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In vitro Screening of Seaweed Extract on the Proliferation of Mouse Spleen and Thymus Cell

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Abstract A total number of 31 types of seaweed were assessed with regard to their effects on the proliferation of mouse spleen and thymus cells in a culture, using an MTT reduction assay. Acetone:dichloromethane (1:1) extracts of three seaweed plants: *Derbesia marina*, *Sargassum* sp., and *Hisikia fuziformis*, exhibited significantly positive effects on the survival of mouse spleen and thymus cells *in vitro*. The acetone:dichloromethane (1:1) extracts of *Sargassum* sp., in particular, much more potent effects on thymus cell activation than did any of the other types of seaweed. However, the methanol extracts of *Sargassum ringgoldianium* and *Chondrus crispus* exerted a stimulatory influence only on the proliferation of mouse spleen cells, whereas the methanol extracts of *Grateloupia lanceolata* exhibited significant cell proliferation properties in both spleen and thymus cells.

Keywords: seaweed, spleen, thymus, MTT

Lymphocytes are known to play pivotal roles in the immune response. Lymphocytes such as T- and B-cells are thought to be wholly responsible for the specific immune recognition of pathogens, and thereby are thought to be responsible for the initiation of adaptive immune responses. Of the two immune organ, the thymus is the principal site at which T-cells differentiate from lymphoid stem cells, later proliferating and maturing into functional cells. The spleen functions as a reservoir for the T-, and B-cells, as well as macrophages which perform immunological functions [1]. Therefore, the regulation of thymus and spleen cell proliferation can be considered an important marker for immune response control. It has been reported that the hot water extract of *Rhaphidophora korthalsii* which had stimulated splenocytes and immunological activity of four phytochemicals isolated from *Stephanotis mucronata* by measuring mice splenocyte proliferation *in vitro* [2,3].

Recently much attention has been paid to seaweeds which have been used traditionally as food. Seaweed is recommended as a food supplement to help the recommended daily intake as it tend to harbor high levels of essential minerals and trace elements [4]. Seaweed also can be a good source of essential fatty acids such as eicosapentaenoic acid which reduce the risk of heart disease, thrombosis and atherosclerosis [5]. It has also been reported that antiviral activity of seaweeds and water-soluble fraction of a methanol extract from *Ulva lactuca* have antitumor and immunostimulating activities [6,7]. However, the effects of seaweed on immune cell proliferation have not been reported so much.

In our continuous search for novel metabolites from marine resources [8-11], we have attempted to characterize the effects of several seaweed extracts on the proliferation of mouse spleen and thymus cells in culture.

Our seaweed samples were collected along the shores of Kijang, Kyungsangnamdo, and Kuiduck on Cheju Island, Korea, between December, 2002 and February, 2003. The taxonomic identifications of seaweed specimens were confirmed by an alga taxonomist, J. S. Yoo, at the Korea Maritime University, in Korea, on the basis of Lee and Kang's classification system [12].

Shade-dried seaweed was extracted with a volumetrically identical solvent of mixed acetone and dichloromethane for 24 h at room temperature. This step was repeated twice. The extracted seaweed solution was then evaporated under vacuum, yielding a dark, sticky, crude extract. The seaweed residue was then extracted once more with methanol, according to the same procedure. Each of the crude extracts was used as experimental material. The prepared samples were then stored in a refrigerator at −20°C, for later study.

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Table 1. Effects of seaweed extracts on mouse spleen cell proliferation

Seaweeds	Proliferation rate (%)	
	MeOH ext.	Acetone/CH_2Cl_2 ext.
Green algae		
Codium adhaerens	9.75 ± 22.98	29.75 ± 5.30
Enteromorpha linza	−10.00 ± 16.97	65.25 ± 26.52
Ulva pertusa	6.50 ± 21.92	118.25 ± 8.84
Derbesia marina	128.75 ± 71.77	239.50 ± 3.54
Brown algae		
*Colpomenia sinuosa**	−47.25 ± 13.08	79.50 ± 15.56
Colpomenia bullosa	−24.50 ± 3.54	144.75 ± 6.72
*Padina arborescens**	150.48 ± 34.09	−64.76 ± 37.29
Hisikia fuziformis	14.50 ± 12.02	369.50 ± 103.24
Dictyota dichotoma	40.00 ± 3.54	74.50 ± 7.78
*Pachydictyon coriaceum**	147.62 ± 10.82	0.95 ± 1.65
Laminaria japonica	141.90 ± 14.66	32.38 ± 46.28
*Scytosiphon lomentaria**	121.90 ± 25.39	118.10 ± 8.25
Sargassum honerii	2.00 ± 2.83	114.50 ± 16.97
*Sargassum ringgoldianium**	200.00 ± 17.38	67.62 ± 83.32
*Sargassum sagamianum**	−37.14 ± 21.82	−85.71 ± 21.63
Sargassum sp.	128.00 ± 21.21	220.00 ± 16.97
Red algae		
Gymngongrus flabelliformis	−28.00 ± 6.36	50.25 ± 13.79
Chondrus crispus	215.24 ± 28.19	−60.95 ± 32.87
Gracillaria textori	−6.67 ± 14.66	−98.10 ± 29.00
*Gelidium amansii**	−21.25 ± 6.72	36.25 ± 30.05
Symphyocladia latiuscula	121.75 ± 15.20	139.75 ± 8.13
Halymenia acuminate	−32.50 ± 7.78	71.50 ± 11.31
plocamium telfairiae	−0.75 ± 16.62	43.25 ± 80.26
Corallina pilulifera	−41.00 ± 4.95	71.50 ± 7.07
Chondria crassicaulis	−46.50 ± 4.95	72.75 ± 11.67
*Laruencia okamurae**	−45.71 ± 5.95	−61.90 ± 23.09
Laurencia intermedia	105.71 ± 15.74	−50.48 ± 30.28
Pachymeniopsis lanceolata	−14.75 ± 11.67	38.25 ± 13.08
Grateloupia lanceolata	202.86 ± 18.59	64.76 ± 39.69
Porphyra suborbiculata	−25.75 ± 6.72	70.50 ± 4.24
Grateloupia turutura	22.75 ± 7.42	−14.50 ± 7.78

Cell viability was evaluated by the MTT colorimetric assay. Each sample dissolved with ethanol. The experiments was tested at the 200 μg/mL concentration and added 2 μL volume to the each wells. Each value indicates the mean ± S.D. of three experiments.
*The sample was collected at Kuiduck on Cheju Island.

BALB/c mice were purchased from the Korea Experimental Animal Center (Seoul, Korea), and were used at 6 weeks of age. These mice were housed in polyethylene cages containing clean wood shavings, and were provided *ad libitum* with rodent chow and tap water. They were kept in a room at constant temperature and humidity, on a 12-h photocycle. The cell culture reagents and chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO, USA).

We used an MTT assay to determine the degree of cell death occurring in the cultures, via measurements of the formation of dark blue formazan dye crystals resulting from the reduction of the tetrazolium ring of MTT [13]. The reduction of MTT is believed to occur primarily in the mitochondria via the activity of succinate dehydrogenase, therefore providing a measure of mitochondrial function. After the incubation of 10^6 spleen or thymus cells in a medium supplemented with either seaweed extract or distilled water for 4 h, the cells were treated with the MTT reagent for 6 h, until purple precipitates formed. These precipitates were then dissolved with a detergent reagent, after which we measured the absorbance at a wavelength of 595 nm, in order to determine the degree of MTT reduction which had occurred, representing the quantity of live cells in the culture. All experiments were performed in triplicate, and repeated twice. The number

Table 2. Effects of seaweed extracts on mouse thymus cell proliferation

Seaweeds	Proliferation rate (%)	
	MeOH ext.	Acetone/CH_2Cl_2 ext.
Green algae		
Codium adhaerens	−2.97 ± 7.97	77.15 ± 1.26
Enteromorpha linza	−12.76 ± 17.63	66.77 ± 20.14
Ulva pertusa	8.31 ± 17.21	141.54 ± 9.23
Derbesia marina	168.84 ± 6.71	224.33 ± 35.67
Brown algae		
*Colpomenia sinuosa**	−41.54 ± 19.72	133.53 ± 21.40
Colpomenia bullosa	−9.20 ± 19.30	138.28 ± 84.35
*Padina arborescens**	65.82 ± 108.28	−131.65 ± 71.28
Hisikia fuziformis	17.80 ± 6.29	252.33 ± 6.29
Dictyota dichotoma	22.26 ± 9.23	−0.89 ± 16.79
*Pachydictyon coriaceum**	172.15 ± 23.72	20.25 ± 18.73
Laminaria japonica	174.68 ± 13.69	11.39 ± 32.30
*Scytosiphon lomentaria**	120.25 ± 10.05	105.06 ± 6.58
*Sargassum ringgoldianium**	98.73 ± 10.05	5.06 ± 17.54
Sargassum honerii	−13.95 ±15.11	91.69 ± 10.07
*Sargassum sagamianum**	−96.20 ± 59.68	−84.81 ± 37.40
Sargassum sp.	104.75 ± 27.70	534.12 ± 7.97
Red algae		
Gymngongrus flabelliformis	−2.37 ± 7.97	91.69 ± 10.07
Chondrus crispus	141.77 ± 19.11	−89.87 ± 100.69
Gracillaria textori	−11.39 ± 13.15	−156.96 ± 118.09
*Gelidium amansii**	−13.35 ± 3.36	67.95 ± 0.84
Symphyocladia latiuscula	157.57 ± 15.95	142.43 ± 16.37
Halymenia acuminata	−18.99 ± 4.62	99.41 ± 30.21
plocamium telfairiae	20.47 ± 20.98	136.80 ± 31.05
Corallina pilulifera	−35.01 ± 1.26	102.97 ± 30.21
Chondria crassicaulis	−28.49 ± 28.12	148.96 ± 14.69
*Laruencia okamurae**	−62.03 ± 27.99	−75.95 ± 13.69
Laurencia intermedia	78.48 ± 16.55	−89.87 ± 62.67
Pachymeniopsis lanceolata	−0.89 ± 16.79	77.15 ± 25.60
Grateloupia lanceolata	245.57 ± 18.73	118.99 ± 30.93
Porphyra suborbiculata	−4.15 ± 14.69	99.70 ± 12.17
Grateloupia turuturu	46.29 ± 16.37	0 ± 13.01

Cell viability was evaluated by the MTT colorimetric assay. Each sample dissolved with ethanol. The experiments was tested at the 200 μg/mL concentration and added 2 μL volume to the each wells. Each value indicates the mean ± S.D. of three experiments.
*The sample was collected at Kuiduck on Cheju Island.

of living cell was calculated using the following equation.

$$\text{Proliferation rate (\%)} = (B - A) \times 100/A$$

Where A is the optical density without sample, and B is the optical density with sample.

A total of 62 crude extracts, obtained from 31 different specimens of seaweed collected in the southern coastal areas of Korea were screened for their effects on the proliferation of mouse spleen and thymus cells in a culture. In mammals, the spleen and thymus are crucial organs, in which lymphocytes, including B- and T-cells, macrophages, and leukocytes, are developed and differentiated into mature cells. The spleen also functions in the direction and control of immune responses, acting as a reservoir for a variety of immune cells. The effects of seaweed extracts on the proliferation of mouse spleen and thymus cells are shown in Table 1 and 2, respectively. Three acetone/dichloromethane extracts and three methanol extracts were determined to be more than two times as effective on mouse spleen cell proliferation as was the control treatment. The extracts which exhibited spleen cell stimulating activity are as follows: the acetone/dichloromethane extracts of *Hisikia fuziformis* (369.5%), *Derbesia marina* (239.5%), and *Sargassum* sp. (220.0%); and the methanol extracts of *Chondrus crispus* (215.2%), *Grateloupia lanceolata* (202.9%), and *Sargassum ringgoldianium* (200.0%). The effects of these seaweed ex-

tracts on thymus and spleen cells were quite similar. However, the methanol extracts of *C. crispus* (98.7%) and *S. ringgoldianium* (141.8%) exhibited feeble influences on the proliferation of thymus than spleen cells. The acetone/dichloromethane extract of *Sargassum* sp. (534.1%) was determined to be five times more effective than the control with regard to the activation of thymus cells. Four crude extracts (*Pachydictyon coriaceum*, *Laminaria japonica*, *Scytosiphon lomentaria*, and *Symphyocladia latiuscula*) were considered as medium stimulating activities between 100 and 200% compared to the control. The majority of the remaining seaweed extracts had weak effects or no discernable effects on the proliferation of the mouse spleen and thymus cells. The acetone/dichloromethane extract of *Gracillaria textori* (−156.9%) was seemed to be cytotoxic against immune cells. These findings indicate that some bioactive components of the crude extracts of some seaweeds do, indeed, exert stimulatory effects on the proliferation of thymus and spleen cells *in vitro*. Liu *et al.* reported that the crude extracts of *Hizikia fusiformis* stimulated markedly normal mouse spleen cells and human lymphocytes to proliferate *in vitro*. These reports are in accordance with our results. It has been also reported that *H. fusiformis* extracts had stimulated immunoglobulin production by B-cells and monocytes to produce tumor necrosis factor. Moreover, it is demonstrated that the active nature of *H. fusiformis* extracts reside in polysaccharide fractions [14,15].

As shown above, some seaweed species might be useful for as immunomodulatory candidates. To the best of our knowledge, our report is the first to address the effects of seaweed variants on the proliferation of mouse spleen and thymus cells *in vitro*. Further study will be required in order to establish methods for the efficient isolation and purification of the bioactive principle, as well as the mechanism by which seaweed extracts regulate immune cell functions.

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REFERENCES

[1] Roitt, I., J. Brostoff, and D. Male (1993) *Immunology.* Mosby-Year Book Europe Ltd. pp. 1.3-1.12.

[2] Wong, K. T. and B. K. H. Tan (1996) *In vitro* cytotoxicity and immunomodulating property of *Rhaphidophora korthalsii*. *J. Ethnopharmacol.* 52: 53-57.

[3] Ye, Y., H. Sun, X. Li, F. Chen, F. Qin, and Y. Pan (2005) Four new C-21 steroidal glycosides from the roots of *Stephanotis mucronata* and their immunological activities. *Steroids* 70: 791-797.

[4] Ruperez, P. (2002) Mineral content of edible marine seaweeds. *Food Chem.* 79: 23-26.

[5] Sanchez-Machado, D. I., J. Lopez-Cervantes, J. Lopez-Hernandez, and P. Paseiro-Losada (2004) Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem.* 85: 439-444.

[6] Kamat, S. Y., S. Wahidulla, L. DSouza, C. G. Naik, V. Ambiye, D. S. Bhakuni, A. K. Goel, H. S. Garg, and R. C. Srimal (1992) Bioactivity of marine organisms VI. Antiviral evaluation of marine algal extracts from the Indian coast. *Botanica Marina* 35: 161-164.

[7] Lee, D. G., J. W. Hyun, K. A. Kang, J. O. Lee, S. H. Lee, B. J. Ha, J. M. Ha, E. Y. Lee, and J. H. Lee (2004) *Ulva lactica*: A potential seaweed for tumor treatment and immune stimulation. *Biotechnol. Bioeng.* 9: 236-238.

[8] Seo, Y., H. J. Lee, K. E. Park, Y. A. Kim, J. W. Ahn, J. S. Yoo, and B. J. Lee (2004) Peroxynitrite scavenging constituents from the brown alga *Sargassum thunbergii*. *Biotechnol. Bioeng.* 9: 212-216.

[9] Lee, H. J., Y. A. Kim, J. W. Ahn, B. J. Lee, S. G. Moon, and Y. Seo (2004) Screening of peroxynitrite and DPPH radical scavenging activities from salt marsh plants. *Kor. J. Biotechnol. Bioeng.* 19: 57-61.

[10] Lee, H. J., J. W. Ahn, B. J. Lee, S. G. Moon, and Y. Seo (2004) Antioxidant activity of *Rosa rugosa*. *Kor. J. Biotechnol. Bioeng.* 19: 67-71.

[11] Park, K. E., Y. A. Kim, H. A. Jung, H. J. Lee, J. W. Ahn, B. J. Lee, and Y. Seo (2004) Three norisoprenoids from the brown alga *Sargassum thunbergii*. *J. Kor. Chem. Soc.* 48: 394-398.

[12] Lee, Y. and S. Kang (2001) *A catalogue of the seaweeds in Korea*. Publishing Department of Cheju National University.

[13] Slater, T. F., B. Sawyer, and U. Struli (1963) Studies on succinate-tetrazolium reductase systems III, points of coupling of four different tetrazolium salts. *Biochem. Biophys. Acta* 77: 383-393.

[14] Liu, J. N., Y. Yoshida, M. Q. Wang, Y. Okai, and U. Yamashita (1997) B cell stimulation activity of seaweed extracts. *Int. J. Immunopharmacol.* 19: 135-142.

[15] Shan, B. E., Y. Yoshida, E. Kuroda, and U. Yamashita (1999) Immunomodulating activity of seaweed extract on human lymphocytes *in vitro*. *Int. J. Immunopharmacol.* 21: 59-70.

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