The residue was dissolved in 0.5 mL of chloroform, and 0.5 mL of acetic acid anhydride was added. Then, added 2 mL of concentrated sulfuric acid through the tube wall. The presence of triterpenoids is characterized by the formation of the brownish or violet ring at the boundary of the solution, whereas the presence of steroids is characterized by the formation of blue-green ring.

#### *Essential oils test*

About 1 mL of test solution was pipetted and then evaporated on porcelain dish to obtain a residue. Positive results of the essential oil are characterized by a distinctive odor that is generated by the residue.

#### *Alkaloids test*

About 2 mL of test solution placed on a porcelain cup was evaporated to give a residue. The residue was added 5 mL of 2 N HCl. The resulting solution is divided into 3 tubes. The first tube serves as a blank, was added HCl 2 N, the second tube was added 3 drops of Dragendorff reagent, and the third tube was added 3 drops of Mayer reagent. Positive results of alkaloids characterized by the formation of an orange precipitate in the second tube and the yellow precipitate in the third tube.

*Tannin test*

Tannin phytochemistry test was conducted by reacting of 1 mL of the test solution with a solution of 10% of iron (III) chloride, the positive result for tannin is shown by the formation of dark blue or greenish black.

*Glycosides test*

Glycoside phytochemistry test was carried out by evaporating of 0.1 mL of test solution over a water bath, then dissolve it with 5 mL of acetic acid anhydride. Add 10 drops of concentrated sulfuric acid, the blue or green product is formed, indicating glycosides.

***In vitro* cytotoxicity assay**

MTT assay is performed to measure the antiproliferation effects of an extract of seaweeds Ulva *U. lactuca and E. cottonii* on the breast cancer MCF-7 and colon cancer HCT-116 cells. The sample extract are diluted and added to target cells in triplicates with final concentrations at 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, and 0.4 µg/mL. The cells are incubated for 48 hrs, and 20 µl of 5 mg/mL solution of MTT in phosphate-buffered saline is added to triplicate samples, and the plates are incubated for additional 4 hrs. The plates are then centrifuged, and the medium is removed. 200 µl of dimethyl sulphoxide is added to each well to dissolve the purple, blue sediment; the absorbance is determined at 590 nm on a microplate reader (Model 550, Bio-Rad, USA). The inhibition rate is calculated as follows:

Inhibition rate (%)=1-(absorbance of treatment group/absorbance of the control group) × 100%.

The 50% inhibitory concentrations ($IC_{50}$) of the 48 hrs are calculated with Bliss assay.

**RESULTS AND DISCUSSION**

**Phytochemical composition**

Phytochemistry test for an extract of *U. lactuca* showed the positive result for metabolites of steroids, flavonoid, tannin, and glycosides, whereas the extract of *E. cottonii* showed positive results for metabolites of flavonoids, steroids, and glycosides (Table 1).

Secondary metabolites contained in extracts *U. lactuca* or *E. cottonii* on each organic solvent showed positive result metabolites of steroid and glycosides, whereas metabolites of flavonoid not all of the extracts showed positive results. Phytochemical test on *U. lactuca* showed positive results for metabolites of steroids, flavonoids, triterpenoids, glycosides, phenol, coumarin, and saponin. The results also indicate that steroid and triterpenoids have greater intensity than other metabolites. Steroid most commonly found is a sterol that is steroids alcohol. Sterol in plants is called phytosterol, which is commonly found in higher plants are sitosterol, stigmasterol, and Kaempferol. These compounds can be used in the manufacture of drugs [23].

**Table 1: Phytochemicals analysis of different extracts of *U. lactuca* and *E. cottonii***

| Metabolites | *U. lactuca* | | | | *E. cottonii* | | | |
|---|---|---|---|---|---|---|---|---|
| | HX | CH | EA | ET | HX | CH | EA | ET |
| Saponin | – | – | – | – | – | – | – | – |
| Flavonoid | – | + | + | + | – | + | + | – |
| Triterpenoid | – | – | – | – | – | – | – | – |
| Steroid | + | + | + | + | + | + | + | + |
| Essential oil | – | – | – | – | – | – | – | – |
| Alkaloid | – | – | – | – | – | – | – | – |
| Tannin | – | + | – | – | – | – | v | – |
| Glycosides | + | + | + | + | + | + | + | + |

*U. lactuca: Ulva lactuca, E. cottonii: Eucheuma cottonii* HX: n-hexane, EA: Ethyl acetate, ET: Ethanol, CH: Chloroform

Similar results were obtained from this study, which contains steroid metabolites, flavonoids, and glycosides, but chloroform extracts of *U. lactuca* showed positive results for metabolites of tannin. Differences of metabolites that are contained in same species of *U. lactuca* can be affected by habitat. In this study, both of seaweeds *U. lactuca* and *E. cottonii* contain metabolite of steroid which are potent as anticancer. Besides that, flavonoids in *E. cottonii* show activity as antiallergic, anti-inflammatory, anticancer, and antibiotic [24].

**Anticancer activity of seaweeds *U. lactuca* and *E. cottonii***

After phytochemical test, extracts of *U. lactuca* and *E. cottonii* were further testing its anticancer activity as an inhibitor of cell growth of MCF-7 breast cancer and colorectal cancer HCT-116. *In vitro* cytotoxicity testing conducted as the first step in the screening of potential anticancer compounds. This test using a cell line that provides advantages, such as test material needed is less and it requires short time. Anticancer activity is represented by percentage of inhibition and $IC_{50}$ value. The higher percentage of inhibition the stronger inhibitory activity. The lower $IC_{50}$ value, the greater anticancer activity. Figs. 2 and 3 display the relationship between the percentage of inhibition with the concentration of the extract *U. lactuca* and *E. cottonii* against MCF-7 and HCT-116 cells.

The results showed the percentage of inhibition increases by increasing concentrations of the extract. There is a difference inhibitory activity between *U. lactuca* extract and *E. cottonii* extract against MCF-7 cell proliferation. Increasing in percentage of inhibition is significant in n-hexane, chloroform, and ethyl acetate extracts of *E. cottonii* but did not occur in ethanolic extract of *E. cottonii* (Fig. 2a, b and d). Whereas the percentage of inhibition of hexane, chloroform, and ethanolic extract of *U. lactuca* are found fluctuative, with exception in ethyl acetate extract. However, it does not affect the $IC_{50}$ value. Overall, the percentage inhibition of *U. lactuca* and *E. cottonii* extracts in ranging from 24% to 58% against MCF-7 cells.

Fig. 3 shows the percentage of inhibition of extracts of *U. lactuca* and *E. cottonii* against HCT-116 cells. Different with the inhibition in MCF-7 cells, the percentage inhibition of *E. cottonii* extract in HCT-116 cells showed no directly proportional to the concentration of the extract (Fig. 3a-d). While the percentage inhibition of *U. lactuca* extract in HCT-116 cells showed directly proportional to the concentration of the extract. *E. cottonii* extract has the percentage of inhibition greater than the percentage of inhibition of *U. lactuca* extract. Overall, the percentage of inhibition of *U. lactuca* and *E. cottonii* extracts in ranging from 24% to 65% against HCT-116 cells.

Anticancer activity of the extracts of *U. lactuca* and *E. cottonii* were evaluated against breast MCF-7 and colorectal HCT-116 cells; the results are summarized in Table 2.

Anticancer activity is represented by an $IC_{50}$ value (µg/mL). $IC_{50}$ value <100 is considered as an active compound with anticancer activity. $IC_{50}$ value in ranging from 100 to 300 is considered as weak anticancer activity, whereas the $IC_{50}$ value over than 300 is considered as inactive compounds.

As shown in Table 2, chloroform extract of *U. lactuca* on MCF-7 has $IC_{50}$ value >300 µg/mL and it is assigned as an inactive extract. While Delirium tremen (DT)-ethanol, ethyl acetate extract of *U. lactuca, and* ethanol, chloroform, DT-hexane, and ethyl acetate extracts of *E. cottonii* which have $IC_{50}$ values ranging from 100 to 300 µg/mL are assigned as extracts with weak activity in inhibiting the growth of cancer cells MCF-7. The best anticancer activity has shown by hexane extract of *U. lactuca* and Du-ethanolic extract of *E. cottonii* which has $IC_{50}$ value of 45.1 µg/mL and 75.7 µg/mL, respectively. This result indicates that hexane extract of *U. lactuca* and Du-ethanolic extract of *E. cottonii are* potential to be developed as an antibreast cancer drug.

Du-ethanolic extract of *E. cottonii* with $IC_{50}$ value over than 300 µg/mL showed no inhibitory activity against colorectal HCT-116 cells. While ethanol,

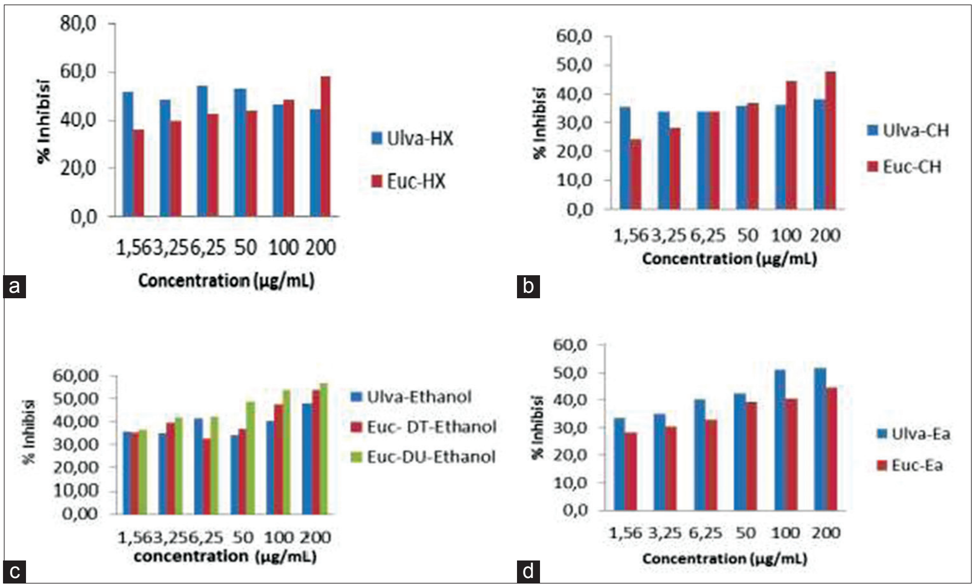


**Fig. 2: Relationship concentration (mg/mL) extract of *Ulva lactuca* and *Eucheuma cottonii* with percent inhibition (%) against the cell line MCF-7 (a) hexane extract (b) chloroform extracts (c) ethanol extract (d) ethyl acetate extract. Euc: *Eucheuma cottonii; Ulva: Ulva lactuca***

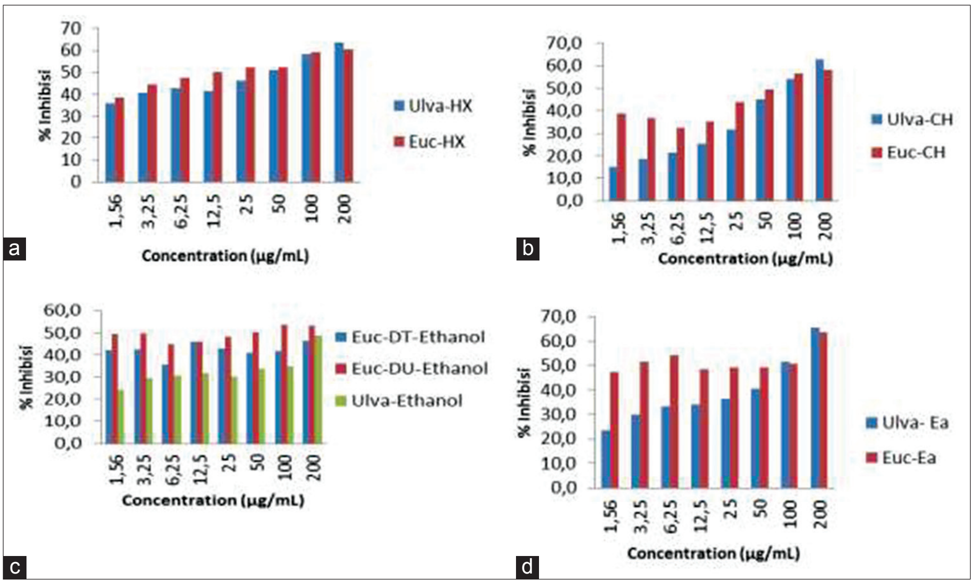


**Fig. 3: Relationship concentration (mg/mL) extract of *Ulva lactuca* and *Eucheuma cottonii* with percent inhibition (%) against the cell line HCT-116 (a) hexane extract (b) chloroform extracts (c) ethanol extract (d) ethyl acetate extract. Euc: *Eucheuma cottonii*, Ulva: *Ulva lactuca***

**Table 2: Anticancer activity of *U. lactuca* and *E. cottonii* ($IC_{50}$ µg/mL) against breast MCF-7 and colorectal HCT-116**

| Tested extract | $IC_{50}$ (µg/mL)* MCF-7 | HCT-116 |
|---|---|---|
| *U. lactuca* | | |
| Ethanol | 246.8±2.5 | 225.0±2.5 |
| Chloroform | 905.0±2.8 | 116.2±1.6 |
| Hexane | 45.1±1.7 | 69.3±1.2 |
| Ethyl acetate | 147.0±1.9 | 106.3±2.8 |
| *E. cottonii* | | |
| DT-ethanol | 149.5±2.8 | 65.3±1.8 |
| Du-ethanol | 75.7±1.3 | 419.1±2.7 |
| Chloroform | 189.0±2.2 | 99.3±1.8 |
| DT-Hexane | 111.5±1.9 | 43.0±1.3 |
| Ethyl acetate | 259.0±2.6 | 21.4±1.4 |

*U. lactuca: Ulva lactuca, E. cottonii: Eucheuma cottonii*. DT: Fractional maceration; DU: Repeated maceration. *$IC_{50}$ is the 50% half maximal inhibitory activity in µg/mL, expressed in mean value (n=3)±SD. SD: Standard deviation

chloroform, and ethyl acetate extracts of *U. lactuca* which have $IC_{50}$ values ranging from 100 to 300 µg/mL are assigned as the extract with weak activity in inhibiting against colorectal HCT-116 cells. DT- ethanol, chloroform, DT-hexane extract of *E. cottonii* and hexane extract of *U. lactuca*, which has $IC_{50}$ value <100 µg/mL against colorectal HCT-116 cells, are assigned as an active extract. The strongest inhibitory activity has shown by ethyl acetate extract of *E. cottonii* with $IC_{50}$ value of 21.4 µg/mL against HCT-116 cells. Thus, ethyl acetate extract of *E. cottonii* should be developed as a promising candidate for anticolorectal cancer drug.

## CONCLUSION

Our results clearly demonstrate seaweeds *U. lactuca* and *E. cotonii* as promising candidates of new antibreast and anticolorectal cancer agents.

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