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Inhibitory Effects of the Methanolic Extract of an Edible Brown Alga, *Ecklonia stolonifera* and Its Component, Phloroglucinol on Aflatoxin B_1 Mutagenicity *In Vitro* (Ames Test) and on Benzo(a)pyrene or N-Methyl N-nitrosourea Clastogenicity *In Vivo* (Mouse Micronucleus Test)

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Abstract – The antimutagenic activity of a methanol extract of *Ecklonia stolonifera* (Laminariaceae) against aflatoxin B_1 (AFB_1) was demonstrated with the *Salmonella typhimurium* assay. The numbers of revertants per plate decreased significantly when this extract was added to the assay system using *S. typhimurium* TA100. The methanol extract also exhibited significant inhibitory effects on the micronuclei formation in mouse peripheral blood reticulocytes and the DNA damage in mouse spleen lymphocytes induced by N-methyl-N-nitrosourea (MMU) and benzo(a)pyrene (B(a)P). The MeOH extract was then sequentially partitioned with CH_2Cl_2, CH_2Cl_2 insoluble intermediate, EtOAc, *n*-BuOH, and H_2O. All fractions possessed antimutagenic activity but the H_2O fraction was inactive. Among active fractions, the EtOAc and CH_2Cl_2 insoluble intermediate fractions showed the highest activity. Column chromatography using SiO_2 and Sephadex LH-20 yielded phloroglucinol from the EtOAc fraction. Phloroglucinol also demonstrated significant antimutagenic activity, and inhibitory effects on the micronuclei formation in mouse peripheral blood reticulocytes and DNA damage in mouse spleen lymphocytes induced by MMU and B(a)P.

Key words – *Ecklonia stolonifera*, phloroglucinol, Ames test, micronucleus test, Comet assay.

Introduction

Humans are exposed to a variety of genotoxins leading to heritable mutations. These genotoxins include food additives, drugs, insecticides, cosmetics, contaminants in the water and air, and various chemical substances used in the home or in industry (Ames, 1983). In addition, a close correlation between mutagenicity and carcinogenicity has also been demonstrated (Brookes and de Serres, 1976). Although it would not be possible to avoid exposure to all genotoxins, it is becoming increasingly clear that the effects of some classes of genotoxins are inactivated or reversed by some anti-genotoxins

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which occur in natural sources such as fruits and vegetables (Block *et al.*, 1992). It has been suggested that the use of anti-genotoxins in everyday life will be the most effective procedure for preventing human cancer and genetic disease. Trials have also been carried out to detect inhibitors of these genotoxic substances from plant and animal sources including marine algae, and to study the mechanism and implications of their action.

In Korea, seaweeds are traditionally used as a foodstuff and an additive or seasoning with various foodstuffs, and are used as a form of medicine in the curing of curare, gout, eczema and gallstones. However, the details of the antigenotoxic activity of edible seaweeds have been poorly elucidated until now. Recently, heterogeneous antimutagenic activities have been identified in the extract of *Laminaria japonica*, *Hijikia fusiforme* and *Undaria pinnatifida* (Okai *et al.*, 1993; Okai and Higashi-Okai, 1994).

We previously reported that the MeOH extracts of different kinds of seaweed as to their antioxidizing activity by measuring lipid peroxides produced when a mouse liver homogenate is exposed to the air at 37℃, using 2-thiobarbituric acid and the radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl radical. MeOH extract of *E. stolonifera* and the phloroglucinol isolated were shown to have a strong antioxidizing activity (Choi *et al.*, 1993; Lee *et al.*, 1996). Since the close correlation between antioxidant activity and antimutagenicity had been demonstrated (Yen and Chen, 1995; Shiraki *et al.*, 1994), we studied the antimutagenic activities of the MeOH extract of the *E. stolonifera*, several fractions purified from the MeOH extract, and phloroglucinol isolated from the EtOAc fraction using the *Salmonella*/microsome assay (Ames test). The antimutagenic activity was assayed against mutagen of AFB_1. We further investigated the effect of the MeOH extract and phloroglucinol on the micronuclei formation in mouse peripheral blood reticulocytes and DNA damage in mouse spleen lymphocytes induced by MMU and B(a)P. *E. stolonifera* is an edible brown algae which belongs to the Laminariaceae. It has been reported that phloroglucinol and ecklonialactones isolated from the algae have a feeding-deterrent effect on the sea urchin and abalone (Kurata *et al.*, 1989 and 1993; Taniguchi *et al.*, 1991 and 1992). The protein-polysaccaride fraction extracted from the algae also has an antitumor effect on Sarcoma-180 cells (Lee *et al.*, 1992). But, no study on the anti-genotoxic activity of has yet appeared in the literature.

Experimental

Algae material – *E. stolonifera* used was collected at Tae Jong Dae, Pusan in July, 1990. The algae was identified by the taxonomist Prof. H. G. Kim, and a voucher specimen is deposited in the author's laboratory. The seaweed was washed with fresh water and air dried in the shade.

Extraction and fractionation – The dried material (2.9 kg) was extracted three times with methanol, and solvent was removed under reduced pressure to a dark blue semisolid (500 g). Successive partitioning yield dichloromethane (80.3 g), dichloromethane insoluble intermediate phase (7.5 g), ethyl acetate (17 g), *n*-butanol (13.8 g) and water soluble (275.5 g) fractions, respectively.

Isolation of phloroglucinol – The ethyl acetate soluble fraction was chromatographed over silica gel using a $CHCl_3$-MeOH mixture and further separated by Sephadex LH-20 using MeOH to yield the phloroglucinol (Fig. 1) in the form of hygroscopic powder, m.p. 218℃, which was identified by direct comparison with an authentic sample (m.m.p., ^{1}H- and ^{13}C-NMR).

Mutagens – AFB_1 and B(a)P were purchased from the Sigma Chemical Co., St.

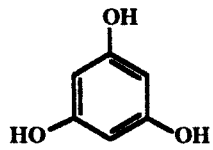

Fig. 1. Chemical structure of purified active compound, phloroglucinol.

Louis, MO, USA. MNU was purchased from the Aldrich Chemical Co., Milwaukee, WI, USA.

Animals and treatment – Male ICR mice (20-25 g) were maintained in chambers with laminar air flow at a temperature of 20±2℃ and relative humidity of 55±5% during the study. Each sample was administered orally once and MMU and B(a)P as mutagen were injected by intraperitoneal route immediately. At forty eight hours after the treatment, five animals per each group for the micronucleus test, and three animals per each group for the Comet test were used.

Bacterial strains – *Salmonella typhimurium* TA100 which is histidine-requiring mutants, was kindly provided by Dr. B. N. Ames, University of Califonia, Berkeley, CA, USA, and maintained as described by Maron and Ames (1983). The genotypes of the test strains were checked routinely for their histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and presence of the R factor.

Antimutagenicity test using Ames test – A modified plate incorporation procedure (Matsushima *et al.*, 1980) was employed to determine the antimutagenic effect of the MeOH extract of *E. stolonifera*, its subfractions and isolated phloroglucinol on AFB_1 -induced mutagenicity. In brief, 0.5 ml of the S9 mix (prepared from the S9 fraction of the liver of Sprague-Dawley rats treated with Aroclor 1254 (Maron and Ames, 1983) was distributed into sterile capped tubes in ice bath, and then 0.1 ml of the test bacterial suspension from an overnight culture (1~2× 10^9 cells/ml) and 0.1 ml of the test compound (50 μl of the mutagen and/or 50 μl of 0.05~5.0 mg test compounds) were added. After gently vortexing and preincubating at 37℃ for 30 min, 2 ml of the top agar kept at 45℃ was added to each tube and vortexed for 3 sec. The resulting complete mixture was overlaid on the minimal agar plate. The plates were incubated at 37℃ for 48 hr and the revertant bacterial colonies on each plate were counted. The inhibition rate (%) of mutagenicity was calculated relative to those in the control group with the mutagen by the following equation: the inhibition rate (%)=100x[(A-B)/(A-C)] where A is the number of revertants in the control group, B is the number of revertants in the test group with the mutagen, and C is the number of spontaneous revertants. Dose dependency of AFB_1 on the test strain was carried out to determine the regions of revealing mutagenicity induced by mutagens. Toxicity tests for the different levels of samples were also carried out, and the sample concentrations employed for the antimutagenic test did not show any toxicity to the tester strain (Maron and Ames, 1983).

Micronucleus test – The animals in each group received different doses of the test sample orally and were injected intraperitoneally with a single dose of MNU or B(a)P as mutagen. From each animal in a group, 5 μl of peripheral blood was collected from tail vein without any anticoagulant 48 hours after the treatment, placed on an acridine orange(AO) coated glass slide, and covered with a coverslip. AO supravitally stained reticulocytes were examined by fluorescence microscope with a blue excitation and a yellow barrier filter. The frequencies of micronucleated peripheral reticulocytes (MNRETs) were recoded based on the observation of 1,000 reticulocytes (RETs) per mouse (Hayashi *et al.*, 1990).

Comet assay – DNA damage was investigated principally using the alkaline single cell gel electrophoresis (the Comet assay)

according to the method of Singh (Singh *et al.*, 1988). At forty eight hr after the treatment, the spleen was removed from the mice which were administered samples. The spleen was suspended in 5 ml of phosphate buffered saline (without Ca^{2+}, Mg^{2+}). 5×10^5 Lymphocytes isolated from spleen were mixed 1 ml of 0.5% low melting point agarose (LMPA) and then placed on a precleaned microscope slide which was already covered with 0.65% normal melting point agarose (NMPA) to promote even and firm attachment of the second layer. The slides were immersed in a lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10, 10% DMSO, 1% Triton X-100) for 30 min to lyse the cells. The slides were removed from the lysis buffer and allowed to set in electrophoretic buffer (300 mM NaOH, 1 mM Na_2 EDTA, pH 13) for 15 minutes to allow unwinding of DNA before electrophoresis. Electrophoresis was conducted for the next 15 minutes at 25, 250 mA. After electrophoresis, the slides were washed gently to remove alkali and detergents[which would interfere with ethidium-bromide staining] by placing them horizontally and flooding them slowly with 0.4 M Tris, pH 7.5. After 15 minutes, the slides were stained by placing 50 μl of 20 μg/ml ethidium-bromide in distilled water solution on each slide, and then covering the slide with a coverglass. Observations were made using a microscope equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. Throughout the experiments, cells were graded into two categories corresponding to the following amount of DNA in the tail : <5%; no damage and >5%; damage (Anderson *et al.*, 1994). One hundred of cells per animal were examined.

Statistics – Data were collected with a mean ± standard deviation of three plates for Ames test, three or five animals for micronucleus/or Comet tests, and their significances were analyzed by Student's *t*- or Variance test.

Results

Antimutagenesis studies – The effects of the MeOH extract and their fractions of *E. stolonifera* and phloroglucinol on the mutagenicity induced by AFB_1 in *S. typhimurium* TA100 are shown in Tables 1, 2 and 3, respectively. All these test samples were not mutagenic or toxic when tested alone at the concentrations indicated (data not shown). As shown in Table 1, AFB_1 proved to be mutagenic in the *S. typhimurium* TA100 with the S9 mix. However, when the concentrations of 0.625, 1.25 and 2.50 mg/plate MeOH extract of *E. stolonifera* were added to the assay system containing *S. typhimurium* TA100, the numbers of revertant per plate decreased by about 39%, 45% and 94% of those obtained in the absence of extract, respectively. Since the MeOH extract exerted antimutagenic activity as shown in Table 1, the effect of each fraction purified from the MeOH extract was examined. As

Table 1. Effect of the methanol extract of *E. stolonifera* on the mutagenicity induced by AFB_1 (1.0 μg/plate) in *Salmonella typhimurium* TA 100 in the presence of S9

Treatments	Concentration (mg/plate)	Revertants per plate
Spontaneous	-	114±9
AFB_1	-	876±95[a]
MeOH extract	0.625	530±69[b] (39)
	1.250	455±35[b] (45)
	2.500	145±16[c] (94)

[a] Significantly different from the spontaneous value, $p<0.001$

[b] Significantly different from the AFB_1-treated value, $p<0.05$

[c] Significantly different from the AFB_1-treated value, $p<0.001$

Figures in parentheses indicate the inhibition rate :

$$\text{Inhibition rate (\%)} = \frac{\text{the number of revertants in the control group - the number of revertants in the test group}}{\text{the number of revertants in the control group - the number of spontaneous revertants}} \times 100$$

Table 2. Effect of fractions obtained from the methanol extract of *E. stolonifera* on the mutagenicity induced by AFB1 (1.0 μg/plate) in *Salmonella typhimurium* TA 100 in the presence of S9

Treatments	Concentration (mg/plate)	Revertants per plate
Spontaneous	-	114± 9
AFB_1	-	876±95[a]
CH_2Cl_2 fr.	0.625	344±91[b] (70)
	1.250	263±17[b] (80)
	2.500	137±13[c] (97)
EtOAc fr.	0.625	223±18[c] (86)
	1.250	197±39[c] (89)
	2.500	111± 9[c] (100)
Intermediate	0.625	256± 4[b] (81)
phase	1.250	203±61[c] (88)
	2.500	107±12[c] (100)
n-BuOH fr.	0.625	444±46[b] (57)
	1.250	433±27[b] (58)
	2.500	188±19[c] (90)
H_2O fr.	0.625	699±86 (23)
	1.250	699±60 (23)
	2.500	665±42 (27)

[a] Significantly different from the spontaneous value, $p<0.001$
[b] Significantly different from the AFB_1-treated value, $p<0.05$
[c] Significantly different from the AFB_1-treated value, $p<0.001$
Figures in parentheses indicate the inhibition rate :

$$\text{Inhibition rate (\%)} = \frac{\text{the number of revertants in the control group} - \text{the number of revertants in the test group}}{\text{the number of revertants in the control group} - \text{the number of spontaneous revertants}} \times 100$$

shown in Table 2, when a 0.625 mg/plate fraction of CH_2Cl_2, EtOAc, intermediate insoluble and *n*-BuOH obtained from the MeOH extract was added to the assay system, the numbers of the TA100 revertants decreased by 70%, 86%, 81% and 57%, respectively. The inhibitory effect was further increased when the concentration of the fractions was raised to 2.50 mg/plate. These antimutagenic activities of the fractions were higher than that of the MeOH extract. Among these fractions, the EtOAc and intermediate insoluble fraction showed the highest activity. On the other hand, the H_2O fraction did not display any activity. The

Table 3. Effect of phloroglucinol on the mutagenicity induced by AFB_1 (1.0 μg/plate) in *Salmonella typhimurium* TA 100 in the presence of S9

Treatments	Concentration (mM)	Revertant /plate	Inhibition rate (%)
Spontaneous	-	103±9.1	-
AFB_1	-	1146±24.1[a]	-
AFB_1+	0.147	742±29.3[b]	39
phloroglucinol	0.734	709±58.9[b]	42
	1.468	626±17.3[b]	50
	3.675	441±27.5[c]	66
	7.350	226±20.7[c]	80

[a] Significantly different from the spontaneous value, $p<0.001$
[b] Significantly different from the AFB1-treated value, $p<0.05$
[c] Significantly different from the AFB1-treated value, $p<0.001$

$$\text{Inhibition rate (\%)} = \frac{\text{the number of revertants in the control group} - \text{the number of revertants in the test group}}{\text{the number of revertants in the control group} - \text{the number of spontaneous revertants}} \times 100$$

present study was also carried out to investigate whether the isolated compound from *E. stolonifera* would be effective on the inhibition of AFB_1 mutagenicity in the *Salmonella* strain. As shown in Table 3, the phloroglucinol significantly reduced the numbers of revertants in dose-dependent manners in comparison with the non-treated group.

Antigenotoxic studies – Since the MeOH extract of *E. stolonifera* and phloroglucinol isolated from MeOH extract showed antimutagenic activity, we further determined the antigenotoxic effect of the MeOH extract and phloroglucinol on mutagen-induced micronucleus or DNA damage of spleen lymphocytes in mice. The effect of the MeOH extract and phloroglucinol on the frequencies of micronucleated reticulocytes induced by MNU or BP in mice is summarized in Tables 4 and 5. The MeOH extract of *E. stolonifera* was orally administered at 0, 50, 100, 200, and 400 mg/kg body weight with a concomitant injection of MNU (40 mg/kg) or B(a)P (150 mg/kg). Peripheral blood was col-

Table 4. Effect of the methanol extract of *E. stolonifera* or phloroglucinol on the frequencies of micronucleated reticulocytes induced by MNU in mouse

Treatments[1]		MNRET/1000RETs[2]		
MNU (mg/kg/i.p.)+MeOH Ext. (mg/kg/p.o.)		Individual value	mean±S.D.	Suppression (%)
40	0	22, 23, 27, 24, 27	24.6±2.30[a]	-
40	50	23, 27, 25, 26, 24	25.0±1.58[a]	-
40	100	20, 22, 26, 25, 23	23.2±2.39[a]	5.7
40	200	14, 17, 16, 19, 21	17.4±2.70[a,**]	29.3
40	400	11, 9, 12, 8, 10	10.0±1.58[a,**]	59.3
MNU (mg/kg/i.p.)+phloroglucinol (mg/kg/p.o.)				
40	0	26, 27, 27, 22, 21	24.6±2.88[a]	-
40	0.1	24, 29, 25, 24, 22	24.8±2.59[a]	-
40	1	23, 27, 26, 25, 23	24.8±1.79[a]	-
40	10	14, 17, 19, 22, 18	18.0±2.91[a,*]	26.8
40	100	7, 7, 9, 8, 7	7.6±0.89[a,*]	69.1

[1]MNU (N-methyl-N-nitrosourea) was intraperitonealy administered and immediately test compound was administered orally. After 48 hours, peripheral blood was collected from mouse tail vein.
[2]1,000 reticulocytes (RETs) per animal were examined and then the frequency of MNRETs was scored.
Significance : *p<0.05, **p<0.01 (Student *t*-test) and [a]p<0.01 (Analysis of Variance test)

lected 48 hours after treatment with clastogens. As shown in Table 4, the frequencies of MNU (40 mg/kg)-induced micronuclei were significantly decreased by the administration of the MeOH extract of *E. stolonifera* (200 mg/kg) or phloroglucinol (10 mg/kg) respectively. Also, both the MeOH extract of *E. stolonifera* and the phloroglucinol exhibited the suppressive effect on the frequencies of micronuclei induced by B(a)P (150 mg/kg) as shown in Table 5.

Since the MeOH extract of *E. stolonifera* suppressed MNU or B(a)P clastogenesis in mouse peripheral blood reticulocytes, we sought to confirm the effect on DNA damage in spleen lymphocytes. The results of the Comet assay with MNU or B(a)P using spleen lymphocytes from mice are summarized in Tables 6 and 7. There were dose-dependently significant decreases in DNA damage in mouse

Table 5. Effect of the methanol extract of *E. stolonifera* or phloroglucinol on the frequencies of micronucleated reticulocytes induced by B(a)P in mouse

Treatments[1]		MNRET/1000RETs[2]		
B(a)P (mg/kg/i.p.)+MeOH Ext. (mg/kg/p.o.)		Individual value	mean ± S.D.	Suppression (%)
150	0	12, 15, 14, 16, 17	14.8±1.92[a]	-
150	50	14, 16, 16, 18, 17	16.2±1.48[a]	-
150	100	14, 16, 17, 17, 16	16.0±1.22[a]	-
150	200	10, 9, 11, 13, 12	11.0±1.58[a,**]	25.7
150	400	7, 9, 9, 8, 6	7.8±1.30[a,**]	47.3
B(a)P (mg/kg/i.p.)+phloroglucinol (mg/kg/p.o.)				
150	0	17, 19, 16, 14, -	16.5±2.08[a]	-
150	0.1	17, 16, 15, 15, 14	15.4±1.14[a]	-
150	1	20, 17, 14, 12, 11	14.8±3.70[a]	10.3
150	10	13, 15, 17, 14, 11	14.0±2.23[a,]	14.8
150	100	9, 12, 11, 9, 7	9.6±1.95[a,**]	41.8

[1]B(a)P (Benzo(a)pyrene) was intraperitonealy administered, and immediately test compound was administered orally. After 48 hours, peripheral blood was collected from mouse tail vein.
[2]1,000 reticulocytes (RETs) per animal were examined and then the frequency of MNRETs was scored.
Significance : *p<0.05, **p<0.01 (Student *t*-test) and ap<0.01 (Analysis of Variance test)

Table 6. Effect of the methanol extract of *E. stolonifera* or phloroglucinol on the DNA damage of spleen lymphocytes induced by MNU in mouse

Treatments[1]		Damaged cell/100 cells[2]		
MNU (mg/kg/i.p.)+MeOH Ext. (mg/kg/p.o.)		Individual value	mean ± S.D.	Suppression (%)
40	0	22, 24, 22	22.7±1.15[a]	-
40	50	23, 22, 23	22.7±0.57[a]	15.0
40	100	19, 18, 21	19.3±1.53[a]	29.5
40	200	15, 17, 16	16.0±1.00[a,**]	45.8
40	400	12, 14, 11	12.3±1.52[a,**]	
MNU (mg/kg/i.p.)+phloroglucinol (mg/kg/p.o.)				
40	0	22, 27, 23	24.0±2.64[a]	-
40	0.1	23, 24, 26	24.3±1.52[a]	-
40	1	17, 18, 14	16.3±2.08[a,*]	32.0
40	10	15, 14, 13	14.0±1.00[a,*]	41.7
40	100	11, 12, 9	10.7±1.53[a,**]	55.4

[1]MNU (N-methyl-N-nitrosourea) was intraperitonealy administered and immediately test compound was administered orally. After 48 hours, spleen was removed from mouse.
[2]100 cells per animal were examined and then damaged cells with tail were scored.
Significance : *p<0.05, **p<0.01 (Student t-test) and [a]p<0.01 (Analysis of Variance test)

Table 7. Effect of the methanol extract of *E. stolonifera* or phloroglucinol on the DNA damage of spleen lymphocytes induced by B(a)P in mouse

Treatments[1]		Damaged cell/100 cells[2]		
B(a)P (mg/kg/i.p.)+MeOH Ext. (mg/kg/p.o.)		Individual value	mean ± S.D.	Suppression (%)
150	0	34, 29, 32	31.7±2.52[a]	-
150	50	30, 32, 31	31.0±1.00[a]	-
150	100	31, 33, 34	32.6±1.53[a]	-
150	200	17, 18, 22	19.0±2.64[a,*]	40.1
150	400	15, 12, 14	13.7±1.53[a,**]	56.8
B(a)P (mg/kg/i.p.)+phloroglucinol (mg/kg/p.o.)				
150	0	34, 37 37	36.0±1.73[a]	-
150	0.1	37, 33, 32	34.0±2.64[a]	-
150	1	30, 33, 37	33.3±3.51[a]	7.5
150	10	22, 25, 27	24.3±2.10[a,**]	32.5
150	100	17, 19, 16	17.3±2.53[a,**]	52.0

[1]B(a)P (Benzo(a)pyrene) was intraperitonealy administered and immediately test compound was administered orally. After 48 hours, spleen was removed from mouse.
[2]100 cells per animal were examined and then damaged cells with tail were scored.
Significance : *p<0.05, **p<0.01 (Student *t*-test), [a]p<0.01 (Analysis of Variance test)

spleen lymphocytes administered the MeOH extract and phloroglucinol with a concomitant injection of MNU (40 mg/kg) or B(a)P (150 mg/kg) respectively.

Discussion

We found that the MeOH extract of *E. stolonifera* and its subsequent fractions, especially EtOAc fraction, have a potent protective activity against mutagenesis induced by AFB_1 in *S. typhimurium* TA 100. And we isolated phloroglucinol as an active component in the EtOAc fraction of the MeOH extract of *E. stolonifera*. The mechanism of antimutagenic activity of these is uncertain. The possible mechanism of these may be mediated through interaction with a microsomal activating system since AFB_1 was shown to be completely dependent upon the

enzyme activation being an ultimate carcinogen.

To clarify the anticlastogenicity of the MeOH extract of *E. stolonifera* and phloroglucinol, the effects of the MeOH extract and phloroglucinol on the micronuclei formation in mouse peripheral blood reticulocytes and DNA damage in mouse spleen lymphocytes were also examined. The micronucleus assay is used widely as a screening method for detecting clastogenicity of chemicals *in vivo*. In the present paper, we show that the MeOH extract, as well as phloroglucinol isolated from the MeOH extract suppressed the formation of micronuclei and DNA damage by MNU or B(a)P. The suppressive effects of *E. stolonifera* and phloroglucinol were observed with both clastogens which require metabolic activation and those that do not. These activities appears to be due to their ability to interfere with the metabolic activation of B(a)P or without the metabolic activation of MNU, and to block the formation of DNA adduct. These results revealed that MeOH extract and phloroglucinol may play a role in antigenotoxic activity.

Marine algae are widely distributed in nature and are shown to have various biological activities. Several research workers (Hiroyuki *et al.*, 1989a and 1989b; Yamamoto *et al.*, 1974; Michico *et al.*, 1984; Terukazu *et al.*, 1988; Yamamoto and Maruyama, 1985; Yamamoto *et al.*, 1987) have revealed that the various kinds of extracts from marine algae showed antitumor activity. Recently, Okai and coresearchers (Okai *et al.*, 1993; Okai and Higashi-Okai, 1994) found that the hot water-soluble extracts of edible brown algae, *Hijikia fusiforme, Laminaria japonica* and *Undaria pinnatifida* are antimutagenic in the *umu* gene expression system in *S. typhimurium* (TA 1535/pSK 1002). They extended the investigation of the antimutagenic properties of these algae, and they indicated that the hot water-soluble extract of these algae contains heterogenous antimutagenic activities against typical genotoxic substances.

Phloroglucinol has been isolated from many plants, for example, *Eucalyptus kino* and *Acacia arabica* (Dictionary of Natural Products, 1994) and marine algae, such as *E, stolonifera, Ecklonia cava, Cystophyllum hakodatense, Sargassum ringgoldianum*, and *Fucus vesiculosus* (Scheuer, 1981; Taniguchi *et al.*, 1994); the antispasmodic, antioxidant and nitrite scavenging effects of phloroglucinol have also been reported (Dictionary of Natural Products, 1994; Choi *et al.*, 1989 and 1997). However, no report on the antigenotoxic activity of the methanol extract of *E. stolonifera* and its component, phloroglucinol, has yet appeared. The findings of the present work would tend to indicate that the methanol extract of *E. stolonifera*, its fractions and active component, phloroglucinol might be proposed as a strong candidate for a potential antigenotoxic material.

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