J Food Sci Nutr
Vol 11, p 17~24 (2006)

J Food Science and Nutrition

Cytotoxic Activities of Green and Brown Seaweeds Collected from Jeju Island against Four Tumor Cell Lines

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Abstract

Methanolic and aqueous extracts from 37 seaweed species (10 green and 27 brown seaweeds) collected from Jeju Island coast were prepared at high (70°C) and room (20°C) temperatures and examined for cytotoxic activity against 4 tumor cell lines: U937 (human monoblastoid leukemia cell line), HL60 (human promyelocytic leukemia cell line), HeLa (woman cervical carcinoma cell line) and CT26 (mouse colon carcinoma line). Both MeOH extracts of *Desmarestia tabacoides* and *Dictyota dichotoma* possessed strong cytotoxic activities against all the tumor cell lines tested, but the aqueous extract exhibited no activity. On the other hand *Ecklonia cava* showed strong cytotoxic activities for the 20°C aqueous extract against the three tumor cells except HeLa cell. *Sagassum coreanum* and *Sagassum siliquastrum* 20°C aqueous extracts also exhibited strong cytotoxic activities against U937, HL60, HeLa cells. Even though green seaweeds showed less activity than brown seaweeds, 20°C aqueous extracts of *Codium contractum* and *Codium fragile* exhibited strong cytotoxic activities against HL60 or CT26 cells, respectively.

Key words: cytotoxic activity, seaweeds extract, Jeju Island, tumor cells

INTRODUCTION

Cancer is a disease characterized by uncontrolled cell growth that presents over 100 distinct clinical pathologies (1). Cancer is the largest single cause of death in both men and women, claiming over 6 million lives each year worldwide. In the last few decades, basic cancer research has produced remarkable advances in understanding the biology and genetics of cancer (2). Recently, many anti-cancer drugs have been developed and applied by clinical doctors. Chemotherapeutic agents and radiation, which cause DNA mutation in actively dividing cells, were intended to selectively kill cancer cells while having limited effect on normal cells. Unfortunately, these cytotoxic agents, while effective in managing certain types of cancer, were limited in their utility due to their toxicity in normal dividing cell populations, resulting in adverse side effects. Therefore, the research into developing new and safe drugs has become a subject of great interest to the pharmaceutical industry (3).

Marine bioresources are known to be attractive as they sometimes yield new compounds showing several kinds of different bioactivities which are not possible in land plants. Screening of algal extracts for biologically active compounds began in the 1950s with simple antibiotic assays and soon expanded to include testing for products with antiviral, antibacterial, antifungal, anti-mitotic or antitumorigenic activities (4-7). Studies on antitumor effects of algal species have been reported by a number of researchers (8-12). Seaweeds contain rich bioactive constituents such as minerals and trace elements identified as bodily requirements, plus they have other nutrients, many of which are known to offer protection against a variety of health complications. The composition of seaweeds is quite different from the land plants. This facilitates the isolation of certain important bioactive materials which are not obviously possible with land plants. Different aspects of biochemical studies have isolated different components of seaweeds which are antioxidants, anticancer, antiangiogenic, anticoagulant, antibacterial, antifungal, anti-inflammatory and immunomodulatory compounds (13-18).

The objective of the present study was to investigate the growth inhibition effects of the methanolic and aqueous extracts from 37 seaweed species on four tumor cell lines (U937, HL60, HeLa and CT26).

MATERIALS AND MHEHODS

Chemicals

RPMI-1640, fetal bovine serum (FBS) and phosphate buffer saline (PBS) were purchased from Gibco BRL Co.

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Table 1. Jeju green and brown seaweeds used in this study

Scientific name	Korea name	Collected space
Green algae		
Monostroma nitidum	참홑파래	Jocheon
Enteromorpha compressa	납작파래	Jocheon
Enteromorpha intestinalis	창자파래	Dodu
Enteromorpha linza	잎파래	Jocheon
Enteromorpha sp.	파래류	Dodu
Ulva conglobata	모란갈파래	Jocheon
Ulva pertusa	구멍갈파래	Jocheon
Chaetomorpha linum	실염주말	Samyang
Codium contractum	몽우리청각	Shinchon
Codium fragile	청각	Kimnyumg
Brown algae		
Papenfussiella kuromo	연두털말	Sasu
Ishige okamurai	패	Jocheon
Ishige sinicola	넓패	Seongsan
Leathesia difformis	바위두룩	Seongsan
Petrospongium rugosum	바위주름	Jocheon
Colpomenia sinuosa	불레기말	Seongsan
Endarachne binghamiae	미역쇠	Seongsan
Hydroclathrus clathratus	그물바구니	Seongsan
Scytosiphon lomentaria	잘록이고리매	Jocheon
Myelophycus simplex	바위수염	Dodu
Desmarestia tabacoides	담배산말	Kimnyumg
Undaria pinnatifida	말미역	Seongsan
Ecklonia cava	감태	Pyoseon
Laminaria ochotensis	다시마	Seongsan
Dictyopteris prolifera	가시뼈대그물말	Sasu
Dictyota dichotoma	참그물바탕말	Jocheon
Pachydictyon sp.	참가죽 그물바탕말	Sasu
Padina arborescens	부챗살	Seongsan
Myagropsis myagroides	외톨개모자반	Seongsan
Hizikia fusiformis	톳	Jocheon
Sargassum coreanum	큰잎모자반	Jocheon
Sargassum fulvellum	모자반	Seongsan
Sargassum horneri	잇바디 괭생이모자반	Seongsan
Sargassum piluliferum	구슬모자반	Seongsan
Sargassum siliquastrum	꽈배기모자반	Seongsan
Sargassum thunbergii	지충이	Jocheon

(Gaithersburg, MD, USA). Dimethyl sulfoxide (DMSO) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT) were purchased from Sigma Co. (St. Louis, MO, USA).

Preparation of seaweed extracts

Seaweeds were collected along the Jeju Island coast (Table 1 and Fig. 1) of Korea during a period from February 2004 to March 2005. Of the Jeju coastal seaweeds, 27 species of brown and 10 species of green seaweeds were collected, and then salt, epiphytes and sand were removed using tap water. Finally the seaweeds were rinsed carefully with freshwater and stored in a medical freezer at -20°C. The frozen samples were lyophilized and homogenized with a grinder before extraction. The powdered samples were then extracted for 24

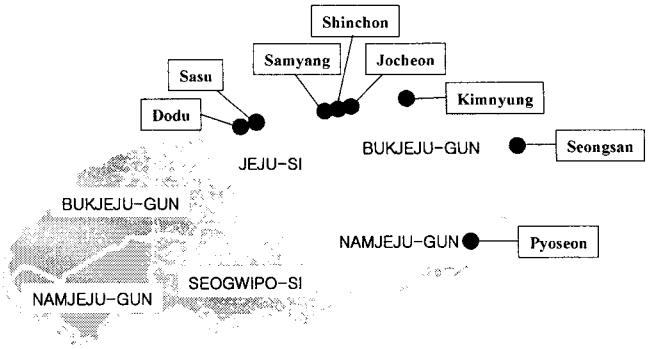

Fig. 1. Map of the sampling stations in the study area, Jeju Island, S. Korea.

h first with 80% MeOH under continuous shaking at 70°C and 20°C, and then aqueous extracts were prepared from the residue. The result was four uinque extracts: 70 ME (methanolic extract at 70°C), 20 ME (methanolic extract at 20°C), 70 AE (aqueous extract at 70°C) and 20 AE (aqueous extract at 20°C). The methanolic extracts were first subjected to evaporation and then dissolved in DMSO and used for experiments adjusting the final concentration of DMSO in culture medium to <0.01%. Respective water extracts were freeze dried and a known amount of the powder was again dissolved in water.

Cell culture

U937 (human monoblastoid leukemia cell line), HL60 (human promyelocytic leukemia cell line), HeLa (woman cervical carcinoma cell line) and CT26 (mouse colon carcinoma line) were grown in RPMI 1640 medium supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL). Cultures were maintained at 37°C in 5% CO_2 incubator.

Cell growth inhibition assay

The cytotoxicity of methanolic and aqueous extracts from marine algae was determined by a colorimetric MTT assay. Suspension cells (U937 and HL60 cells) were seeded at a concentration of 2×10^4 cells/mL together with the extracts (100 μg/mL) and incubated up to 72 h before MTT treatment. Attach cells (HeLa and CT26 cells) were seeded in a 96-well plate at a concentration of 2×10^4 cells/mL. Sixteen hours after plating, the cells were treated with the extract samples (100 μg/ mL). The cells were then incubated for an additional 72 h at 37°C. MTT stock solution (50 μL; 2 mg/mL in PBS) was then added to each well for a total reaction volume of 250 μL. After incubating for 4 h, the plate was centrifuged at 2,000 rpm for 5 min and the supernatants were aspirated. The formazan crystals in each well were dissolved in 150 μL of DMSO. The amount of purple formazan was

determined by measuring the absorbance at 540 nm.

Cell growth inhibition calculation

The percentage of cancer cell growth inhibition was calculated according to the following equation.

$$\% \text{ growth inhibition} = 1 - (CS/CC) \times 100$$

Where CS is the cells treated with seaweed extracts; CC is the untreated control cells grown with the same amount of distilled water or DMSO added as used as the solvent in the sample treatment.

Statistical analysis

The overall growth inhibition activities of methanolic and aqueous extracts on tumor cell lines are expressed as the means ± SD. These data were analyzed for significance using Student's *t*-test. $p<0.05$ was considered to be a significant difference.

RESULTS AND DISCUSSION

Although many anticancer agents have been developed and used, their side effects and resistance to anticancer drugs are serious problems to be overcome in the treatment of cancer. There is a need, therefore, to develop of safer and better therapeutic drugs from natural bioresorces. Recently, there has been increasing interest in the cancer therapeutic potential of natural plants, suggesting that many plants have anticancer activities that

Table 2. Growth inhibition activity of methanolic and aqueous extracts (100 μg/mL) from Jeju seaweeds on U937 cells

Scientific name	U937 cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Green seaweeds				
Monostroma nitidum	17.98±12.08	21.12±6.00	16.89±11.57	10.26±4.35
Enteromorpha compressa	32.33±7.05	60.31±3.52	18.84±1.80	28.73±6.46
Enteromorpha intestinalis	44.03±5.34	48.58±0.37	29.55±2,61	24.74±0.47
Enteromorpha linza	25.68±15.12	42.82±5.41	45.03±1.55	26.65±7.05
Enteromorpha sp.	39.22±3.36	60.90±6.82	30.35±6.29	26.39±11.01
Ulva conglobata	24.53±5.63	51.78±3.41	30.80±6.25	14.05±4.94
Ulva pertusa	23.99±7.37	35.23±4.92	30.01±0.28	13.79±21.15
Chaetomorpha linum	27.40±7.29	27.99±14.82	31.21±10.63	14.51±3.54
Codium contractum	47.80±6.47	52.36±1.70	62.08±0.98	36.35±7.00
Codium fragile	44.32±0.25	51.80±16.71	45.03±1.55	26.65±7.05
Brown seaweeds				
Papenfussiella kuromo	39.94±4.15	34.96±0.07	28.76±11.12	14.45±5.32
Ishige okamurai	34.47±5.00	20.05±5.24	43.24±2.94	36.36±5.48
Ishige sinicola	2.95±1.07	15.01±7.13	29.02±0.82	26.76±2.86
Leathesia difformis	1.74±5.24	26.73±7.71	34.89±14.46	48.90±7.23
Petrospongium rugosum	8.46±1.80	15.83±1.93	11.54±2.52	15.21±9.14
Colpomenia sinuosa	16.72±15.05	25.00±17.57	6.66±0.47	26.19±6.25
Endarachne binghamiae	35.40±2.21	44.50±2.13	25.55±2.13	33.93±1.21
Hydroclathrus clathratus	48.11±2.82	63.53±5.78	38.19±10.23	24.01±6.16
Scytosiphon lomentaria	25.56±4.98	18.60±11.06	20.06±1.88	19.36±13.16
Myelophycus simplex	26.14±12.57	23.35±13.19	22.20±8.83	26.07±11.53
Desmarestia tabacoides	86.37±5.34	79.43±5.43	*[2)]	*
Undaria pinnatifida	44.61±5.90	59.15±0.41	22.60±2.37	20.75±13.79
Ecklonia cava	37.66±9.83	33.31±1.39	74.39±1.72	33.87±3.76
Laminaria ochotensis	33.78±5.82	12.80±2.04	26.42±3.19	41.94±5.26
Dictyopteris prolifera	51.83±13.27	14.36±12.16	23.48±11.75	9.17±0.47
Dictyota dichotoma	84.33±0.52	76.47±1.26	37.14±7.00	20.45±11.75
Pachydictyon sp.	69.18±1.93	42.40±5.11	18.21±7.46	22.30±1.68
Padina arborescens	34.65±7.37	20.44±10.31	19.19±3.27	21.62±5.89
Myagropsis myagroides	20.55±12.60	25.68±12.75	48.75±10.73	9.37±4.10
Hizikia fusiformis	47.45±5.65	45.13±1.39	29.77±1.06	25.61±7.28
Sargassum coreanum	20.02±8.45	21.49±1.93	80.80±3.45	10.63±4.35
Sargassum fulvellum	11.53±3.56	17.77±3.15	46.83±0.19	26.03±5.02
Sargassum horneri	29.61±8.08	31.08±2.00	58.27±10.92	19.85±7.00
Sargassum piluliferum	31.29±11.47	26.99±0.52	27.51±5.69	36.02±6.34
Sargassum siliquastrum	15.79±7.49	24.27±4.45	72.03±3.92	18.19±6.13
Sargassum thunbergii	23.60±11.12	25.20±4.67	32.20±6.29	29.71±4.90

[1)]20 ME: methanolic extract at 20°C, 70 ME: methanolic extract at 70°C, 20 AE: aqueous extract at 20°C, 70 AE: aqueous extract at 70°C.

[2)]*Not determined.*

could be therapeutically useful. The objective of the present study was to investigate the growth inhibition effects of the methanolic and aqueous extracts of 37 marine alga species on four tumor cell lines (U937, HL60, HeLa and CT26 cell).

The U937 cell growth inhibitory effects of seaweed extracts are shown in Table 2. Ten brown seaweeds and five green seaweeds inhibited the growth of U937 cells by more than 50%. Of those extracts, *Desmarestia tabacoides* 20 ME, *Dictyota dichotoma* 20 ME, *Sargassum coreanum* 20 AE, *Ecklonia cava* 20 AE and *Sargassum siliquastrum* 20 AE exhibited growth inhibitions of 86.37%, 84.33%, 80.80%, 74.39% and 72.03%, respectively, on U937 cells.

The HL60 cell growth inhibitory effects of the seaweed extracts are shown in Table 3. Growth inhibition activity of eleven brown seaweeds and seven green seaweeds on HL60 cells exceeded 50%. Excellent growth inhibitory activities in this assay was exhibited by *D. tabacoides* 20 ME (89.27%), *D. dichotoma* 20 ME (85.85%), *S. siliquastrum* 20 AE (75.78%), *S. coreanum* 20 AE (73.94%) and *E. cava* 20 AE (72.24%).

The growth inhibitory effects of seaweed extracts against HeLa and CT26 cells are shown in Table 4 and 5. Growth inhibition activity of six brown seaweeds and two green seaweeds on HeLa cells exceeded 50% and

Table 3. Growth inhibition activity of methanolic and aqueous extracts (100 μg/mL) of Jeju seaweeds on HL60 cells

Scientific name	HL60 cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Green seaweeds				
Monostroma nitidum	10.93±4.77	31.38±4.71	46.49±0.83	37.27±0.79
Enteromorpha compressa	20.73±4.13	63.97±1.23	42.30±7.99	33.01±6.54
Enteromorpha intestinalis	33.17±9.43	35.16±4.89	35.53±8.78	45.06±0.75
Enteromorpha linza	26.91±2.99	45.65±12.02	58.35±0.22	40.31±3.34
Enteromorpha sp.	25.76±8.21	64.23±2.76	51.52±2.59	49.91±0.04
Ulva conglobata	4.88±2.37	16.67±3.27	51.83±4.61	43.14±5.49
Ulva pertusa	24.23±7.01	27.52±6.27	45.06±4.88	35.28±10.54
Chaetomorpha linum	16.50±4.79	5.98±2.51	60.90±4.35	57.24±0.57
Codium contractum	43.61±0.90	49.92±13.57	71.80±10.54	53.51±0.66
Codium fragile	68.94±0.09	33.21±1.09	67.76±1.23	59.72±1.45
Brown seaweeds				
Papenfussiella kuromo	26.46±3.05	32.76±0.46	27.33±3.60	37.45±.2.02
Ishige okamurai	36.10±14.81	19.09±5.41	46.30±3.38	53.17±2.28
Ishige sinicola	17.11±4.62	18.92±2.99	30.50±3.34	49.01±8.34
Leathesia difformis	23.72±2.18	31.63±5.52	50.86±072	49.19±5.53
Petrospongium rugosum	25.26±2.56	24.51±8.80	14.38±3.25	28.54±.5.93
Colpomenia sinuosa	22.89±3.16	21.79±1.03	34.19±2.33	47.20±2.20
Endarachne binghamiae	20.33±0.33	27.07±2.75	44.22±0.00	40.78±1.36
Hydroclathrus clathratus	43.41±2.99	0.85±1.09	48.94±6.50	50.25±1.49
Scytosiphon lomentaria	4.19±0.57	17.98±3.56	29.75±2.11	52.76±6.10
Myelophycus simplex	14.02±3.18	23.45±2.85	47.11±3.65	46.40±4.48
Desmarestia tabacoides	89.27±1.38	74.29±7.94	*[3)]	*
Undaria pinnatifida	30.80±8.11	49.25±0.62	42.08±5.23	35.43±0.40
Ecklonia cava	33.65±7.12	21.79±3.68	72.24±1.23	33.98±8.78
Laminaria ochotensis	20.80±3.27	13.92±3.20	37.14±1.84	38.11±0.22
Dictyopteris prolifera	46.34±0.92	35.73±5.23	38.01±10.69	48.54±0.83
Dictyota dichotoma	85.85±3.33	76.06±10.06	52.95±0.13	42.20±5.50
Pachydictyon sp.	45.61±15.86	47.68±4.31	43.01±5.49	34.53±1.49
Padina arborescens	-[2)]	1.34±4.79	36.37±15.50	45.03±0.18
Myagropsis myagroides	13.82±3.96	24.76±0.76	25.28±10.54	44.16±4.13
Hizikia fusiformis	63.94±13.52	39.72±4.31	39.19±2.20	52.20±1.54
Sargassum coreanum	27.52±12.25	26.42±8.16	73.94±5.40	39.81±1.76
Sargassum fulvellum	22.03±0.00	29.07±3.97	46.68±0.48	38.42±5.05
Sargassum horneri	28.78±0.23	30.53±5.00	38.48±2.15	48.54±3.03
Sargassum piluliferum	27.31±4.98	24.11±10.52	37.22±10.64	58.20±9.31
Sargassum siliquastrum	9.88±6.50	29.07±7.42	75.78±2.20	40.62±1.32
Sargassum thunbergii	18.75±7.40	8.22±3.99	21.83±1.71	36.43±10.23

[1)]Samples are the same as in Table 2.
[2)]Not detected. [3)]Not determined.

Table 4. Growth inhibition activity of methanolic and aqueous extracts (100 μg/mL) of Jeju seaweeds on HeLa cells

Scientific name	HeLa cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Green seaweeds				
Monostroma nitidum	-[2)]	-	18.28±4.54	17.20±3.14
Enteromorpha compressa	40.79±1.52	72.00±4.89	35.45±1.66	20.37±0.23
Enteromorpha intestinalis	33.52±1.18	34.19±6.48	24.81±0.64	25.44±2.96
Enteromorpha linza	24.67±2.24	23.52±2.37	8.57±4.92	12.76±3.74
Enteromorpha sp.	43.33±1.07	49.74±0.31	11.90±1.48	11.38±9.53
Ulva conglobata	16.56±1.99	14.76±5.05	24.89±2.91	19.20±4.76
Ulva pertusa	22.92±1.74	31.10±5.34	21.29±4.12	7.59±2.68
Chaetomorpha linum	20.65±0.90	12.60±2.84	32.96±5.15	34.14±3.42
Codium contractum	30.54±0.84	51.81±4.61	19.59±4.65	23.38±4.49
Codium fragile	47.14±3.20	33.72±3.09	21.26±1.64	25.81±1.87
Brown seaweeds				
Papenfussiella kuromo	27.00±4.80	20.75±0.56	8.60±1.07	28.61±2.17
Ishige okamurai	5.12±1.56	18.15±1.97	15.63±0.59	19.20±0.64
Ishige sinicola	15.65±1.46	17.12±5.00	4.19±1.00	15.98±1.73
Leathesia difformis	9.53±1.90	25.59±4.17	24.13±4.53	28.32±6.75
Petrospongium rugosum	6.39±1.18	21.72±2.43	30.05±3.10	16.81±6.20
Colpomenia sinuosa	9.82±12.15	24.58±12.09	3.38±1.09	28.68±0.23
Endarachne binghamiae	23.19±1.74	32.05±2.53	2.90±1.10	2.84±0.91
Hydroclathrus clathratus	34.63±0.25	32.16±3.74	1.43±1.09	28.68±0.23
Scytosiphon lomentaria	18.51±3.03	16.56±4.44	-	7.09±0.00
Myelophycus simplex	16.56±4.10	19.94±1.68	25.81±1.32	11.79±1.64
Desmarestia tabacoides	90.93±1.62	71.76±9.12	*[3)]	*
Undaria pinnatifida	22.32±7.30	53.14±8.20	5.35±2.55	1.68±1.01
Ecklonia cava	48.57±8.03	25.33±2.18	38.24±0.23	-
Laminaria ochotensis	6.04±3.37	6.20±2.58	-	6.77±2.11
Dictyopteris prolifera	35.77±0.12	21.89±6.04	8.14±1.07	28.61±2.17
Dictyota dichotoma	81.37±1.12	72.25±2.62	6.84±1.26	22.04±5.92
Pachydictyon sp.	63.74±0.64	35.95±6.11	3.70±1.31	18.08±5.04
Padina arborescens	11.32±1.85	14.02±0.06	7.35±4.56	10.60±1.78
Myagropsis myagroides	14.23±2.68	-	14.98±2.77	20.70±0.42
Hizikia fusiformis	25.02±8.20	26.61±6.85	6.98±3.84	16.91±6.52
Sargassum coreanum	56.12±4.36	48.72±9.22	78.74±12.12	16.74±2.87
Sargassum fulvellum	20.79±0.75	8.46±3.61	19.00±5.69	14.52±3.70
Sargassum horneri	17.05±9.78	10.00±1.93	24.26±3.51	18.61±1.16
Sargassum piluliferum	6.20±2.74	11.23±1.81	13.87±4.72	22.17±0.55
Sargassum siliquastrum	12.56±0.44	13.57±1.37	84.02±1.20	20.34±8.23
Sargassum thunbergii	17.51±4.77	13.42±2.92	-	12.76±3.74

[1)]Samples are the same as in Table 2.
[2)]Not detected. [3)]Not determined.

twenty brown seaweeds and nine green seaweeds species on inhibited the growth of CT26 cells by over 50%. Especially, the *D. tabacoides* 20 ME, *S. siliquastrum* 20 AE, *D. dichotoma* 20 ME and *S. coreanum* 20 AE showed highly potent inhibitory effects with 90.93%, 84.02%, 81.37% and 78.74% growth inhibition on HeLa, respectively. Among all the seaweed extracts, tested *D. tabacoides* 20 ME (87.23%), *E. cava* 20 AE (85.72%), *D. dichotoma* 20 ME (84.45%) and *Codium fragile* 20 AE (73.41%) exhibited the greatest growth inhibition on CT26 cells.

The growth inhibitory potential of the four tumor cells were over 80% in the 20 ME of *D. tabacoides* and *D. dichotoma* among brown seaweeds. The growth inhibitory potential of U937, HL60 and HeLa cell were over 70% in the 20 AE of *S. siliquastrum* and *S. coreanum* among brown seaweeds. The growth inhibitory potential of U937, HL60 and CT26 cell were over 70% in the 20 AE of *E. cava*.

Taking into consideration all of the results regarding the tumor cell growth inhibitory acitivties of the algal extracts, we have tried to analyze significant differences between the extraction solvents (methanol and water) and between the extracting temperatures (20 and 70°C). As shown in Table 6, there was no significant difference between extracts at either temperature. However a significant difference between the extraction solvents was observed in HL60, CT26 and HeLa cell. Aqueous ex-

Table 5. Growth inhibition activity of methanolic and aqueous extracts (100 μg/mL) of Jeju seaweeds on CT26 cells

Scientific name	CT26 cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Green seaweeds				
Monostroma nitidum	29.93±10.49	52.74±6.20	46.14±13.01	51.12±8.94
Enteromorpha compressa	47.67±4.84	66.85±1.74	46.42±4.66	47.17±2.78
Enteromorpha intestinalis	38.63±6.58	59.45±0.58	32.87±5.16	35.11±0.40
Enteromorpha linza	44.59±12.49	54.86±1.07	54.63±1.99	25.35±5.46
Enteromorpha sp.	48.01±5.33	53.29±9.59	69.83±1.80	58.50±2.68
Ulva conglobata	27.33±11.78	50.14±3.68	53.37±2.78	32.44±1.99
Ulva pertusa	40.34±8.33	42.26±9.20	40.73±3.38	36.94±5.96
Chaetomorpha linum	31.85±4.17	25.67±0.89	61.50±2.13	52.32±3.48
Codium contractum	32.33±6.20	49.73±1.16	65.61±0.74	62.78±0.79
Codium fragile	42.05±0.19	35.27±0.68	73.41±6.21	69.36±4.90
Brown seaweeds				
Papenfussiella kuromo	37.60±8.04	53.22±3.39	55.55±6.65	53.44±0.30
Ishige okamurai	30.89±4.36	37.05±5.33	50.46±3.68	61.04±0.82
Ishige sinicola	34.59±3.39	37.88±4.55	50.29±0.98	46.65±3.02
Leathesia difformis	44.59±4.36	47.19±2.62	58.50±3.67	60.32±2.28
Petrospongium rugosum	42.12±1.07	35.41±7.85	48.74±1.99	46.00±2.28
Colpomenia sinuosa	47.40±3.49	35.55±11.72	50.98±7.15	65.03±2.78
Endarachne binghamiae	27.40±2.42	31.23±6.01	39.13±11.04	46.59±0.65
Hydroclathrus clathratus	50.55±4.07	53.63±6.10	65.45±3.38	64.12±2.09
Scytosiphon lomentaria	19.11±6.88	21.37±0.19	53.29±4.25	72.60±1.14
Myelophycus simplex	27.33±9.20	29.59±4.65	24.80±6.46	39.19±1.80
Desmarestia tabacoides	87.23±3.23	77.32±4.98	*[2)]	*
Undaria pinnatifida	44.45±1.26	59.45±0.39	28.73±3.84	27.63±5.40
Ecklonia cava	51.44±0.87	35.21±2.71	85.72±0.90	41.04±10.13
Laminaria ochotensis	37.19±1.84	66.92±4.17	45.20±8.67	58.38±8.99
Dictyopteris prolifera	65.21±2.52	57.74±7.65	29.78±9.73	52.32±3.28
Dictyota dichotoma	84.45±2.03	50.48±5.91	62.08±1.59	55.76±0.40
Pachydictyon sp.	48.08±9.02	45.14±8.21	50.77±5.26	30.41±7.85
Padina arborescens	20.62±0.29	23.63±4.75	50.40±5.89	50.35±4.50
Myagropsis myagroides	27.81±6.35	40.89±9.59	53.37±3.58	50.42±1.79
Hizikia fusiformis	67.95±2.13	41.03±1.07	52.25±7.15	41.15±6.75
Sargassum coreanum	38.97±15.80	48.90±14.92	7.87±0.20	14.96±3.67
Sargassum fulvellum	25.75±2.97	24.99±9.78	46.77±3.58	20.22±9.53
Sargassum horneri	35.82±8.56	33.15±1.74	50.63±0.30	23.10±4.67
Sargassum piluliferum	41.51±6.59	33.01±1.74	51.05±11.02	51.47±0.10
Sargassum siliquastrum	36.85±6.38	34.59±2.23	27.74±7.85	11.94±4.57
Sargassum thunbergii	41.99±1.65	62.12±6.49	35.90±6.29	62.60±1.39

[1)]Samples are the same as in Table 2.
[2)]Not determined.

Table 6. The overall growth inhibition activity of methanolic and aqueous extracts on tumor cell lines

Sample[1)]	U937	HL60	CT26	HeLa
Methanolic extract	33.42±2.02[NS2)]	29.05±2.08[a3)]	42.03±1.55[a]	25.69±2.12[b]
Aqueous extract	29.38±1.80	44.72±1.41[b]	47.68±1.83[b]	18.03±1.75[a]
Extracts at 20°C	33.43±2.06	36.71±2.22[a]	44.75±1.76[a]	21.88±2.24[a]
Extracts at 70°C	29.37±1.74	36.94±1.8[a]	44.96±1.6[a]	21.84±1.72[a]

[1)]The mean of tumor cell growth inhibitory activity values of total methanolic extracts at 20 and 70°C, total aqueous extracts at 20 and 70°C, total methanolic and aqueous extracts at 20°C and total methanolic and aqueous extracts at 70°C.
[2)]Not significant.
[3)]Means with different letters within a column are significantly different ($p<0.05$).

tracts were superior to methanolic extracts for inhibiting cell growth of HL60 and CT26, and the opposite was true for HeLa.

The activities reported in methanolic extracts can be attributed to the pigments and phlorotannis of the brown seaweeds (19,20). The predominant pigments of brown seaweeds have been reported to be fucoxanthine and chlorophyll (21,22). The polyphenolic compounds of the

brown seaweeds are referred to as phlorotannis and those are readily soluble in polar solvents like methanol and water (19). This suggests that the tumor cell growth inhibitory activities of the aqueous extracts of brown seaweeds can be attributed to the phlorotannis. Previous studies have reported that fucoxanthin inhibits the growths of human neuroblastoma GOTO cells, human leukemia cells, and prostate cancer cells (21-24). Several phlorotannins of brown seaweeds have been identified for their potential bioactivities such as antioxidant, antibacterial and antihyluranase (25-36). Therefore these results suggest that further intensive studies on the anticancer activities of those extracts are needed. The aqueous extract of *Codium contractum* exhibited the highest tumor cell growth inhibition activity (71.80%) of the green seaweed extracts. This activity can not be due to the chlorophylls, because they are hydrophobic and relatively insoluble in water. Thus, it might be suggested that it can be due to some other water soluble constituents of the seaweed such as protein or low molecular weight polysaccharide. Seaweeds contain many different kinds of compounds which could possess potent anticancer activity. As has been reported previously, other seaweed compounds such as β-carotin and lutein as well as chlorophyll related compounds have documented high anticancer activities (37). Those compounds quench radical species and thereby reduce cancer cell formation *in vitro* and *in vivo* (38). Hence, it is clear that the similar kinds of compounds may be associated with the high antiproliferative effect of the present study.

In conclusion, methanolic and aqueous extracts from 37 marine algal species collected from Jeju Island were evaluated for their cytotoxic activities against four tumor cells (U937, HL60, CT26 and HeLa). Most seaweed species tested in this study showed potential anticancer activities. Of the seaweed species *D. tabacoides*, *D. dichotoma*, *S. siliquastrum* and *S. coreanum* showed excellent cytotoxic activities. However, further studies are essential to purify anticancer active compounds to elucidate relation ships between structure and activity which might help with future drug design. Therefore, seaweeds present in Jeju Island are possible candidates for future anticancer drug discovery.

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (2004-F00038) and the Brain Korea 21 Project in 2005.

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(Received January 20, 2006; Accepted March 6, 2006)