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Cytotoxic Activities of Red Algae Collected from Jeju Island Against Four Tumor Cell Lines

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Abstract

Methanolic and aqueous extracts of 26 red algae species collected from Jeju Island coast were prepared at a high (70°C) and a room temperature (20°C) and were examined for their cytotoxic activity against 4 tumor cell lines: U-937 (human monoblastoid leukemia cell line), HL-60 (human promyelocytic leukemia cell line), B-16 (murine melanoma cell line) and HeLa (woman cervical carcinoma cell line). 20°C methanolic extract of *Polysiphonia japonica* showed cytotoxic activity of over 50% against U-937, HL-60 and B-16 cells. On the other hand, the 20°C aqueous extract of *Scinaia okamurae* and 70°C aqueous extract of *Chondrus crispus* showed cell growth inhibition activity of more than 50% against HL-60 and B-16 cells. The highest cytotoxic activity was observed in the 20°C aqueous extract of *Scinaia okamurae* against B-16 cells (80.55%).

Key words: red algae, cytotoxic activity, extract, Jeju, tumor

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 researches have 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, although effective in managing certain types of cancer, are 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 safer 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 anti-tumorigenic 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 known to be essential nutrients, furthermore 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 land plants. This facilitates the isolation of certain important bioactive materials which are not obviously possible with land plants. Biochemical studies employing a variety of methods 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 26 red algae species on four tumor cell lines (U-937, HL-60, B-16 and HeLa).

MATERIALS AND MHEHODS

Chemicals

RPMI-1640, fetal bovine serum (FBS) and phosphate buffer saline (PBS) were purchased from Gibco BRL Co. (Gaithersburg, MD, USA). Dimethyl sulfoxide (DMSO) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma Co. (St. Louis, MO, USA).

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Preparation of red algal extracts

Red algae 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 algae, 26 species of red algae were collected, and then salt, epiphytes and sand were removed using tap water. Finally the red algae 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 hr 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 unique extracts from each alga: 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 dissolved in DMSO, and then 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.

Table 1. Jeju red algae used in this study

Scientic name	Korean name	Collected space
Porphyra tenera	김	Jocheon
Scinaia okamurae	매끈껍질	Kimnyung
Bonnemaisonia hamifera	참갈고리풀	Sasu
Gelidium amansii	우뭇가사리	Shinchon
Pterocladiella capillacea	큰개우미	Jocheon
Lithophyllum okamurae	흑돌잎	Seongsan
Carpopeltis affinis	참까막살	Hallim
Prionitis cornea	붉은까막살	Hallim
Grateloupia filicina	빈참지누아리	Seongsan
Sinkoraena lancifolia	털지누아리	Seongsan
Halymenia dilatata	넓왕지누아리	Seongsan
Grateloupia elliptica	참도박	Shinchon
Grateloupia lanceolate	가는개도박	Seongsan
Gloiopeltis furcata	불등풀가사리	Jocheon
Schizymenia dubyi	갈래잎	Seongsan
Phacelocarpus sp.	꿩꼬리풀	Seongsan
Gracilaria textorii	잎꼬시래기	Seongsan
Gracilaria verrucosa	꼬시래기	Hallim
Ahnfeltiopsis flabelliformis	부챗살	Seongsan
Chondrus crispus	주름진두발	Jocheon
Lomentaria catenata	마디잘록이	Seongsan
Martensia denticulate	비단망사	Bukchon
Chondria cassicaulis	개서실	Seongsan
Laurencia okamurae	쌍발이서실	Seongsan
Chondrophycus undulates	흑서실	Seongsan
Polysiphonia japonica	왜떨기나무붉은실	Sasu

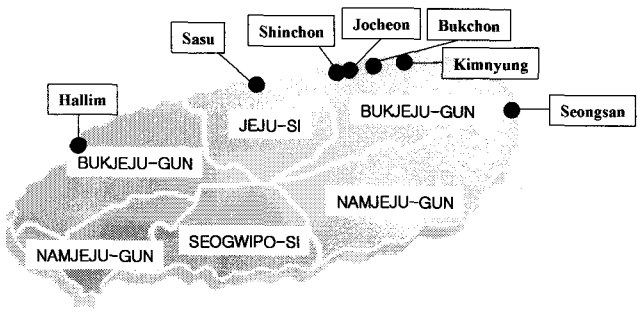

Fig. 1. Map of the sampling stations in the study area, Jeju Island, Korea.

Cell culture

U937 (human monoblastoid leukemia cell line), HL-60 (human promyelocytic leukemia cell line), B-16 (murine melanoma cell line) and HeLa (woman cervical carcinoma cell line) were grown in RPMI 1640 and DMEM 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 a 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. Suspended cells (U-937 and HL-60 cells) were seeded at a concentration of 2×10^4 cells/mL together with the extracts (100 μg/mL) and incubated up to 72 hr before MTT treatment. Attached cells (B-16 and HeLa 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 hr 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 hr, 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.

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. Therefore, there is a need to develop 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 could be therapeutically useful. The objective of the present study was to investigate the growth inhibition effects of the methanolic and aqueous extracts of 26 red algal species on four tumor cell lines (U-937, HL-60, B-16 and HeLa cell).

The U-937 cell growth inhibitory effects of red algal extracts are shown in Table 2. Among the tested red algae, *Laurencia okamurae*, 70 ME showed the highest U-937 cells growth inhibitory activity. Moreover, *Ahnfeltiopsis flabelliformis*, *Laurencia okamurae* and *Polysiphonia japonica* methanolic extracts inhibited the growth of U-937 cells over 50%. Taken together, the aqueous extracts of red algal species have less growth inhibitory effect on U-937 cells than the methanolic extracts.

The HL-60 cell growth inhibitory effects of the red algal extracts are shown in Table 3. *Bonnemaisonia hamifera* 20 AE, *Gracilaria verrucosa* 70 ME, *Ahnfeltiopsis flabelliformis* 20 ME, *Chondria cassicaulis* 70 AE, *Lomentaria catenata* 70 AE and *Polysiphonia japonica* 20 ME showed highly potent inhibitory effects with 55%, 58%, 56%, 53% and 54% growth inhibition on HL-60, respectively. In this study, MeOH extracts of algal species exhibited better antitumor activities than their aqueous extract counterparts. However, extraction temperature has no clear effect for tumor cell growth inhibitory activity.

The growth inhibitory effects of red algal extracts against B-16 and HeLa cells are shown in Table 4 and 5. Growth inhibitory activity of eight red algal extracts (*S. okamurae, B. hamifera, L. okamurae, prionitis cor-*

Table 2. Growth inhibitory activity of methanolic and aqueous extracts from Jeju seaweeds on U-937 cells

Scientific name	U-937 cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Porphyra tenera	10.66±5.33[2)]	11.82±2.00	8.16±5.30	13.41±3.53
Scinaia okamurae	13.91±8.48	5.83±1.06	-[3)]	-
Bonnemaisonia hamifera	11.60±5.47	18.58±6.00	-	-
Gelidium amansii	6.70±2.80	12.92±7.34	-	-
Pterocladiella capillacea	12.24±5.18	9.30±3.00	5.58±2.12	12.16±6.48
Lithophyllum okamurae	7.24±5.07	-	8.41±0.08	9.30±0.17
Carpopeltis affinis	11.51±2.13	17.36±6.14	-	4.27±1.89
Prionitis cornea	9.43±5.67	11.70±8.27	-	-
Grateloupia filicina	43.21±4.40	32.75±6.01	11.34±3.71	9.40±2.12
Sinkoraena lancifolia	15.00±4.94	20.66±4.67	-	-
Halymenia dilatata	20.48±9.19	18.23±8.19	-	-
Grateloupia elliptica	24.43±1.73	31.89±4.54	-	-
Grateloupia lanceolate	42.22±2.96	49.34±9.48	28.45±0.96	22.04±8.64
Gloiopeltis furcata	12.41±6.59	14.24±4.00	6.74±0.71	8.41±2.83
Schizymenia dubyi	*[4)]	27.64±4.36	-	-
Phacelocarpus sp.	20.75±2.13	11.60±4.40	-	-
Gracilaria textorii	45.21±2.96	32.75±6.01	25.75±2.37	29.10±2.71
Gracilaria verrucosa	46.05±9.91	40.30±9.23	23.41±9.68	25.27±4.06
Ahnfeltiopsis flabelliformis	54.79±1.27	35.93±2.03	13.17±5.29	39.04±0.85
Chondrus crispus	12.82±8.66	11.57±9.60	33.64±1.53	49.46±8.12
Lomentaria catenata	15.65±8.72	-	*	43.21±6.83
Martensia denticulate	21.48±8.12	18.73±5.42	-	-
Chondria cassicaulis	41.26±5.34	37.13±7.28	23.47±2.37	25.33±1.44
Laurencia okamurae	57.60±2.71	67.78±2.37	29.22±7.28	25.15±2.54
Chondrophycus undulates	2.17±0.40	6.84±1.89	31.70±3.20	9.91±5.67
Polysiphonia japonica	50.09±8.41	36.42±5.87	-	-

[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)]Mean±SE of determinations was made in triplicate experiments.
[3)]Not detected. [4)]Not determined.

Table 3. Growth inhibitory activity of methanolic and aqueous extracts from Jeju seaweeds on HL-60 cells

Scientific name	HL-60 cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Porphyra tenera	15.39±0.14	18.04±2.50	13.53±3.33	19.61±0.13
Scinaia okamurae	2.16±1.82	4.16±1.76	55.00±8.73	18.21±4.23
Bonnemaisonia hamifera	22.51±2.96	33.86±1.48	-[2)]	-
Gelidium amansii	27.84±2.59	24.52±2.10	40.92±0.12	33.07±3.09
Pterocladiella capillacea	15.49±0.83	6.96±5.96	7.49±2.16	11.37±1.76
Lithophyllum okamurae	11.57±0.37	7.94±5.68	29.61±0.28	-
Carpopeltis affinis	24.17±0.86	39.97±1.97	17.36±0.37	36.13±0.25
Prionitis cornea	13.18±0.86	21.64±3.46	8.20±4.44	9.38±3.84
Grateloupia filicina	30.84±5.36	29.42±0.67	3.46±2.97	-
Sinkoraena lancifolia	22.69±3.21	40.14±1.23	-	5.55±3.75
Halymenia dilatata	8.04±4.71	7.45±2.77	-	-
Grateloupia elliptica	44.68±0.49	43.11±1.73	13.26±6.42	9.87±5.76
Grateloupia lanceolate	37.79±2.23	34.08±9.63	8.71±6.94	-
Gloiopeltis furcata	25.10±2.22	11.57±3.60	-	-
Schizymenia dubyi	*[3)]	20.78±8.60	-	-
Phacelocarpus sp.	41.01±9.63	29.23±8.96	-	-
Gracilaria textorii	28.81±9.81	29.42±0.67	38.73±2.60	20.52±9.01
Gracilaria verrucosa	48.76±5.47	58.87±1.23	18.60±11.99	-
Ahnfeltiopsis flabelliformis	51.37±3.13	37.47±4.47	11.26±9.17	-
Chondrus crispus	9.61±6.38	7.16±0.97	41.27±6.52	56.57±5.68
Lomentaria catenata	5.98±0.14	7.06±5.82	*	53.33±0.83
Martensia denticulate	15.78±6.24	23.24±6.52	-	-
Chondria cassicaulis	37.31±0.45	33.84±5.81	20.97±4.80	19.76±5.65
Laurencia okamurae	38.50±11.95	49.94±7.02	27.05±4.47	15.57±3.31
Chondrophycus undulates	17.80±0.99	17.89±5.31	26.00±0.99	35.86±5.08
Polysiphonia japonica	54.10±0.99	44.42±0.49	15.62±3.58	18.59±2.84

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

Table 4. Growth inhibitory activity of methanolic and aqueous extracts from Jeju seaweeds on B-16 cells

Scientific name	B-16 cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Porphyra tenera	23.16±1.31	32.61±2.36	36.19±4.08	27.74±3.81
Scinaia okamurae	29.95±1.59	17.97±3.22	80.55±0.41	26.39±1.08
Bonnemaisonia hamifera	20.39±8.11	13.31±2.42	67.76±0.10	10.84±4.59
Gelidium amansii	31.98±5.11	13.53±0.36	20.61±0.77	3.06±0.21
Pterocladiella capillacea	17.36±5.89	31.10±2.04	45.55±2.08	14.51±2.94
Lithophyllum okamurae	14.19±0.14	27.39±0.59	69.35±0.59	46.22±14.27
Carpopeltis affinis	22.10±11.45	27.79±2.27	13.46±4.18	-
Prionitis cornea	21.41±10.16	4.08±2.37	30.23±6.34	62.18±3.87
Grateloupia filicina	-[2)]	-	15.32±0.99	11.95±7.22
Sinkoraena lancifolia	24.47±3.46	14.73±3.09	-	5.98±4.23
Halymenia dilatata	24.12±7.11	27.55±4.39	49.07±5.80	20.50±1.90
Grateloupia elliptica	24.11±8.30	23.71±1.03	19.29±1.91	8.79±6.03
Grateloupia lanceolate	-	4.03±3.93	-	13.66±3.96
Gloiopeltis furcata	27.64±0.32	31.90±3.53	16.14±4.80	21.17±4.76
Schizymenia dubyi	*[3)]	33.02±5.21	59.48±10.19	18.10±5.21
Phacelocarpus sp.	26.40±10.68	11.43±7.72	-	17.54±4.80
Gracilaria textorii	-	-	8.11±2.32	-
Gracilaria verrucosa	-	-	-	-
Ahnfeltiopsis flabelliformis	-	-	-	-
Chondrus crispus	9.32±1.77	22.29±7.07	55.80±5.53	50.99±7.61
Lomentaria catenata	29.95±1.59	6.82±4.39	*	35.91±0.23
Martensia denticulate	17.68±8.52	12.00±7.98	70.69±1.49	22.23±6.89
Chondria cassicaulis	-	-	5.82±3.28	8.24±6.61
Laurencia okamurae	65.59±2.48	53.15±2.97	-	9.66±3.85
Chondrophycus undulates	18.78±8.51	8.13±0.26	20.13±1.13	6.16±2.63
Polysiphonia japonica	78.15±7.99	68.05±3.92	-	-

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

Table 5. Growth inhibitory activity of methanolic and aqueous extracts from Jeju seaweeds on HeLa cells

Scientific name	HeLa cell growth inhibition rate (%)			
	20 ME[1)]	70 ME	20 AE	70 AE
Porphyra tenera	16.14±2.36	17.84±9.46	-	-
Scinaia okamurae	18.27±8.01	-	12.45±5.40	-
Bonnemaisonia hamifera	9.42±2.60	11.13±7.53	19.33±3.64	7.66±2.33
Gelidium amansii	-[2)]	-	22.79±1.77	11.39±1.78
Pterocladiella capillacea	15.49±4.50	13.13±11.11	0.83±0.33	3.74±0.43
Lithophyllum okamurae	14.90±6.85	-	17.66±7.02	-
Carpopeltis affinis	9.68±6.09	14.11±6.55	5.89±0.55	7.38±1.07
Prionitis cornea	11.33±9.05	-	23.12±5.57	16.58±4.84
Grateloupia filicina	18.54±10.47	3.28±2.25	10.97±8.61	-
Sinkoraena lancifolia	-	-	15.39±0.28	-
Halymenia dilatata	-	-	-	-
Grateloupia elliptica	19.31±0.18	21.67±10.19	-	-
Grateloupia lanceolate	20.79±9.37	15.20±7.55	14.16±9.03	-
Gloiopeltis furcata	-	-	-	-
Schizymenia dubyi	*[3)]	-	-	-
Phacelocarpus sp.	-	11.88±6.03	-	-
Gracilaria textorii	19.68±1.68	22.01±2.49	29.07±13.22	12.62±9.75
Gracilaria verrucosa	25.70±1.79	23.02±5.64	20.56±10.01	11.01±1.79
Ahnfeltiopsis flabelliformis	32.77±4.34	24.94±4.60	9.06±7.38	9.82±2.31
Chondrus crispus	8.97±7.58	-	-	19.21±5.13
Lomentaria catenata	-	-	*	-
Martensia denticulate	19.00±9.96	18.10±6.46	-	-
Chondria cassicaulis	15.02±3.95	21.62±2.00	22.07±8.54	19.54±7.09
Laurencia okamurae	15.47±1.97	11.44±0.61	19.09±7.15	18.41±7.52
Chondrophycus undulates	-	-	22.08±5.69	8.90±1.68
Polysiphonia japonica	-	8.96±3.37	11.58±4.07	-

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

nea, S. dubyi, C. crispus, M. denticulate and P. japonica) on B-16 cells exceeded 50%. Excellent growth inhibitory activities in this assay were observed in *Scinaia okamurae* 20 AE (80.55%), *Lithophyllum lkamurae* 20 AE (69.35%), *Martensia denticulate* 20 AE (70.69%) and *Polysiphonia japonica* 20 ME (78.15%). As shown in Table 5, all the methanolic and aqueous extracts of red seaweed showed poor growth inhibitory activity (less than 40%) on HeLa cells. Therefore, the tested red algal samples showed the least inhibitory activity against HeLa cell growth among the four tumors examined.

Polysiphonia japonica 20 ME was showed cell growth inhibition activity of more than 50% against U-937, HL-60 and B-16 cells. On the other hand *Scinaia okamurae* 20 AE and *Chondrus crispus* 70 AE showed cell growth inhibitory activity of more than 50% against HL-60 and B-16 cells. *Ahnfeltiopsis flabelliformis* and *Laurencia okamurae* also indicated cell growth inhibition activities of more than 50% in 20°C methanolic extracts against U-937 and HL-60 or U-937 and B-16 cells, respectively.

Based on the inhibitory activities of the red algal extracts on the tumor cell growth, we further examined the effect of different extraction solvents (methanol and water) and extracting temperature (20 and 70°C) on the tumor cell growth. As shown in Table 6, there was no difference in the inhibitory activity on the tumor cell growth between the extracts prepared at 20°C and 80°C. However, methanolic extracts were more effective than aqueous extracts for inhibiting the cell growth of U-937 and HL-60.

According to our previous experiments (19) brown and green algae also have high tumor cell growth inhibitory activities, however, in this study red algae exhibited moderate/low activities towards tumor cell growth inhibition. In this study aqueous extracts were less potent than methanolic extracts on tumor cell growth inhibition.

The activities reported in aqueous extracts can be attributed to the polysaccharides of the red algal species (20,21). Yamamoto and Maruyama (22) demonstrated that partially purified polysaccharide fraction from seaweeds, the activity component of which was suggested to be a sulfated glycuronoglycan, was effective against sarcoma-180 cells and that the aqueous fraction of seaweeds decreased the growth of Meth-A, B-16 melanoma and L-1210 leukemia cells. Other studies have shown that daily injection with polysaccharide fraction from red algae after tumor cell inoculation resulted in tumor growth inhibition of the mice (20,21). Therefore, the high antitumor activity of red algal species on the B-16 cell

Table 6. The overall growth inhibitory activity of methanolic and aqueous extracts on tumor cell line

Sample[1)]	U-937	HL-60	B-16	HeLa
Methanolic extracts	23.89±2.40[a2)]	27.06±2.12[a]	20.03±2.63	11.01±1.34
Aqueous extracts	11.07±1.90[b]	14.73±2.22[b]	21.08±3.27	8.80±1.28
Extracts at 20°C	17.55±2.40[NS3)]	21.70±2.29	23.15±3.34	9.41±1.36
Extracts at 70°C	17.42±2.31	20.09±2.40	16.88±2.44	8.01±1.21

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

line is probably due to their polysaccharide constituents.

In this study, high antitumor activity was recorded from methanol fractions. As it has been reported previously, polyphenolic compounds can be effectively extracted into organic solvent fractions. Polyphenols have been reported to inhibit telomerase activity in cell free studies as well as leukemia and HT-29 colon adenocarcinoma cells (23). Polyphenols of brown algal extracts have been identified for their potential bioactivities such as antioxidant, antibacterial and antihyluranase (24-35). Moreover, leutin, carotein, pheophytin, chlorophyllin-a and chlorophyll-a are also possible antitumor compounds isolated from algal species (36). These compounds can be easily extracted using organic solvents, therefore similar compounds may associated with the methanol fractions of red algae and responsible for their antitumor activity. Cho et al. (37) have also reported that methanolic extracts of red algae exhibited dose-dependent inhibition of the growth of human gastric and HT-29 colon cancer cells. However, there are few studies related to antitumor activity of red algae compared to that of brown and green algae. Therefore, systematically designed experiments are required to investigate the main active compounds of red algae for their good antitumor activity. Most red algae species tested in this preliminary screening showed potential antitumor activities. Of the algae species *S. okamurae*, *M. denticulate* and *P. japonica* showed excellent antitumor activities. Therefore, red algae being present in Jeju Island are also possible candidates for future antitumor drug discovery. Further studies are being carried out to purify antitumor active compounds from those species to elucidate structure function relationships for their high antitumor activity.

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