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Anti-inflammatory activities of methanol extracts from various seaweed species

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Abstract: *Thirty-seven species of common seaweeds from the coast of Korea were screened for anti-inflammatory activity. Methanol extracts of the seaweeds were tested against mouse ear edema and erythema induced by phorbol myristate acetate. At 40 mg ml^{-1} of extract, edema was strongly suppressed by the seaweeds Undaria pinnatifida and Ulva linza, with relative inhibition of 85 and 84%, respectively. These two seaweeds also showed the greatest suppression of erythema, with inhibition of 78 and 70%, respectively. IC_{50} values of U. pinnatifida were 10, 15, and 18 mg ml^{-1} when inflammation symptoms of edema, erythema, and blood flow, respectively, were measured. The IC_{50} of U. linza was 20, 26, and 31 mg ml^{-1} when edema, erythema, and blood flow, respectively, were measured. A linear correlation among inhibition rates of edema, erythema, and blood flow was observed with high confidence.*

Key words: *Anti-inflammation, Seaweed, Ulva linza, Undaria pinnatifida*
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Introduction

Numerous studies have concentrated on the contribution of marine organisms, including seaweeds and marine microorganisms, in the search for new drugs from natural products (Smit, 2004). One important approach to drug development involves assaying folk remedies for active ingredients, and several seaweed species are used as traditional medicines, foods and health care products in various regions of the world. The use of seaweed species to treat fever, lumps, and swelling is recorded in the Oriental medical textbook *Donguibogam* published in 1613 (Donguibogam Committee, 1999). Many seaweed species also have been used as herbal medicines in China (Tseng and Chang, 1984). For development of pharmaceutical compounds from a marine source, supply issues will always be a critical problem, and a major obstacle to drug development is the lack of sufficient material. Among marine organisms, seaweed is a promising candidate for drug production because it is relatively easy to obtain adequate, reliable, and most importantly, renewable supplies by aquaculture. In 2006, commercial seaweed production by aquaculture in Korea was estimated at 699,000 tons (wet wt.) of at least 10 species (MOMAF, 2007). Thus, such an abundant species with immense aquaculture potential will have a better chance of being developed on a large scale as a commercial food and drug producer. Seaweed aquaculture has become widespread, and production sometimes exceeds consumption. Seaweeds are generally rich in polyunsaturated fatty acids (PUFAs) and are capable of metabolizing various PUFAs *via* oxidative pathways (Gerwick *et al.*, 1993). The metabolized products of PUFAs, called oxylipins, resemble human eicosanoid hormones, which carry out a range of physiologically important functions. The anomalous production of these compounds underlies several diseases related to inflammation and thus eicosanoids and their derivatives have received wide attention in the search for anti-inflammatory drugs (Smit, 2004; Serhan, 2005). Accordingly, we have measured anti-inflammatory activities of 37 prevalent seaweed species to detect the anti-inflammatory substances against mouse ear edema (swelling) and erythema (redness).

Materials and Methods

Seaweed extracts: Thalli of prevalent seaweed species (6 Chlorophyta, 17 Phaeophyta, and 14 Rhodophyta) were collected along the coast of Korea from October 2003 to June 2006. Epiphytes and salts were removed from the thalli by washing in tap water. The thalli were dried completely for one week at room temperature and then ground to a powder for 5 min, using a grinder (HMF-340, Hanil Co., Seoul, Korea). To extract the methanol-soluble fraction, 20 g of each seaweed powder was mixed with 1 liter methanol for one day. The methanol-soluble extract was re-extracted into 100% methanol several times until the amount of salt was negligible upon visual inspection, using a rotary evaporator (Eyela Co., Tokyo, Japan). For a stock solution of the seaweed extracts, 1 ml of 100% ethanol was added to 40 mg of each dried extract.

Anti-inflammatory bioassays: BALB/c mice (8-10 weeks old; 20-25 g body weight) were used for anti-inflammatory assays. The animals were housed at 24 ± 1°C on a 12 hr light/dark cycle, with free access to food and water. Animal experiments were performed in accordance with the US NIH Guidelines for the Care and Use of Laboratory Animals (Bethesda, MD, USA). Phorbol 12 myristate 13 acetate (PMA; Sigma, St. Louis, MO, USA) was topically applied to the inner side of the mouse ear at 0.2 μg in 10 μl acetone with an equal volume of seaweed extract in ethanol (0.4 mg 10 μl^{-1}). As a reference, indomethacin was also prepared in 10 μl of ethanol and applied with PMA. Ear edema (swelling) was measured 10 hr after

the PMA application using a spring-loaded micrometer (Mitutoyo Co., Tokyo, Japan) (Griswold *et al*., 1998). The edema value (AU) was expressed as $(S_{10} - S_0)/S_0$, where S_{10} is the ear thickness 10 hr after PMA application and S_0 is the ear thickness at 0 hr. Ear erythema (redness) was determined at 10 hr using digital photography adjusted to balance white and Photoshop 7.0 program (Adobe, San Jose, CA, USA) to measure the magenta value (Khan *et al*., 2008). The erythema value (AU) was expressed as $(R_{10} - R_0)/R_0$, where R_{10} is ear redness 10 hr after PMA application, and R_0 is ear redness at 0 hr. To confirm the anti-inflammatory activity of the seaweeds, local blood flow in the mouse ear was measured using laser speckle flowgraphy (Inflameter LFG-1; SoftCare, Fukuoka, Japan). The ear skin was scanned by moving the laser beam over the inner surface of the mouse ear. The distance between the scanner and ear surface was 0.5 cm. Blood flow was analyzed for a skin area 5 mm in diameter following the method of Lee *et al.* (2003). Blood flow (AU) was calculated as $(B_{10} - B_0)/B_0$, where B_{10} is blood flow 10 hr after PMA application, and B_0 is blood flow at 0 hr. Relative inhibition rate (%) was expressed as [1 – (inflammatory value of the extract / inflammatory value of the ethanol control)] × 100. Acetone or ethanol provided minimum effects in the inflammation assays at 10 μl per ear (data not shown). Therefore, solvent controls were always used to the mouse ear at concentrations of 10 μl per ear in all assays.

Statistical analysis: All animal experiments were performed with at least seven mice in each group and the highest and lowest values were discarded. Data are reported as the means ± SE, and the statistical analysis was performed using linear correlation and Student's *t*-test.

Results and Discussion

Mouse ear inflammation was caused by topical application of PMA (0.05-1.0 μg in 10 μl acetone). Edema and erythema responses were noted 10 hr later following application with a concentration of 0.2 μg or lower; the responses persisted for 15 hr with 0.5 μg and for 20 hr with 1.0 μg, exhibiting increasingly higher edema and erythema values (data not shown). To assay inflammatory suppression in the short term, the ear thickness and redness were measured 10 hr after 0.2 μg PMA application. Edema and erythema values of the vehicle at 10 hr reached 0.81 ± 0.04 and 0.27 ± 0.01, respectively. To measure the inhibition rate of the seaweed extracts, each extract was mixed and applied to the ear. One (*Ulva linza*) of the six Chlorophyta, four (*Colpomenia sinuosa*, *Ecklonia stolonifera*, *Sargassum thunbergii* and *Undaria pinnatifida*) of 17 Phaeophyta, and two (*Gracilaria verrucosa* and *Pachymeniopsis elliptica*) of 14 Rhodophyta showed potent activity more than 70% inhibition against edema (Table 1). Of the 37 seaweed species tested, *U. pinnatifida* and *U. linza* showed the highest inhibition activity (85% and 84%, respectively). Then, against erythema, one (*U. linza*) of the six Chlorophyta, four (*Costaria costata*, *Ecklonia cava, E. stolonifera* and *U. pinnatifida*) of 17 Phaeophyta, and three (*Carpopeltis cornea, P. elliptica* and *Porphyra yezoensis*) of 14 Rhodophyta showed potent activity more than 55% inhibition (Table 1). Of the 37 species, *U. pinnatifida* produced the greatest inhibition against erythema (78%).

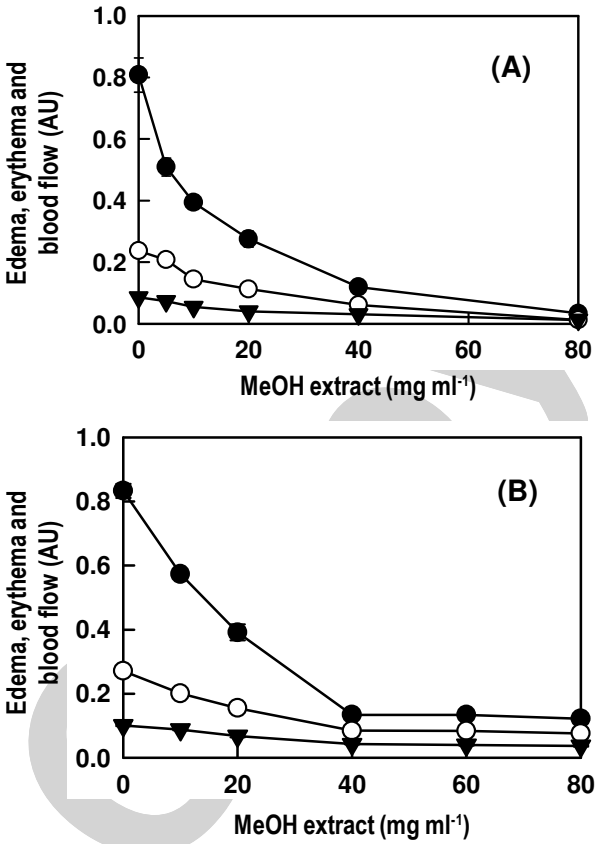

Fig. 1: Suppression of edema (•), erythema (o), and blood flow (▼) by different concentrations of methanol extracts from *Undaria pinnatifida* (A), and *Ulva linza* (B). Values represent the mean ± SE ($n \geq 5$)

The methanol extracts of *U. pinnatifida* and *U. linza*, the most effective at inhibiting edema and erythema, were tested at different concentrations in the assay. The concentration of *U. pinnatifida* giving a 50% inhibition (IC_{50}) was 10, 15, and 18 mg ml^{-1} when inflammation symptoms of edema, erythema, and blood flow, respectively, were measured (Fig. 1A). A concentration of 40 mg ml^{-1} inhibited 85, 78, and 82% of edema, erythema, and blood flow, respectively. The IC_{50} values of *U. linza* were 20, 26, and 31 mg ml^{-1} when edema, erythema, and blood flow, respectively, were measured (Fig. 1B). A concentration of 40 mg ml^{-1} inhibited 84, 69, and 58% of edema, erythema, and blood flow, respectively.

When we compared the inhibition rates of edema and erythema by the *U. pinnatifida* extract, a linear correlation of erythema values (y) with those of edema (x) was observed; the relationship is described by $y = 0.29x + 0.02$ with a confidence of 0.94 (Fig. 2A). The linear relationship of edema (y) with blood flow (x) was $y = 10.26x - 0.15$ with a confidence of 0.95 (Fig. 2B). The linear relationship of blood flow (y) with erythema (x) was $y = 0.26x + 0.01$ with a confidence of 0.96 (Fig. 2C). With the *U. linza* extract,

Table - 1: Comparison of anti-edema and anti-erythema activities in methanol extracts of various seaweed species. Mean ± SE ($n \geq 5$); Student's *t*-test *p < 0.001

	Edema		Erythema	
	Edema value (AU)	Relative inhibition (%)	Erythema value (AU)	Relative inhibition (%)
Chlorophyta				
Capsosiphon fulvescens	0.40 ± 0.05	51	0.21 ±0.00	22
Codium fragile	0.53 ± 0.04	33	0.20 ± 0.01	26
Scytosiphon lomentaria	0.26 ± 0.04*	68	0.16± 0.02	41
Ulva compressa	0.28 ± 0.01*	65	0.15 ± 0.02	44
Ulva linza	0.13 ± 0.00*	84	0.08 ± 0.00*	70
Ulva pertusa	0.37 ± 0.02	54	0.15 ± 0.03	44
Phaeophyta				
Colpomenia bullosa	0.39 ± 0.03	52	0.13 ± 0.02	52
Colpomenia sinuosa	0.23 ± 0.01*	72	0.13 ± 0.02	52
Costaria costata	0.38 ± 0.04	53	0.12 ± 0.00	56
Dictyota dichotoma	0.39 ± 0.03	52	0.16 ± 0.00	41
Ecklonia cava	0.28 ± 0.04*	65	0.12 ± 0.00	56
Ecklonia stolonifera	0.20 ± 0.03*	75	0.11 ± 0.00	59
Hizikia fusiformis	0.30 ± 0.01*	63	0.17 ± 0.02	37
Ishige okamurae	0.45 ± 0.03	44	0.19 ± 0.00	30
Ishige sinicola	0.70 ± 0.04	14	0.16 ± 0.00	41
Laminaria japonica	0.79 ± 0.10	01	0.23 ± 0.02	15
Sargassum confusum	0.48 ± 0.04	41	0.13 ± 0.01	52
Sargassum fulvellum	0.72 ± 0.10	11	0.20 ± 0.02	26
Sargassum horneri	0.58 ± 0.03	28	0.15 ± 0.03	44
Sargassum ringgoldianum	0.62 ± 0.03	23	0.21 ± 0.00	22
Sargassum sagamianum	0.32 ± 0.04*	60	0.13 ± 0.00	52
Sargassum thunbergii	0.20 ± 0.01*	75	0.13 ± 0.01	52
Undaria pinnatifida	0.12 ± 0.00*	85	0.06 ± 0.00*	78
Rhodophyta				
Carpopeltis cornea	0.21 ± 0.02	25	0.12 ± 0.00	56
Chondrus ocellatus	0.33 ± 0.02	59	0.14 ± 0.04	48
Corallina pilulifera	0.66 ± 0.02	19	0.22 ± 0.01	19
Gigartina tenella	0.38 ± 0.20	53	0.15 ± 0.00	44
Gracilaria verrucosa	0.22 ± 0.02*	73	0.13 ± 0.00	52
Gymnogongrus flabelliformis	0.56 ± 0.02	31	0.18 ± 0.02	33
Hypnea charoides	0.70 ± 0.02	14	0.17 ± 0.02	37
Helminthocladia australis	0.31 ± 0.06*	62	0.14 ± 0.02	48
Lomentaria catenata	0.76 ± 0.00	06	0.23 ± 0.03	15
Meristotheca papulosa	0.50 ± 0.00	38	0.13 ± 0.01	52
Pachymeniopsis elliptica	0.18 ± 0.02*	78	0.10 ± 0.02	63
Pachymeniopsis lanceolata	0.42 ± 0.00	48	0.14 ± 0.01	48
Porphyra yezoensis	0.29 ± 0.04*	64	0.12 ± 0.02	56
Symphyocladia latiuscula	0.60 ± 0.07	30	0.17 ± 0.00	37
Ethanol control	0.81 ± 0.04	0	0.27 ± 0.01	0

a linear correlation of erythema values (y) and edema (x) values was observed; $y = 0.27x + 0.05$ with a confidence of 0.99 (Fig. 2A). The linear relationship of edema (y) with blood flow (x) was $y = 10.67x - 0.30$ with a confidence of 0.98 (Fig. 2B). The linear relationship of blood flow (y) with erythema (x) was $y = 0.34x + 0.01$ with a confidence of 0.98 (Fig. 2C). Thus, linear correlations among inhibition rates of edema, erythema, and blood flow by *U. pinnatifida* and *U. linza* were observed with high confidence. The inflammatory symptoms were directly related and simultaneously suppressed by extracts of *U. pinnatifida* and *U. linza*.

Of the 37 seaweed extracts screened, the one with the highest activity, *U. pinnatifida* (Harvey) Suringar (known as miyok in Korea), is an edible brown seaweed common along temperate coastal regions of the northeast Pacific including Korea, Japan, and northern China (Ohno and Matsuoka, 1993). It is an annual seaweed belonging to the family Alariaceae and grows on rocks and reefs to a depth of 1 to 10 m below the tide level in open seas or within bays near the open sea. The length of the mature fronds is 1–2 m. In 2006, production by aquaculture in Korea was estimated at 305,000 tons (wet wt.) (MOMAF, 2007). *Undaria pinnatifida* is well-known

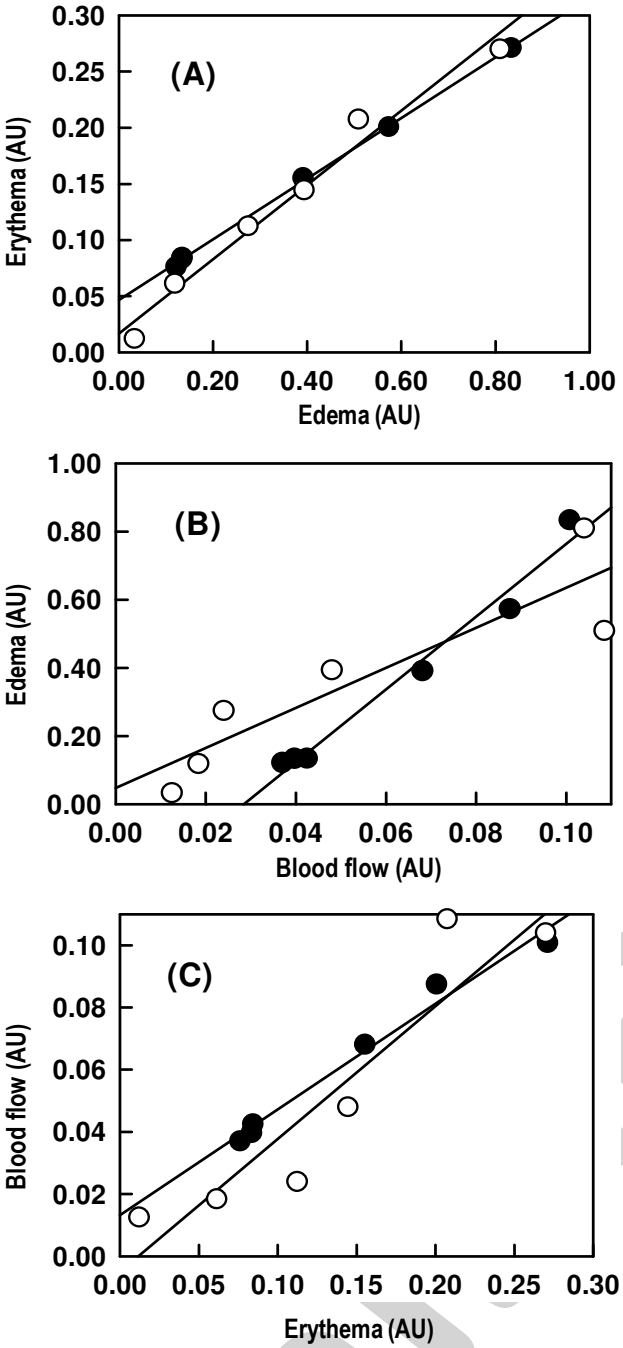

Fig. 2: Linear correlations of erythema with edema (A), edema with blood flow (B), and blood flow with erythema (C) during suppression of these inflammatory symptoms by *Undaria pinnatifida* (•) and *Ulva linza* (o) extracts.

as a desired food of nursing mothers in Korea. Almost all Korean women consume *U. pinnatifida* soup for a month or so after childbirth in the belief that it helps postpartum convalescence and cleanses the blood. It has also been used traditionally to treat fever, urination problems, lumps, or swelling (Donguibogam Committee, 1999). In herbal medicine in China, *U. pinnatifida* is used to treat urinary diseases, dropsy, stomach ailments, hemorrhoids, anal fistulas, leukorrhea in women and other conditions (Tseng and Chang, 1984). Most of the purported beneficial effects are thought to be directly or indirectly related to the anti-inflammatory properties of the seaweed. The seaweed is also known to affect contraction of the uterus (Huh *et al.*, 1992), to stimulate hepatic fatty acid oxidation (Murata *et al.*, 1999), and to have antioxidant (Yoo *et al.*, 2004), antitumor (Hosokawa *et al.*, 2004), antivirus (Thompson and Dragar, 2004), and anti-obesity properties (Maeda *et al.*, 2005). The other highly active species, *U. linza* (L.) *J. agardh*, is a green seaweed common throughout the world on rocks and quay walls in the upper intertidal zone (Tokuda *et al.*, 1994). It is well-known as one of the bloom-forming green seaweeds in the eutrophic area. It is an annual seaweed belonging to the family Ulvaceae. The frond is membranous and distromatic in cross section with a 5-10 cm height and 1-7 cm width, but is tubular only at the base. The species grows luxuriantly from late winter to early summer. In 2006, production of *Ulva* species by aquaculture in Korea was estimated at 395 tons (wet wt.) (MOMAF, 2007). This species showed microalgal growth enhancement (Cho *et al.*, 1999), antiviral activity (Hudson *et al.*, 1999), and an allelopathic effect on the red tide *Prorocentrum micans* (Jin *et al.*, 2005).

Many seaweed species are known to have diverse polyunsaturated fatty acids (PUFAs) (Ishihara *et al.*, 1998; Kaneniwa *et al.*, 1987). The n-3 PUFAs are mainly known for their anti-inflammatory effects, which are related to their competition as substrates for cyclooxygenase and lipoxygenase that leads to decreased production of prostaglandins and leukotrienes (James *et al.*, 2000). Dietary supplementation with n-3 PUFAs causes a reduction in the expression and activity of aggrecanases, inflammation-inducible cytokines, and cyclooxygenase-2, but not the constitutively expressed cyclooxygenase-1 (Curtis *et al.*, 2000). Of the 37 seaweed extracts screened, *U. pinnatifida* and *U. linza* showed the highest inhibition activity against edema and erythema, and therefore may contain substantial amounts of PUFAs. Isolation of the main anti-inflammatory compounds is now in progress.

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