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Original Article

Comparison the cytotoxic effects of *Ulva fasciata* and *Ulva lactuca* on the MCF-7 and MDA-MB-231 breast cancer cell lines

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ABSTRACT

Introduction: Marine seaweeds has received increased attention in the protection or treatment of cancer, because of their bioactive compounds. The aim of the present study was to evaluate the anti-cancer capacity of two green seaweeds, *Ulva fasciata* and *Ulva lactuca*.

Methods: The phenolic and flavonoid content of the hydro-methanolic extracts was measured, respectively by Folin-Ciocateu and aluminum chloride methods. The antioxidant activity of the extracts was evaluated by FRAP and DPPH assay and were compared to ascorbic acid. Cytotoxic effect of the extracts on the MCF-7 (ER^+) and MDA-MB-231 (ER^-) breast cancer cell lines was also evaluated by MTT assay after 48 and 72 hours of incubation.

Results: The phenolic and flavonoid content of *Ulva fasciata* was respectively 14.92±1.38 µgGAE/mg and 72.15±15.4 µgQE/mg which was significantly higher than *Ulva lactuca*. The reducing power and radical scavenging activity of *Ulva fasciata* was also higher. The cytotoxic effects of *Ulva fasciata* on the MCF-7 and MDA-MB-231 cell lines was more than *Ulva lactuca*, in concentration and time dependent manner. The cytotoxic effects of the both seaweeds were more potent on the MDA-MB-231 compared to the MCF-7 cell line and indicated an estrogen and progesterone receptor independent manner of cellular growth inhibition.

Conclusion: It is appeared that the *Ulva fasciata* extract was a better drug candidate in treatment of triple negative breast cancer due to higher antioxidant activity, phenolic and flavonoid content. Further studies in fractionation and bioactive extraction of *Ulva fasciata* are recommended.

Keywords:
Ulva fasciata
Ulva lactuca
Breast cancer
MTT assay
Antioxidant

Introduction

Cancer has become a serious crisis, which has led to the great mortality all over the world. Cancer is still one of the major obstacles of many countries despite of the vast improvement in the health care system. the number of new cancer cases is expected to rise by approximately 70% over the next 20 years, based on WHO evaluation (Arem and Loftfield, 2018). The latest statistic which

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is published by American Cancer Society reveals that 1,762,450 new cancer cases and 606,880 cancer deaths in 2019 are occurred in the United States (Siegel et al., 2019). Breast cancer is one of the most common malignancy, which accounts for about 14,000 deaths each year. Currently, the breast cancer therapy depends highly on the stage and tumor's subtypes. Surgical resection, chemotherapy, radiotherapy and hormone therapy are among the most common therapies, which are vastly being used. Although due to drug resistant, side effects and their high costs, new and more therapeutic drug should be developed (Waks and Winer, 2019).

Marine seaweeds are one of the traditional products which has recently received attention in the protection and treatment of cancer (Liu et al., 2012). *Ulva fasciata* and *Ulva lactuca* are among these traditional products and belong to Chlorophyceae family. These seaweeds contain rich resources of many minerals, amino acids and vitamins and produce a wide range of secondary antioxidant metabolites (Langeswaran et al., 2019; Lee et al., 2013). The aim of the present study was to evaluate the capacity of *Ulva fasciata* and *Ulva lactuca* in development of future new anti-cancer drugs. Therefore, the phenolic and flavonoid content of the *Ulva fasciata* and *Ulva lactuca* extracts was evaluated and their antioxidant activity and cytotoxic effects on the MCF-7 and MDA-MB-231 breast cancer cell lines was compared.

Materials and methods

Collection and extraction of the seaweeds

The seaweed samples were collected from the Persian Gulf in the coast of Bandar-Abbas, Hormozgan province, Iran. The collected seaweed species were identified by experts of Fasa Medicinal Plants Research Center (FMPRC) as *Ulva fasciata* and *Ulva lactuca*. The voucher numbers of FMPRC-100-5 and FMPRC-100-6 was assigned for the *Ulva fasciata* and *Ulva lactuca* respectively in the herbarium of FMPRC, Fasa University of Medical Sciences, Iran. This study was approved by Medical Ethics Committee of Fasa University of Medical Sciences (Code: IR.FUMS.REC.1396.314).

Extraction was done by hydro-methanolic maceration method. The powders of *Ulva fasciata* and *Ulva lactuca* (100 g) was immersed in methanol (70:30 v/v) and kept at room temperature and darkness for one week with continuous stirring. The solid and insoluble particles were removed by centrifugation (500 g) and filtration (wattman filter 14). The excess solvent was evaporated at 55°C and the concentrated extract was incubated at 45°C for 24h to dry (Hoseinzadeh and Shirazinejad, 2019; Meshkibaf et al., 2010). Dried extracts (500 mg) was dissolved in 5 mL of dimethylsulfoxide (DMSO) (Merck, Germany) to prepare a 100 mg/mL stock solution of the extracts. The solution was sterilized with a 0.22 µm filter and diluted 1:100 (v/v) to prepare the working solution of 1 mg/mL with distilled water and complete Dulbecco's modified Eagle medium (DMEM, containing 10% fetal bovine serum) for biochemical and cell culture experiments respectively.

Measurement of phenolic content of the extracts

The phenolic content of the *Ulva fasciata* and *Ulva lactuca* extracts was measured using the Folin− Ciocateu method. Accordingly, 500 µL of the Folin− Ciocateu reagent (10% v/v) was added to the 100 µL of the each extract (1 mg/ml) and incubated for 5 min at room temperature and darkness. Then 400 µL of sodium carbonate (7.5% w/v) was added to the sample and was kept at room temperature and darkness for 60 min. Finally, absorbance of the samples was measured at 765 nm by Synergy HTX multi-mode reader. Gallic acid was also used as standard and the phenol content of the extracts was reported in microgram gallic acid equivalent (GAE) per milligram of dry weight (µg GAE/ mg) (Hoseini et al., 2019). All measurements were done in duplicate.

Measurement of flavonoid content of the extracts

Aluminum chloride method was used to measure the flavonoid content of the extracts. Accordingly, 50 µL of the aluminum chloride (10% w/v) and 50 µL of the sodium nitrite (10% w/v) were added to 200 µL of the each extract (1 mg/mL). The solution was incubated 5 min at room temperature and darkness, then 700 µL of sodium hydroxide (4% w/v) was added and the total volume of the solution was 1 mL. After complete stirring, the solution was again incubated at room temperature and darkness for 15 min and the sample absorbance was read at 510 nm using a Synergy HTX multi-mode reader. Quercetin was also used as standard and the flavonoid content of the extracts was reported in microgram quercetin equivalent (QE) per milligram of dry weight (µg QE/ mg) (Moulazadeh et al., 2021). All measurements were done in duplicate.

Evaluation of antioxidant activity

In the present study, ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) methods were used to compare the antioxidant activity of the *Ulva fasciata* and *Ulva lactuca* extracts. The FRAP assay was used to investigate the potency of monovalent antioxidants that capable of reducing Fe^{3+} to Fe^{2+} ions. Whereas in the DPPH method, the total radical scavenging activity is determined with reducing the stable nitrogen radical (DPPH radical) (Valverde Malaver et al., 2015).

Evaluation of the monovalent reducing power

As mentioned, the FRAP assay measures the antioxidant capacity of the extracts by reducing the Fe^{3+} ions (present in the Fe-TPTZ complex) to Fe^{2+} ions, so it's called monovalent reduction (Gallego et al., 2013). According to the previous study (Wang et al., 2016), 50 µL of the seaweed extracts (1 mg/mL) were added to the FRAP working solution (1.5 mL) and mixed. The absorbance of the colored solution was read at 593 nm after 10 min of incubation at 37°C. Serial dilution of $FeSO_4$ solution (1 mM) was used as standard and the antioxidant activity of the extract was reported in µmolFe^{2+}/g. All measurements were done in duplicate.

Determination of total radical scavenging activity

Total radical scavenging activity of the extracts is determined by DPPH assay. The DPPH radicals has a purple color that turns to yellow after reduction by antioxidants in the herbal extracts. The intensity of colorlessness indicates the antioxidant activity of the extracts. Accordingly, serial dilution of the extracts (100, 200, 500 and 1000 µg/mL) was prepared using ethanol 70%. The prepared extracts (40 µL) was added to 160 µL of the DPPH radicals (0.3 mM) and kept at room temperature and darkness for 30 min. Finally, the optical absorption changes of the samples were determined at 517 nm using Synergy HTX multi-mode reader. All measurements were done in duplicate. Ascorbic acid, a potent antioxidant, was used as a basis for comparing the results (Wang et al., 2016). The antioxidant activity was calculated as the percentage of the free radicals reduction relative to the control group using the following equation:

Antioxidant activity = [(optical absorption of the control group- optical absorption of the experimental group)/ optical absorption of the control group]× 100

Cell culture

Two invasive breast ductal carcinoma cell lines, the human MDAMB231 (estrogen receptor negative [ER−] with aggressive phenotype) and MCF7 (ER+, less aggressive) cell lines were purchased from the National Cell Bank of Pasteur Institute of Iran. Cells were cultured in DMEM (Gibco, USA) with 10% fetal bovine serum (Gibco, USA) and 1% penicillinstreptomycin (Bio idea, Iran) at 37°C in a humidified 5% CO_2 incubator. Cells with confluency of 70% were used for subsequent experiments (Galavi et al., 2016).

Morphology alteration and cytotoxic effects

Cell viability and cytotoxic effect of the extracts were carried out through the MTT assay as described previously (Galavi et al., 2016). The MDA-MB-231 and MCF-7 cell lines with a density of 10,000 cells per well (150 µL) were seeded in the 96-well plates and incubated overnight to reach the confluency of 70% approximately. The confluent cells were treated with the concentrations of 500, 750 and 1000 µg/mL of *Ulva fasciata* and *Ulva lactuca*, and incubated for 48 and 72 hours. The untreated wells were assigned as the negative control. The morphology alteration of different groups was evaluated by the invert microscope at the end of incubation time.

For MTT assay, the culture medium was changed with fresh media and 20 µL of the MTT solution (0.5 mg/mL) was added to the each well (1:10 v/v) and incubated for 4h in CO_2 incubator. Next, the supernatant was removed carefully and 200 µL of DMSO was added to the each well for solubilization of the formazan crystals. Plates were shacked for 15 min and read at 540 nm using the Synergy HTX multi-mode reader. The results were reported as cell viability percentage that calculated by dividing the percentage of absorbance in the treated cells (test) by the absorbance of untreated (control) cells: [%viability= (test/ control)× 100%]. The cytotoxic effects of the extracts also calculated as reduction rate in the cell viability. Finally, the minimum concentration of the extracts that giving at least 50% of the cancer cell viability was calculated as IC50 value with linear regression (Galavi et al., 2016 ; Moulazadeh et al., 2020).

Statistical analysis

Data are expressed as the mean±SD and compared between the experimental groups by t-test and one-way

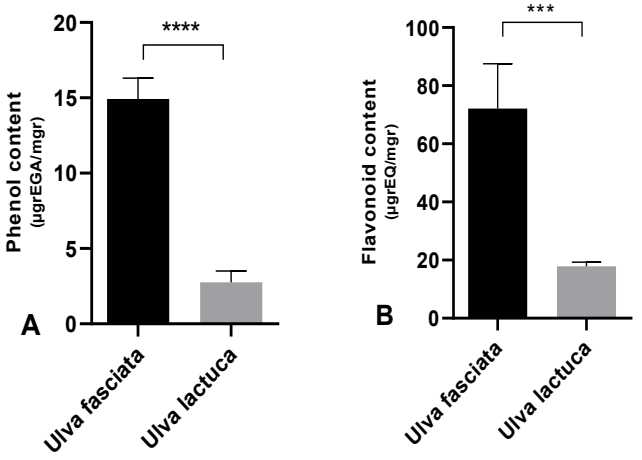

FIGURE 1. Phenolic (A) and flavonoid (B) content of *Ulva fasciata* and *Ulva lactuca*. Data are expressed as mean± SD. Statistical difference between the groups was investigated by t-test and *P*-value <0.05 was considered significant. The bullet of (***) means *P*<0.001 and the bullet of (****) means *P*<0.0001.

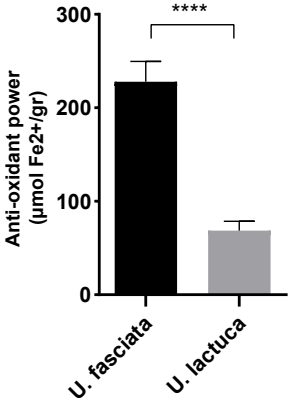

FIGURE 2. Monovalent reducing power of the *Ulva fasciata* and *Ulva lactuca* extract. Data are expressed as mean± SD. Statistical difference between the groups was investigated by t-test and *P*-value <0.05 was considered significant. The bullet of (****) means *P*<0.0001.

TABLE 1: Total phenolic and flavonoid content and antioxidant activity of *Ulva fasciata* and *Ulva lactuca*. Data are shown as mean±SD. Statistical difference between the groups was investigated by t-test and *P*-value <0.05 was considered as significant.

	Phenol content (µg GAE/ mg)	Flavonoid content (µg QE/mg)	Antioxidant activity	
			FRAP assay (µmolFe^{2+}/g)	DPPH IC50 (µg/mL)
Ulva fasciata	14.92±1.38	72.15±15.4	228 ± 21.73	>1000
Ulva lactuca	2.75±0.74	1.43 ± 17.88	68.69 ± 9.98	>1000
***P*-value**	**<0.0001**	**0.0004**	**< 0.0001**	-

analysis of variance (ANOVA). Significant difference was determined at the level of *P*<0.05. The IC50 value was calculated by linear and four parametric logistic regressions, respectively for MTT assay and total radical scavenging activity (DPPH assay). All analyses were performed using GraphPad Prism 8.0.2 (Chen et al., 2013).

Results

Phenolic and flavonoid content of the extracts

The phenolic content of the extracts was calculated by Folin-Ciocateu method based on the standard line equation of gallic acid (y= 278.59x- 48.578, R^2=0.9902). As it is shown in the Table 1, the phenolic content of the *Ulva fasciata* extract was 14.92±1.38 µg GAE/mg, which was significantly (*P*<0.0001) higher than the phenolic content of *Ulva lactuca* (2.75±0.74 µg GAE/mg). The flavonoid content of the extracts was also calculated by the aluminum chloride method based on the standard line equation of quercetin (y= 278.59x- 48.578, R^2=0.9968). Accordingly, the flavonoid content of the *Ulva fasciata* extract was 72.15±15.4 µg QE/mg which was significantly (*P*=0.0004) higher than the flavonoid content of *Ulva lactuca* (17.88± 1.43 µg QE/mg).

Monovalent reducing power

The monovalent reducing power of the extracts was calculated by the FRAP assay based on the FeSO4 standard line equation (y=1642.1x- 272.29, R^2=0.9982). According to the Table 1 and Figure 2, the antioxidant activity of the *Ulva fasciata* extract was 228± 21.73 µmol Fe^{2+}/g which was significantly (*P*<0.0001) higher than *Ulva lactuca* (68.69± 9.98 µmol Fe^{2+}/g).

Total radical scavenging activity

The total radical scavenging activity of the extracts was determined by DPPH assay. The highest radical scavenging activity of the extracts was related to ascorbic acid (IC50= 30.99 µg/mL), the *Ulva fasciata* (IC50>1000 µg/mL) and *Ulva lactuca* extracts (IC50>1000 µg/mL), respectively. According to the Table 2, the antioxidant activity of *Ulva fasciata* was

TABLE 2: Total radical scavenging activity (%) of the *Ulva fasciata* and *Ulva lactuca* extracts and ascorbic acid in the different concentrations. Data are expressed as mean± SD. The *P*-value column indicate statistical differences between *Ulva fasciata* and *Ulva lactuca* by t-test analysis. The *P*-value <0.05 was considered as significant.

CONC (µg/mL)	*Ulva fasciata*		*Ulva lactuca*		*P*-value	Ascorbic Acid	
	Mean± SD	IC50	Mean± SD	IC50		Mean± SD	IC50
100	8.88 ± 2.42	>1000	4 ± 2.54	>1000	**0.049**	84.10± 4.68	30.99
200	11.32 ± 2.20		4.78 ± 2.59		**0.017**	92.42± 0.03	
500	14.18 ± 1.94		5.70 ± 2.07		**0.002**	92.62± 0.05	
1000	18.75 ± 1.51		17.80 ± 3.03		0.653	93.09± 0.40	

TABLE 3: The viability of the MCF-7 and MDA-MB 231 breast cancer cell lines after treatment (48 and 72 hours) with the *Ulva fasciata* and *Ulva lactuca* extract. Data were expressed as mean±SD and analyzed by t-test. The *P*-value <0.05 was considered as significant.

Cell line	Time	CONC (µg/mL)	Ulva fasciata			Ulva lactuca			*P*-value
			Viability %		IC50	Viability %		IC50	
			Mean	STD		Mean	STD		
MDA-MB-231	48 h	500	85.71	1.69	>1000	89.21	2.23	>1000	0.09
		750	70.09	7.85		76.89	5.52		0.28
		1000	55.98	4.05		65.05	2.02		**0.02**
	72 h	500	72.54	9.01	**804.1**	79.75	4.19	**848.6**	0.27
		750	51.52	3.78		55.99	1.22		0.18
		1000	27.33	3.81		31.75	0.82		0.12
MCF-7	48 h	500	98.56	5.17	>1000	103.04	5.91	>1000	0.21
		750	98.53	11.25		96.20	11.15		0.81
		1000	77.74	5.33		96.06	4.97		**0.003**
	72 h	500	99.58	1.78	>1000	100	5.32	>1000	0.90
		750	78.27	6.97		98.79	3.38		**0.01**
		1000	68.72	1.44		97.75	1.23		**0.0002**

significantly higher than the *Ulva lactuca* extract on the concentrations of 100, 200 and 500 µg/mL. However, at concentration of 1000 µg/mL, there was no significant difference between the extracts (Table 2). There was a direct relationship between the concentration of the extracts and their antioxidant activity. The antioxidant activity of the *Ulva fasciata* and *Ulva lactuca* extracts in concentration of 1000 µg/mL compared to 100 µg/mL increased by 1.11 and 3.45 fold, respectively.

Analysis of cell morphology

The morphology of the MDA-MB-231 cells changed in a concentration and time dependent manner in treatment with the *Ulva fasciata* and *Ulva lactuca* extracts. According to the Figure 4, the MDA-MB-231 cells treated with *Ulva fasciata* were ruptured. The cell debris, granulated cellular contents, dropsy and shrinkage were increased clearly. Whereas, the MDA-MB-231 cells after treatment with *Ulva lactuca* were narrowed and slightly granulated. This cellular morphology intensified with concentration and time increasing, and the highest rate of cell granulation, shrinkage and cell rupture was seen after 72 h of treatment with the concentration of 1000 µg/mL *Ulva lactuca* extract (Figure 4). The MCF-7 cell line also showed morphological changes in treatment with the *Ulva fasciata* extract. These morphological changes were concentration and time dependent with shrinkage and disruption of cell integrity. Nevertheless, the *Ulva lactuca* extract did not show any specific morphological changes on the MCF-7 cell line

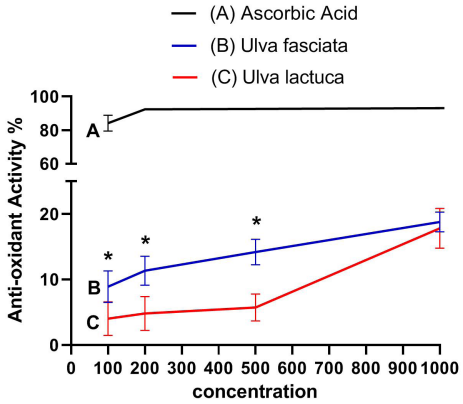

FIGURE 3. Total radical scavenging activity of the *Ulva fasciata* and *Ulva lactuca* extract and ascorbic Acid. Data are expressed as mean±SD and analyzed by t-test. The *P*-value <0.05 was also considered as significant. The bullet (*) Indicates a statistically significant difference between *Lawsonia inermis* and *Haplophyllum vermiculare* as *P*-value < 0.05 by t-test.

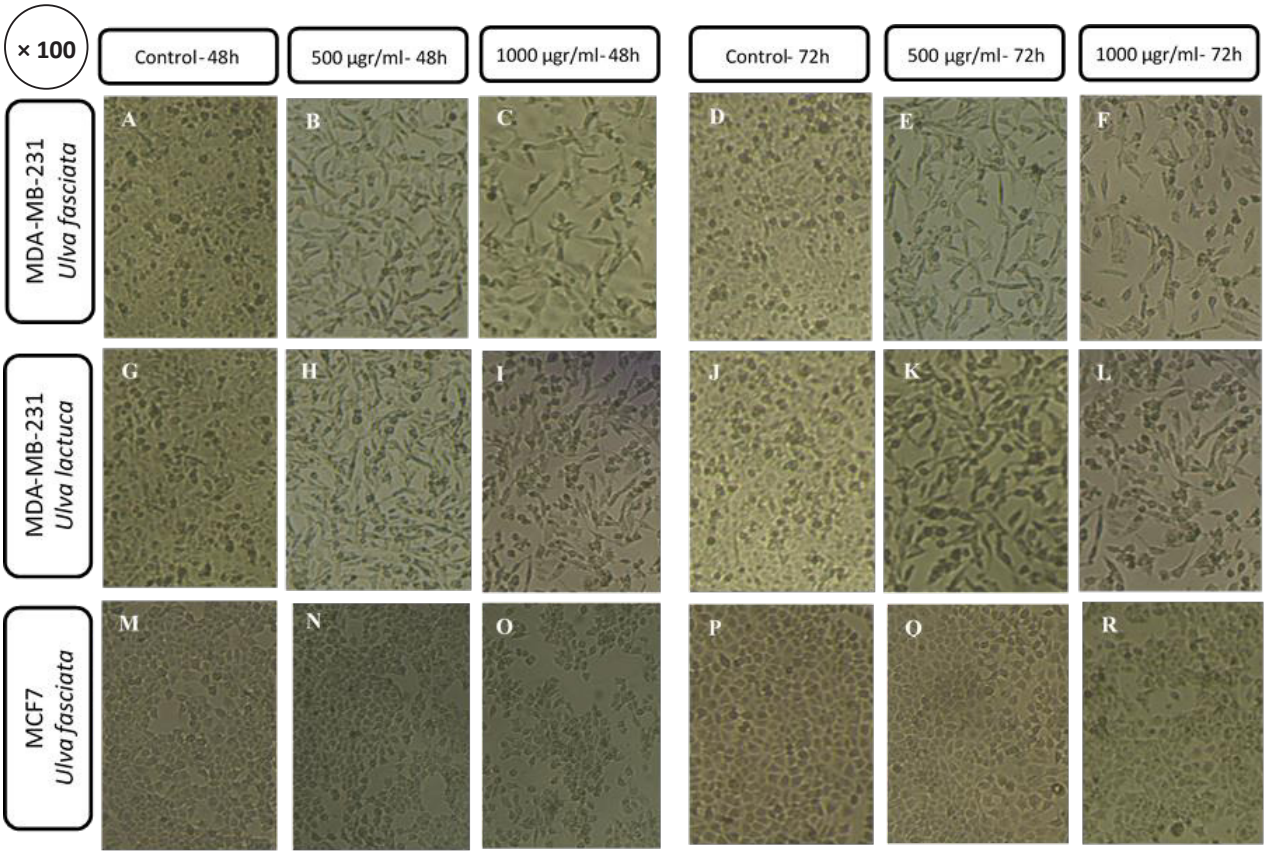

FIGURE 4. The images (×100) of morphological alterations of the MDA-MB-231 (A-L) and MCF-7 (M-R) cell lines in treatment with 500 and 1000µg/ml concentrations of *Ulva fasciata* (A-F, M-R) and *Ulva lactuca* (G-L) extract after 48 and 72 hours of incubation. For brevity, the image of the MCF-7 and MDA-MB-231 cell lines treated with the concentration of 750µg/ml of the extracts is not shown. Also, the image of MCF-7 treated with *Ulva lactuca* is not demonstrated due to no morphological changes.

(Images not reported).

Cytotoxic effects

According to the Figure 5, treatment of the MDA-MB-231 cell line with the *Ulva fasciata* extract resulted to a significant reduction of the cell viability by 14.29% (*P*=0.01), 29.91% (*P*=0.0001) and 44.02% (*P*<0.0001) after 48h of incubation, respectively on the concentrations of 500, 750 and 1000 µg/mL. The viability of the MDA-MB-231 cell line after 72h of incubation with the *Ulva fasciata* extract also decreased by 27.46% (*P*=0.009), 48.48% (*P*<0.0001) and 72.67% (*P*<0.0001) on the concentrations of 500, 750 and 1000 µg/mL respectively, which was statistically significant. Treatment

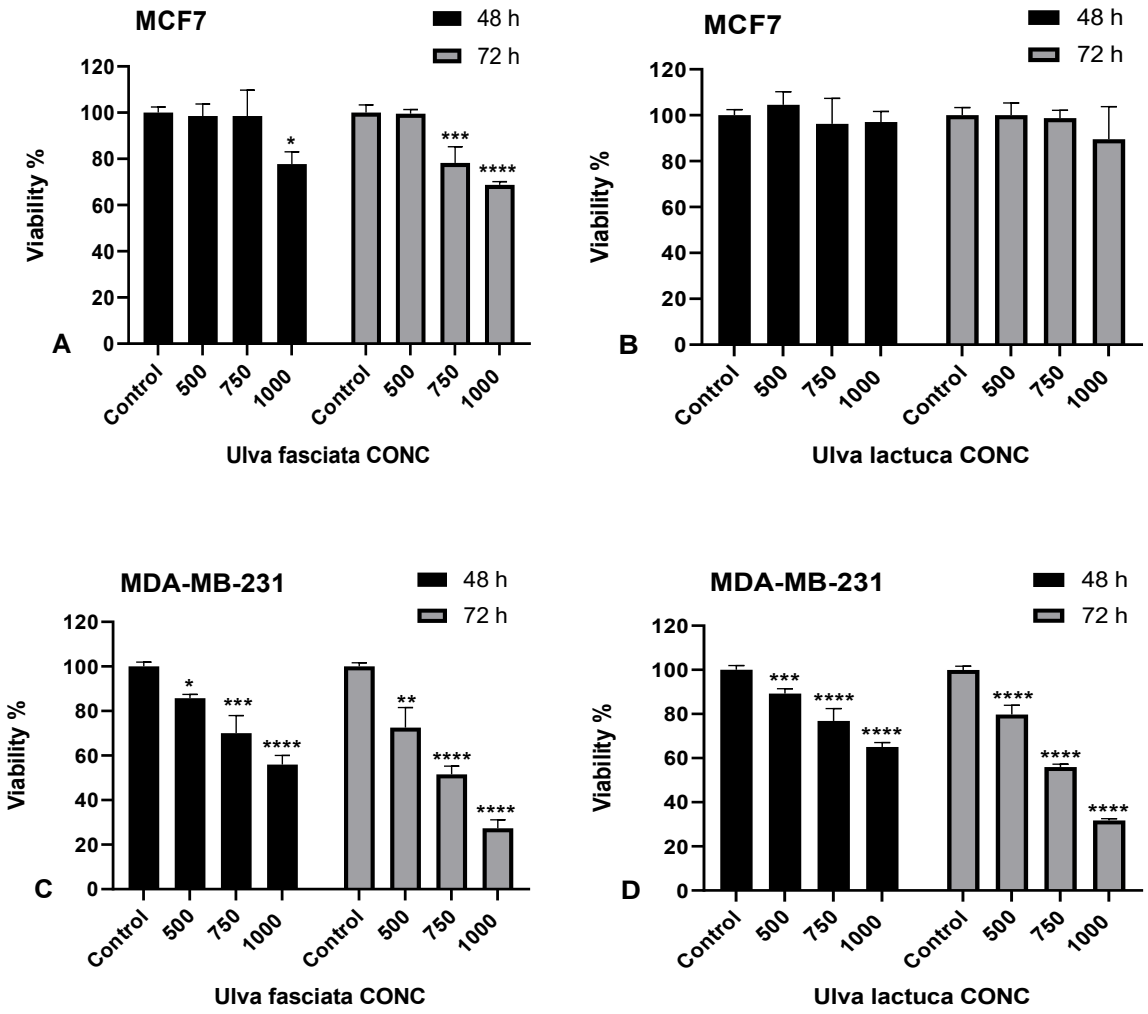

FIGURE 5. The viability of the MCF-7 and MDA-MB 231 breast cancer cell lines after treatment (48 and 72 hours) with the *Ulva fasciata* and *Ulva lactuca* extract on the concentrations of 500, 750 and 1000µg/ml. $^{*}P<0.05$, $^{**}P<0.01$,$^{***}P<0.001$ and $^{****}P<0.0001$

of the MDA-MB-231 cell line with *Ulva lactuca* also resulted in a significant reduction of 10.79% (P=0.0006), 23.11% (P<0.0001) and 34.95% (P<0.0001) in cell viability 48h of incubation, respectively on the concentrations of 500, 750 and 1000 µg/mL after. The viθ ability of the MDA-MB-231 cell line after 72h of incubation with the *Ulva lactuca* extract also decreased by 20.25% (P<0.0001), 44.01% (P<0.0001) and 68.25% (P<0.0001) in the concentrations of 500, 750 and 1000 µg/mL respectively, which was statistically significant. According to the Table 3, the cytotoxic effects of *Ulva fasciata* was significantly higher than *Ulva lactuca* in the concentrations of 1000 µg/mL (P=0.02) after 48h of incubation. The IC50 value of the *Ulva fasciata* and *Ulva lactuca* extracts after 48h of incubation on the MDA-MB-231 was more than 1000 µg/mL and after 72h of incubation was 804.1 and 848.6 µg/mL respectively.

Treatment of the MCF-7 cell line with the *Ulva fasciata* extract (1000 µg/mL) reduced the viability of the cells by 22.26% after 48h of incubation, which was statistically significant (P=0.01). After 72h of incubation, the *Ulva fasciata* extract reduced the viability of the MCF-7 cell line by 21.73% (P=0.0005) and 31.28% (P<0.0001) in the concentrations of 750 and 1000 µg/mL, respectively. However, treatment of the *Ulva lactuca* extract with the MCF-7 cell line did not lead to a significant reduction in cell viability. According to the Table 3, the IC50 value of the *Ulva fasciata* and *Ulva lactuca* extracts after 48 and 72 hours of incubation was more than 1000 µg/mL. The cytotoxic effects of the *Ulva fasciata* and *Ulva lactuca* extracts (1000 µg/mL) had a statistically significant difference (P=0.003) after 48h of incubation. After 72h of incubation, the cytotoxic effects of *Ulva fasciata* was also significantly higher than the *Ulva lactuca* extract in the concentrations of 750 µg/mL (P=0.01) and 1000 µg/mL (P=0.0002).

Discussion

Seaweeds have achieved a special place in traditional medicine research. They have active ingredients which have made them effective in the treatment of non-communicable diseases (El Gamal, 2010; Moulazadeh et al., 2021). Seaweed has shown great potential in the production of new drugs especially in cancer, and has been called "the sea, the future pharmacy" (Mohebbi et al., 2014). In the present study, the capacity of *Ulva fasciata* and *Ulva lactuca* seaweeds was evaluated in the production of future new anticancer drugs. The present study demonstrated the cytotoxic effect of *Ulva fasciata* and *Ulva lactuca* hydro-methanolic extract in a time and dose dependent manner. However, the *Ulva fasciata* extract had a stronger cytotoxic effect than *Ulva lactuca* extract on the MCF-7 and MDA-MB-231 breast cancer cell lines.

This stronger cytotoxic effects of *Ulva fasciata* was probably due to the potent antioxidant activity and more rich phenolic and flavonoid content of *Ulva fasciata* (Langeswaran et al., 2019; Nordin et al., 2018). Polyphenols have potent antioxidant activity and are able to modulate molecular targets and signaling pathways in cell survival, proliferation, migration and angiogenesis. As the best of our knowledge, the antioxidant activity of *Ulva lactuca* was not evaluated so far, and limited study evaluated the antioxidant activity of *Ulva fasciata* (Nordin et al., 2018). Langeswaran et al. (2019) was reported the high DPPH radical scavenging activity of *Ulva fasciata*. They also reported that the methanolic extract of *Ulva fasciata* has the ability to trap nitric oxide and superoxide radicals and has the protective effect on DNA sugar damage. In the present study, the higher antioxidant activity of the *Ulva fasciata* extract was double confirmed by DPPH and FRAP assay, and indicated that *Ulva fasciata* contains higher radical scavenging activity and reducing power. So *Ulva fasciata* with high phenolic and flavonoid content and higher antioxidant activity probably is effective in cancer prevention and treatment (Zhou et al., 2016).

As mentioned, the IC50 value of the *Ulva fasciata* and *Ulva lactuca* extracts on the MDA-MB-231 cell line after 72h of incubation was 804.1 and 848.6 μg/mL, respectively. The IC50 value of these extracts in treatment to the MCF-7 (48 and 72 hours of incubation) and MDA-MB-231 (48h of incubation) cell lines was more than 1000 μg/mL. The cytotoxic effect of the plant extracts and traditional medicine products are divided into four categories according to the concentration of IC50. In this category, plants with the IC50 values of 0-20 μg/mL, 20-100 μg/mL, 100-1000 μg/mL and more than 1000 μg/mL respectively are considered as very active, moderately active, weakly active and finally inactive drugs (Atjanasuppat et al., 2009; Baharum et al., 2014). Therefore, the *Ulva fasciata* and *Ulva lactuca* total extracts are assigned as weakly active compounds on the MDA-MB-231 (in 72h of incubation) and inactive drugs on the MCF-7 cell lines. Similar studies on other cell line categories have shown similar results. For example, a study by Guedes et al. (2013) showed that the various extracts of *Ulva lactuca* did not have cytotoxic effect on the NCI-H292, HEp-2 and K562 cell lines.

So far limited studies have evaluated the cytotoxic effects of Chlorophyceae family total extracts on the breast cancer cell lines. Most of the previous study indicated relatively weak cytotoxic effects of the *Ulva fasciata* and *Ulva lactuca* extracts. For example, Mosaddegh et al. (2014) showed that the Persian Gulf native *Ulva fasciata* had little cytotoxic effect on the MCF-7 cell line after 72h of treatment and was in the range of inactive compounds. Arsianti et al. (2016) showed that the ethanolic extract of *Ulva lactuca* with the IC50 value of 246.8 μg/mL is weakly active on the MCF-7 cell line. It was also shown in the study of Mashjoor et al. (2016) that the methanolic extract of *Ulva flexuosa* with the IC50 value of 107.48 μg/mL, is in the range of weak active compounds on the MCF7 cell line. The study of Namvar et al. (2014) also showed that the *Ulva fasciata* methanolic extract with IC50 value of 104 μg/mL on the MDA-MB-231 cell line (after 24h of incubation) was in the range of weakly active compounds. But its IC50 value after 48 and 72 hours of incubation was lower than 100 μg/mL and assigned as moderately active products, unlike the present study. This different cytotoxic effect is probably due to different extraction methods, as the hydro-methanolic extract of the seaweeds was prepared in the present study unlike the pure methanolic extracts of the Namvar et al. (2014) study. Arsianti et al. (2016) was also showed that the hexan fraction of *Ulva lactuca* has the highest cytotoxic effects. So it is appeared that the cytotoxic effects of the *Ulva fasciata* and *Ulva lactuca* depends on the non-polar ingredients.

Breast cancer is a heterogeneous tumor with a wide range of clinical behavior. Patients with the tumor that

lack the estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 is called triple negative tumor. These patients are highly resistant to chemotherapy and their disease does not have a good prognosis (Moses et al., 2016). *Ulva fasciata* and *Ulva lactuca* seaweeds probably are an appropriate option for the future studies on the triple negative tumors, because of stronger effects of the extracts on the MDA-MB-231 compared to the MCF-7 cell line. Also, it is appeared that their cytotoxic effects is independent of the ER receptor (Banerjee et al., 2017; Erfani et al., 2015). In the study of Erfani et al. (2015) was also showed that the cytotoxic effect of cladophoropsis spp and *Gracilaria foliifera* seaweeds ethanolic extract was independent of ER receptor. The previous studies have reported a relatively weak cytotoxic effect for the total extract of *Ulva fasciata* and *Ulva lactuca* seaweed. So it is recommended that researchers should far from the holistic view of traditional medicine in the future studies and focus on the fractionation and extracting of the bioactive compounds of the *Ulva fasciata* and *Ulva lactuca* such as sulfated polysaccharide (Suresh et al., 2012). In addition, it is recommended that the cytotoxic effects of the extracted bioactive was also evaluated on non-tumor cells for monitoring their side effects.

Conclusion

The *Ulva fasciata* extract has more cytotoxic effect than *Ulva lactuca*, which is probably due to the higher phenolic and flavonoid content and its antioxidant activity. The crude hydro-methanolic extracts of *Ulva fasciata* and *Ulva lactuca* are assigned as weakly active compounds on the breast cancer cell lines. However, they indicated an estrogen receptor independent manner of cellular growth inhibition that effective in treatment of triple negative tumors. Therefore, the crude extract of the seaweeds (especially *Ulva fasciata*) was an appropriate drug candidate for fractionation and bioactive extraction.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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