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Effects of Several Salt Marsh Plants on Mouse Spleen and Thymus Cell Proliferation Using MTT Assay

Youngwan Seo[1]*, Hee-Jung Lee[2], You Ah Kim[1], Hyun Joo Youn[3], and Burm-Jong Lee[4]

[1]*Division of Marine Environment and Biosciences, Korea Maritime University, Busan 606-791, Korea*
[2]*Research Institute of Marine Science and Technology(RIMST), Korea Maritime University, Busan 606-791, Korea*
[3]*Department of Microbiology and School of Biotechnology & Biomedical Science, Inje University, Gimhae 621-749, Korea*
[4]*Department of Chemistry, Inje University, Gimhae 621-749, Korea*

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Abstract – In the present study, we have tested the effects of 21 salt marsh plants on cell proliferation of mouse immune cells (spleen and thymus) using MTT assay in culture. The methanolic extracts of six salt marsh plants (*Rosa rugosa, Ixeris tamagawaensis, Artemisia capillaris, Tetragonia tetragonoides, Erigeron annus,* and *Glehnia littoralis*) showed very powerful suppressive effects of mouse immune cell death and significant activities of cell proliferation *in vitro*. Especially, the methanolic extract of *Rosa rugosa* was found to have fifteen times compared to the control treatment, demonstrating that *Rosa rugosa* may have a potent stimulation effect on immune cell proliferation. These results suggest that several salt marsh plants including *Rosa rugosa* could be useful for further study as an immunomodulating agent.

Key words – salt marsh, spleen, thymus, MTT

1. Introduction

Immune response is produced primarily by leucocytes, of which there are several different types. One important group of leucocytes is the lymphocytes like T and B-cell group, which is wholly responsible for the specific immune recognition of pathogens. The T-cell is developed in the thymus, a major primary lymphoid organ and the B-cell also in the bone marrow in the case of mammals. These immune cells then migrate to the spleen, the secondary lymphoid tissue, where they can respond to antigens (Roitt *et al.* 1993; Fleisher *et al.* 2004). Therefore, the regulation of thymus and spleen cell proliferation can be considered an important marker for immune response control. This has been demonstrated in some literature. For example, the hot water extract of *Rhaphidophora korthalsii* and four phytochemicals isolated from *Stephanotis mucronata* have been established immunomodulating properties by measuring mice splenocyte proliferation *in vitro* (Wong *et al.* 1996; Ye *et al.* 2005).

Although salt marsh plants were recognized environmentally as one of the most important ecosystems in a tidal zone because they play vital roles in the tidal ecology, such as serving as buffers, protecting the shorelines from erosion by the force of waves, and filtering contaminants from the land, their biological activities as well as their secondary metabolites have been little reported until now (Bang *et al.* 2002; Choi and Kim 1998; Han *et al.* 2003; Jo *et al.* 2002).

Therefore, as a part of our search for new immunomodulatory compounds from natural sources (Seo *et al.* 2004; Lee *et al.* 2004; Park *et al.* 2004) , we tried to screen the effects of halophytes extracts, recognized as new sources, on the proliferation of mouse spleen and thymus cell in culture.

2. Materials and Methods

The salt marsh plants were collected at Daebudo, Korea in October, 2003 and Yangpori, Pohang and Geojedo,

*Corresponding author. E-mail: ywseo@hhu.ac.kr

Korea in September, 2002. The taxonomic identification of salt marsh plants was confirmed by a botanist, S. G. Moon, in the Kyungsung University, Korea. The shade-dried salt marsh plant was chopped, then extracted with dichloromethane for 24 hrs at room temperature. This step was repeated twice. The combined solutions were evaporated under a vacuum to give a dark sticky crude extract. The salt marsh plant was extracted with methanol by the same procedure. Each crude extract was completely dried by a lyophilizer and then used as experimental material. The prepared samples were stored in a refrigerator at -25°C until further study.

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-hour 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 (Slater *et al.* 1963). The reduction of MTT is believed to occur primarily in the mitochondria via the activity of succinate dehydrogenase, thereby providing a degree 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 hours, the cells were treated with the MTT reagent for 6 hours, 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 that had occurred, representing the quantity of live cells in the culture. All experiments were performed in triplicate, and repeated twice. The number of living cells was calculated using the following equation. Proliferation rate (%) = (B-A) × 100/A. Where A is the optical density

Table 1. Effects of salt marsh plants on mouse spleen and thymus cell proliferation

Salt Marsh plants	Cell Proliferation (%)			
	spleen		thymus	
	MeOH ext.	CH_2Cl_2 ext	MeOH ext.	CH_2Cl_2 ext
Artemisia capillaries	630.70 ± 70.71	226.02 ± 22.74	504.77 ± 21.03	188.19 ± 44.31
Aster spathulifolius	145.03 ± 0	120.18 ± 2.07	121.36 ± 53.26	116.83 ± 5.82
Calystegia soldanella	43.27 ± 8.27	267.25 ± 10.75	49.50 ± 2.24	227.39 ± 7.61
Carex scabrifolia	5.85 ± 6.62	50.88 ± 7.44	7.29 ± 21.03	12.81 ± 85.48
Corydalis heterocarpa	11.43 ± 2.27	−95.24 ± 11.44	65.38 ± 20.35	−69.23 ± 7.36
Erigeron annus	322.81 ± 7.44	145.61 ± 2.48	611.06 ± 56.39	114.57 ± 14.32
Glehnia littoralis	564.91 ± 363.89	214.33 ± 11.99	350.29 ± 54.60	182.66 ± 29.09
Imperata cylindrical	90.35 ± 8.68	171.64 ± 37.63	68.34 ± 0.9	136.68 ± 0.9
Ixeris tamagawaensis	535.09 ± 97.59	60.23 ± 19.02	469.85 ± 14.32	57.04 ± 3.13
Lactuca indica Linne	−54.09 ± 6.20	16.37 ± 0	−34.17 ± 11.64	24.62 ± 2.69
Lathyrus japonicus Willdenow	13.16 ± 9.51	235.67 ± 46.31	7.79 ± 20.14	130.40 ± 21.93
Limonium tetragonum	32.46 ± 7.86	235.09 ± 40.52	17.34 ± 26.40	174.62 ± 43.41
Messerschmidia sibirica	77.49 ± 21.92	157.02 ± 2.89	66.33 ± 10.74	121.11 ± 20.59
Persicaria lapathifolia	−28.65 ± 2.48	87.13 ± 16.54	−6.53 ± 28.64	61.06 ± 1.34
Polygonum aviculare	135.61 ± 14.79	104.55 ± 6.94	98.47 ± 22.08	140.46 ± 9.98
Rosa rugosa	466.37 ± 317.16	83.33 ± 52.52	1538.69 ± 26.06	146.98 ± 49.68
Salsola komarovii	44.74 ± 1.24	192.40 ± 65.34	42.71 ± 3.58	168.84 ± 15.22
Suaeda asparagoides	−9.36 ± 0	133.92 ± 11.58	−8.79 ± 7.61	93.72 ± 3.13
Suaeda japonica	−53.22 ± 11.58	164.33 ± 61.20	−32.41 ± 6.71	120.10 ± 25.06
Tetragonia tetragonoides	502.92 ± 202.62	161.70 ± 115.37	502.76 ± 98.91	261.31 ± 17.01
Vitex rotundifolia	115.24 ±19.68	−65.71 ± 13.70	150 ± 22.94	−67.95 ± 38.93

Cell viability was evaluated by the MTT colorimetric assay. Each sample was dissolved with ethanol. The experiments were conducted at concentrations of 200 μg/ml and a 2μl volume was added to each of the wells. Each value indicates the mean ± S.D. of the three experiments.

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

3. Results and Discussion

The effect of salt marsh plant extracts on cell proliferation of mouse spleen and thymus was presented in Table 1. Cell proliferation was determined by MTT assay, which is useful for measuring living cells by the mitochondrial dehydrogenase activity (Mossman 1983). Among 21 species of salt marsh plants, six methanolic extracts - *Artemisia capillaries* (630.7%, 504.8%), *Erigeron annus* (322.8%, 611.1%), *Glehnia littoralis* (565.0%, 350.3%), *Ixeris tamagawaensis* (535.1%, 469.9%), *Rosa rugosa* (466.4%, 1538.7%) and *Tetragonia tetragonoides* (503.0%, 502.8%) - were found to have very significant effects on spleen and thymus cell activation, about three to six times compared to that of the control group, in fact. In particular, the methanolic extract of *Rosa rugosa* showed fifteen times more activity on mouse thymus cell proliferation *in vitro* than the control treatment. However, the dichloromethane extract of these plants exhibited far more feeble spleen and thymus cell proliferation effects than in methanolic extracts. Thus, it may be supposed to be due to the polar components of salt marsh plants, even though it is not clear what the cause of cell proliferation effect by these methanolic extracts is.

On the other hand, three dichloromethane extracts (*Lathyrus japonicus Willdenow* (235.7%), *Limonium tetragonum* (235.1%), *Salsola komarovii* (192.4%)) were considered as medium spleen cell stimulating activities, about 200% compared to that of the control group. And the dichloromethane extract of *Calystegia soldanella* significantly exhibited proliferation effects on both spleen (267.2%) and thymus (227.4%) cells. The majority of the remaining salt marsh plants had weak effects or no discernable effects on the proliferation of the mouse spleen and thymus cells. The datum presented as negative figures seemed to be related to cytotoxic effects against immune cells.

Regulation of the proliferation of spleen and thymus cell is known to be closely related to maintaining immune homeostasis (Ohta *et al.* 1996; Park *et al.* 2004). It has been reported that the polysaccharide fraction showing spleen cell proliferation activity is closely related to the anti-apoptotic effect of mouse spleen and thymus (Hwang *et al.* 2003; Koo *et al.* 1994). Some marine plants have also been reported to have stimulatory activities on normal mouse spleen cells and human lymphocytes to proliferate *in vitro* with immunoglobulin production showing increases as a result of B cells and monocytes (Lee *et al.* 2004; Liu *et al.* 1997; Shan *et al.* 1999). Therefore, in this study, some plant extracts possessing significant cell proliferation activity might be useful as immunomodulatory candidates. To the best of our knowledge, our report is the first to address the effects of several salt marsh plants on the proliferation of mouse spleen and thymus cells *in culture*. These results suggest that some salt marsh plants could be valuable sources for the modulation of immune responses.

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References

Bang, M.A., H.A. Kim, and Y.J. Kim. 2002. Hypoglycemic and antioxidant effect of dietary hamcho powder in streptozotocin-induced diabetic rats. *J. Kor. Soc. Food Sci. Nutr.*, **31**, 840-846.

Choi, H.K. and H.J. Kim. 1998. A distributional patten of halophytes around Shiwha lake in Korea. *J. Ajou Nat. Sci.*, **3**, 1-5.

Fleisher, T.A. and J.B. Oliveira. 2004. Functional and molecular evaluation of lymphocytes., *J. Allergy Clin. Immunol.*, **114**, 227-234.

Han, S.K., S.M. Kim, and B.S. Pyo. 2003. Antioxidative effect of glasswort (*Salicornia herbacea* L.) on the lipid oxidation of pork. *Kor. J. Food Sci. Ani. Resour.*, **23**, 46-49.

Hwang, J.S., H.K. Chung, E.K. Bae, A.Y. Lee, H.J. Ji, D.W. Park, H.J. Jung, C.W. Cho, D. S. Lee, K. R. Kee, and H. J. Youn. 2003. The polysaccharide fraction AIP1 from *Artemisia iwayomogi* suppresses apoptotic death of the mouse spleen cells in culture. *Arch. Pharm. Res.*, **26**, 294-300.

Jo, Y.C., J.H. Ahn, S.M. Chon, K.S. Lee, T.J. Bae, and D.S. Kang. 2002. Studies on pharmacological effects of glasswort (*Salicornia herbacea L.*). *Kor. J. Medicinal Crop Sci.*, **10**, 93-99.

Koo, K.A., J.H. Kwak, K.R. Lee, O.P. Zee, E.R. Woo, H.K. Park, and H.J. Youn. 1994. Antitumor and immunomodulating activities of the polysaccharide fractions from Artemisia selengensis and Artemisia iwayomogi. *Arch. Pharm. Res.*, **17**, 371-374.

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.

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.

Liu, J. N., Y. Yoshida, M.Q. Wang, Y. Okai, and U. Yamashita. 1997. B cell stimulation activity of seaweed extracts. *Int. J. Immunopharmaco.*, **19**, 135-142.

Mossman, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. *J. Immunol. Methods,* **65**, 55-63.

Ohta, Y., S. Fukuda, A. Baba, H. Nagai, T. Taskuda, T. Sohda, and H. Makino. 1996. Immunomodulating and articular protecting activities of a new anti-rheumatic drug, TAK-603. *Immunopharmacology*, **34**, 17-26.

Park, J.H., J.K. Lee, H.S. Kim, S.T. Chung, J.H. Eom, K.A. Kim, S.J. Chung, S.Y. Paik, and H.Y. Oh. 2004. Immunomodulatory effect of caffeic acid phenethyl ester in Balb/c mice. *Int. Immunopharmacol.*, **4**, 429-436.

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.

Roitt, I., J. Brostoff, and D. Male. 1993. *Immunology.* Mosby, London. 423 p.

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.

Shan, B.E., Y. Yoshida, E. Kuroda, and U. Yamashita. 1999. Immunomodulating activity of seaweed extract on human lymphocytes *in vitro*. *Int. J. Immunopharmaco.*, **21**, 59-70.

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.

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

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.