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# *In vitro* antibacterial and anti-inflammatory properties of seaweed extracts against acne inducing bacteria, *Propionibacterium acnes*

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**Abstract**

This study was conducted to evaluate the antimicrobial activities of common seaweeds from the coast of South Korea against the etiologic agents of acne vulgaris. Fifty-seven species of seaweed were screened for potential antimicrobial activity. Methanol extracts of 13 species (22.8%) showed inhibitory effects against Propionibacterium acnes. The aqueous extracts of only two species (3.5%) showed antimicrobial activity. When tested with the agar disk diffusion method, *Ecklonia cava, E. kurome, Ishige sinicola*, and *Symphyocladia latiuscula* had the strongest inhibitory effects. However, these four seaweed extracts showed no antibacterial activity against Staphylococcus epidermidis at 5 mg disk$^{-1}$. The minimum inhibitory concentration (MIC) values of *E. cava* and *E. kurome* were both 0.31 mg ml$^{-1}$ and the MIC values of *I. sinicola* and *S. latiuscula* were 0.26 and 0.21 mg ml$^{-1}$, respectively. Among whole plants of *E. cava* and *E. kurome*, extracts of the pinnate blade had the highest inhibitory activity on bacterial growth. In cytotoxicity assays, methanol extracts of *E. cava*, *E. kurome*, and *I. sinicola* showed no effect on cell viability at concentrations of 200 μg ml$^{-1}$. However, the methanol extracts of *S. latiuscula* reduced cell viability rates to 50% at the same concentration. Additionally, methanol extracts of *E. cava, E. kurome*, and *I. sinicola* potently inhibited the *in vitro* production of nitric oxide. These results suggest that the methanol extracts from these three species may be useful in the development of therapeutic agents for acne vulgaris. Further investigations to determine the bioactive compound are in progress.

**Key words**

Seaweed extracts, *Propionibacterium acnes,* Antibacterial, Anti - inflammatory

## Introduction

*Acne vulgaris* is a common disease that affects most adolescents and adults at some time in their lives. The skin bacteria commonly implicated in the clinical course of acne include *Propionibacterium acnes* and *Staphylococcus epidermidis*. *Propionibacterium acnes,* an obligate anaerobic organism, has been implicated in the development of inflammatory acne because of its ability to activate complement proteins and to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. In contrast, *S. epidermidis*, an aerobic organism, is usually involved in superficial infections within the sebaceous unit (Burkhart *et al.*, 1999).

Topical therapy is the standard of care in acne treatment (Tan, 2003). Topical antimicrobial agents include benzoyl peroxide, clindamycin, erythromycin, tetracycline, azelaic acid, triclosan, salicylic acid, and various combinations of these ingredients. Benzoyl peroxide and combination agents containing erythromycin or clindamycin are effective acne treatments, whereas salicylic acid is moderately effective against acne (Shalita, 1981).

However, it has been known for many years that topical treatment is not universally successful and, in patients at risk for scarring of the skin and pigmentary changes, systemic antibiotics are indicated. The use of these agents alone can be associated with the development of bacterial resistance (Eady, 1998; Swanson, 2003), which has led to extensive research to find novel antimicrobial compounds.

Interest in marine organisms as potentially promising natural sources of pharmaceutical agents has increased in recent years

(Mayer and Hamann, 2002; Newman *et al*., 2003). Seaweeds, as sources of bioactive compounds, produce a great variety of secondary metabolites with varying activities. Compounds with antiviral, antihelminthic, antifungal, and antibacterial activities have been detected in green, brown, and red algae (Newman *et al.*, 2003; Del Val *et al*., 2001). There are many reports on the biological activities of macroalgae against human pathogens, fungi, and yeasts, but only a few contain describe effects against *P. acnes*.

Thus, we conducted this study to examine the antimicrobial and anti-inflammatory effects of seaweed extracts against *P. acnes*.

## Materials and Methods

**Seaweed extracts:** In total, 57 species of seaweed were collected from various locations in South Korea between November 2005 and April 2006. Collected seaweed tissues were washed with tap water to remove salt, epiphytes, and sand, and then dried for 1 day at room temperature. The dried tissues were ground into a powder using a coffee grinder for 5 min. To extract methanol-soluble components, 1 lit. of methanol was added for 1 d to 20 g of each powder. This was repeated three times, and the combined extracts were evaporated to dryness. Distilled water (1 lit.) was then added to the remaining powder to extract the water-soluble components. Stock solutions were prepared by the addition of 1 ml of methanol, distilled water, or ether to 100 mg of each dried extract. Stock solutions were filtered through a 0.22 μM filter and stored at -20°C until use (Jin *et al*., 1997).

**Culturing of microorganisms:** *Propionibacterium acnes* (KCTC 3314) and *Staphylococcus epidermidis* (KCTC 3958), obtained from the Korean Collection for Type Cultures (KCTC; Daejeon, Korea), were used for the study. *Propionibacterium acnes* was incubated in brain–heart infusion (BHI) agar (Oxoid) with 1% glucose at 37°C for 72 hr under anaerobic conditions using the Bactron Anaerobic Chamber system (SHELLAB, USA). Gas conditions were $H_2$ 5%, $CO_2$ 5%, and $N_2$ 90%. *Staphylococcus epidermidis* was incubated in tryptic soy broth (TSB) agar (Difco) at 37°C for 24 hr under aerobic conditions.

**Disk diffusion method:** *Propionibacterium acnes* was incubated in BHI medium with 1% glucose for 24 hr under anaerobic conditions and then adjusted to approximately $2.0\times10^8$ CFU $ml^{-1}$. *Propionibacterium acnes* solution (1 ml) was spread on the BHI agar plate. Uniform-sized (8 mm diameter) filter-paper disks were impregnated with seaweed extracts and then placed on the surface of an agar plate that had been seeded with the organism to be tested. Plates were then incubated at 37°C for 72 hr under anaerobic conditions (Chomnawang *et al*., 2005). *Staphylococcus epidermidis* was incubated in TSB for 24 hr at 37°C and then adjusted to yield approximately $2.0 \times 10^8$ CFU $ml^{-1}$. Plates were incubated at 37°C for 24 hr under aerobic conditions (Chomnawang *et al*., 2005). Antimicrobial activity was defined by measuring the diameter of the growth inhibition zone (mm). Controls were also run simultaneously. The antimicrobial agent erythromycin (Sigma E-5389) was included in the assays as a positive control (Park *et al*., 2004). All disk diffusion tests were performed three times, independently.

**Determination of MIC values:** The antimicrobial activity was determined by the broth microdilution assay, following the guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS) for anaerobic bacteria M11-A6 (NCCLS, 2004) in 96-well U-shaped microplates. Inocula of *P. acnes* were prepared from 24 hr broth cultures and suspensions were adjusted to 0.5 McFarland standard solution turbidity. The seaweed extracts were first diluted to the highest concentration (10 mg $ml^{-1}$) to be tested, and then serial two-fold dilutions were made in a concentration range from 19.5 μg to 10 mg $ml^{-1}$.

The 96-well plates were prepared by dispensing 100 μl of the inoculum and 100 μl of each sample into wells. The first well, containing 100 μl BHI broth with no compound and 100 μl of the inoculum on each strip, was used as a negative control. The second well, containing 90 μl BHI broth, 10 μl methanol and 100 μl of the inoculum on each strip, was used as a positive control. The final volume in each well was 200 μl. The plates were then incubated at 37°C for 48 hr under anaerobic conditions. The procedures for *S. epidermidis* were the same as mentioned above except for the use of TSB. The MIC value was defined as the lowest concentration that yielded no bacterial cell growth. All MIC tests were performed three times, independently.

**Cytotoxicity assay:** Cytotoxicity was determined as described by Mosmann (1983) with some modifications. The murine macrophage cell line RAW264.7 (American Type Culture Collection, Rockville, MD, USA) was grown in Nunc flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U $ml^{-1}$ penicillin, 100 μg $ml^{-1}$ streptomycin, 10 mM HEPES, 2 mM L-glutamine, 0.2% $NaHCO_3$, 1 mM sodium pyruvate, and 10% (v/v) heat-inactivated fetal bovine serum (FBS), in a humidified chamber with 5% $CO_2$ / 95% air at 37°C. The cellular toxicity of several seaweed extracts was assessed by the MTT assay, which is based on the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan by mitochondrial dehydrogenases. Cells were incubated with each compound for 24 hr and MTT was added to the cultures to a final concentration of 0.5 mg $ml^{-1}$. After incubation at 37°C in 5% $CO_2$ for 2 hr, the supernatant was removed and the cells were solubilized in dimethyl sulfoxide (DMSO). The extent of the reduction of MTT to formazan within the cells was quantified by measuring absorbance at 570 nm with a Spectra Max 250 ELISA Reader (Molecular Devices, USA). Cell viability is expressed as a percentage of the control value.

**Measurement of NO production:** Nitric oxide (NO) production was assayed by measuring the accumulation of the stable oxidative metabolite, nitrite ($NO_2^-$), in culture supernatants (Green *et al*., 1982). Briefly, RAW264.7 cells were seeded in 24-well culture plates at a density of $1 \times 10^6$ cells $well^{-1}$ and incubated for at least 2 hr to allow

them to adhere to the plates. After washing three times with medium, various concentrations of seaweed extracts were added and the cells were cultured for the indicated times, after which culture supernatants were collected. Griess reagent (100 µl; 1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride, 2.5% phosphoric acid; Sigma G-4410) was added to equal volumes of culture supernatants in a 96-well flat-bottomed microtiter plate and left at room temperature for 10 min. Optical densities at 540 nm were read with a Spectra Max 250 ELISA Reader, and nitrite concentrations were calculated from a standard curve established with serial dilutions of $NaNO_2$ (Sigma S-3421) in culture medium.

**Constituent separation:** For constituent separation, seaweed powders (20 g) were extracted three times with 1 lit. of methanol water (4:1). Crude extracts were evaporated under vacuum and then successively fractioned according to polarity into different classes: saccharides, lipids, phenolics, alkaloids, and nitrogen compounds (Harborne, 1998).

## Results and Discussion

**Screening for antimicrobial activity:** Of the 57 species of seaweed screened for their potential antimicrobial (= anti *P. acnes*) activity, only 14 species (24.6%) showed activity by the disk diffusion method (Table 1). In the Chlorophyta (10 species), none of the methanol or aqueous extracts exhibited antimicrobial activity. The Phaeophyta showed the highest activity (44.8%) among the three classes of seaweeds screened. Among the 29 species of brown algae screened, the methanol extracts of 12 species and the aqueous extracts of 2 species (*E. cava*, *E. stolonifera*) inhibited microbial pathogens. In the Rhodophyta (18 species), except for the *S. latiuscula* methanol extract, none of the red algae exhibited antimicrobial activity. The methanol extracts of *E. cava*, *E. kurome*, *I. sinicola*, and *S. latiuscula* showed the highest activity against *P. acnes*. In contrast, a 13.0 mm inhibition zone was produced against *P. acnes* by erythromycin, the positive control at 0.5 µg $disk^{-1}$.

We examined the antimicrobial activities of different parts of *E. cava* and *E. kurome* (Table 2). A mature thallus of these seaweeds

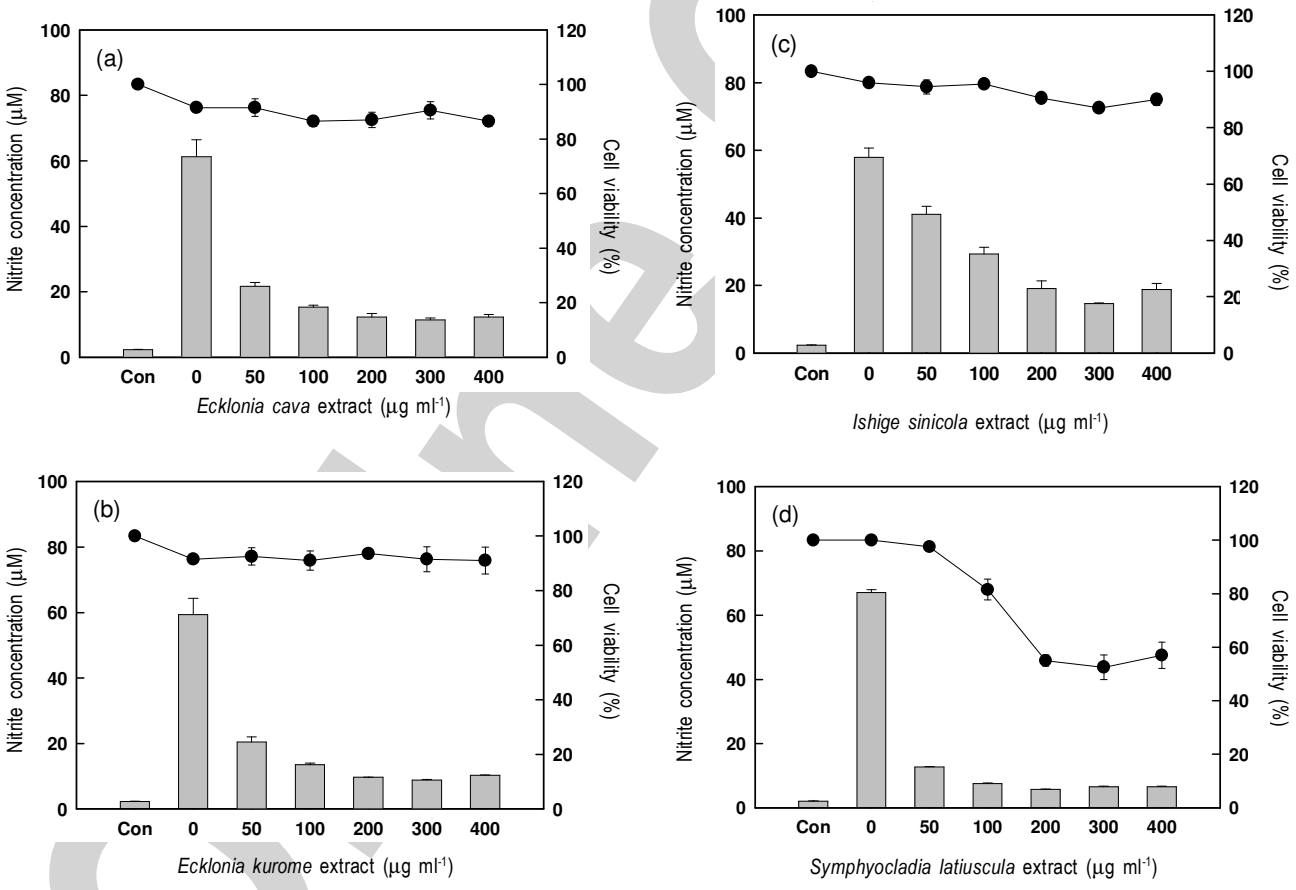


**Fig. 1:** The inhibitory effects of NO synthesis ( □ ) and cytotoxic effects (●) of *Ecklonia cava* (a), *Ecklonia kurome* (b), *Ishige sinicola* (c) and *Symphyocladia latiuscula* (d) methanol extracts. The inhibitory effects of NO production were examined in an activated macrophage-like cell line, RAW 264.7 cells in a dose-dependent manner. RAW264.7 cells were pretreated with the indicated concentration of each seaweed extracts before 24 hr incubation. Supernatants were removed after 24 hr and RAW264.7 cells were assessed by the MTT assay. Results are expressed as means ± standard deviation of four experiments. Significant at $p<0.01$

**Table - 1:** Antimicrobial activity by methanol and aqueous extracts of seaweed against *Propionibacterium acnes* by disk diffusion method. After 72 hr of incubation, the inhibition zone was measured in mm

| Scientific name | Collection site | Methanol extracts | | Aqueous extracts |
|---|---|---|---|---|
| | | 1 mg disk$^{-1}$ | 5 mg disk$^{-1}$ | 5 mg disk$^{-1}$ |
| **Phaeophyta** | | | | |
| *Dictyota dichotoma* | Cheongsapo, Busan | - | 0.5±0.0 | - |
| *Ecklonia cava* | Unseong, Jeju | 2.3±0.3 | 5.3±0.3 | 2.8±1.0 |
| *Ecklonia kurome* | Bangeori, Pohang | 3.0±0.0 | 5.7±0.3 | - |
| *Ecklonia stolonifera* | Daebyeon, Busan | - | - | 2.5±0.5 |
| *Eisenia bicyclis* | Sinyang, Jeju | - | 1.5±0.7 | - |
| *Ishige okamurae* | Sachon, Namhae | - | 1.0±0.0 | - |
| *Ishige sinicola* | Cheongsapo, Busan | 2.2±0.3 | 6.3±0.8 | - |
| *Laminaria japonica* | Cheongsapo, Busan | 0.5±0.7 | 1.0±0.0 | - |
| *Pachydictyon coriaceum* | Iho, Jeju | - | 1.0±0.0 | - |
| *Sargassum horneri* | Cheongsapo, Busan | - | 1.3±0.4 | - |
| *Sargassum sagamianum* | Cheongsapo, Busan | 2.3±0.6 | 3.3±0.6 | - |
| *Sargassum thunbergii* | Cheongsapo, Busan | - | - | - |
| *Sargassum sp.* | Iho, Jeju | 2.5±0.7 | 3.5±0.7 | - |
| *Scytosiphon lomentaria* | Cheongsapo, Busan | - | 1.0±0.0 | - |
| **Rhodophyta** | | | | |
| *Symphyocladia latiuscula* | Cheongsapo, Busan | 3.5±1.3 | 8.8±0.8 | - |

Data are the averages of triplicate experiment. Significant at p<0.01

**Table - 2:** The antimicrobial(=*Propionibacterium acnes*) activity of methanol extracts from different parts of *Ecklonia cava* and *E. kurome* by disk diffusion method. After 72hr of incubation, the inhibition zone was measured in mm.

| | *Ecklonia cava* | | *Ecklonia kurome* | |
|---|---|---|---|---|
| | 1 mg disk$^{-1}$ | 5 mg disk$^{-1}$ | 1 mg disk$^{-1}$ | 5 mg disk$^{-1}$ |
| Blade | 1.8 ± 0.5 | 5.7 ± 1.2 | 2.0 ± 0.0 | 5.3 ± 0.4 |
| Midrib | 1.2 ± 0.6 | 5.7 ± 1.5 | 1.3 ± 0.4 | 4.0 ± 0.7 |
| Stipe | 0.0 ± 0.0 | 2.5 ± 1.0 | 0.1 ± 0.1 | 3.2 ± 0.0 |
| Holdfast | 0.0 ± 0.0 | 1.7 ± 0.6 | 0.0 ± 0.0 | 2.5 ± 0.5 |

Data are the averages of triplicate experiment. Significant at p<0.01

**Table - 3:** The MIC values of *Ecklonia cava*, *Ecklonia kurome*, *Ishige sinicola* and *Symphyocladia latiuscua* methanol extracts against *Propionibacterium acnes* and *Staphylococcus epidermidis* tested by broth microdilution assay

| | *Propionibacterium acnes* | *Staphylococcus epidermidis* |
|---|---|---|
| | MIC (mg ml$^{-1}$) | MIC (mg ml$^{-1}$) |
| *Ecklonia cava* | 0.31 | 2.50 |
| *Ecklonia kurome* | 0.31 | 2.50 |
| *Ishige sinicola* | 0.31 | - |
| *Symphyocladia latiuscula* | 0.16 | 0.63 |

The results are shown as average values from three separate experiments (MIC in mg ml$^{-1}$) and "-" is no inhibition at 10 mg ml$^{-1}$

consists of a pinnately divided blade with midrib, stipe, and a fibrous holdfast (= rhizoid). In both species, the blade part exhibited the highest activity against bacterial growth, with 5.7 and 5.3 mm at 5 mg disk$^{-1}$, respectively. The holdfast extracts exhibited the lowest activity, with 1.7 and 2.5 mm at 5 mg disk$^{-1}$, respectively.

In acne, the aerobic *S. epidermidis* is usually involved in superficial infections within the sebaceous unit (Burkhart *et al.*, 1999). The extracts tested were found to have no inhibitory effects against *S. epidermidis* at 5 mg disk$^{-1}$. As a positive control, the antimicrobial activity of erythromycin against *S. epidermidis* at 0.5 µg disk$^{-1}$ produced a 4.0 mm inhibition zone.

**MIC value determination:** The antimicrobial activities of *E. cava*, *E. kurome*, *I. sinicola* and *S. latiuscula* were further evaluated by determining the MIC, which is the lowest concentration of antimicrobial that will visibly inhibit the growth of a microorganism. The microbroth dilution assay was conducted to determine the MIC values of four selected seaweed extracts (Table 3). MIC values were determined using a two-fold serial dilution method. The MIC values of *E. cava*, *E. kurome,* and *I. sinicola* were equal (0.31 mg ml$^{-1}$) against *P. acnes*. The MIC for *S. latiuscula* was 0.16 mg ml$^{-1}$. As a positive control, the MIC value of erythromycin against *P. acnes* was 0.019 µg ml$^{-1}$.

The antimicrobial activities of the selected four species were also evaluated by determining MIC values against *S. epidermidis*. The MIC values of *E. cava* and *E. kurome* were equal (2.5 mg ml$^{-1}$) against *S. epidermidis* and the MIC value of *S. latiuscula* was 0.63 mg ml$^{-1}$. For *I. sinicola,* no inhibitory effect was seen, even at 10 mg ml$^{-1}$. As a positive control, the MIC value of erythromycin against *S. epidermidis* was 0.312 µg ml$^{-1}$.

**Cytotoxicity assay:** For possible future applications in therapeutic agents and cosmetic products for acne, the effects of each seaweed extracts on the cell viability were determined with the MTT assay, using murine macrophage-derived RAW264.7 cells (Fig. 1).

**Table - 4:** Comparison of different compound fraction from *Ecklonia cava*, *Ecklonia kurome*, and *Ishige sinicola* on the antimicrobial activity against *Propionibacterium acnes* by MIC test

| | Saccharides | Lipids | Phenolics | Alkaloides | Nitrogen compounds |
|---|---|---|---|---|---|
| *Ecklonia cava* | 5.0 | - | - | 12.5 | 0.31 |
| *Ecklonia kurome* | - | - | 1.25 | - | 0.16 |
| *Ishige sinicola* | - | - | 0.31 | - | - |

Data are the averages of triplicate experiment on each test material and "-" is no inhibition at 10 mg $ml^{-1}$

Treatment of RAW 264.7 cells with *E. cava*, *E. kurome* and *I. sinicola* at the concentrations used had no significant effect on cell viability after 24 hr incubation, whereas *S. latiuscula* reduced cell viability by 55% at 200 $\mu g\ ml^{-1}$, 52% at 300 $\mu g\ ml^{-1}$ and 57% at 400 $\mu g\ ml^{-1}$.

**Inhibitory effects on NO production:** To investigate the anti-inflammatory effects of the *E. cava*, *E. kurome,* and *I. sinicola* extracts, the inhibitory effects on NO production were examined in an activated macrophage like cell line (RAW 264.7 cells). The methanol extracts from these three species at 50-400 $\mu g\ ml^{-1}$ potently inhibited NO production, in a dose-dependent manner (Fig. 1). The methanol extract of *S. latiuscula* also strongly inhibited NO production. However, the inhibition of the *S. latiuscula* extract was likely caused by the cytotoxicity of the extract itself. For this reason, we selected *E. cava*, *E. kurome* and *I. sinicola*, but not *S. latiuscula,* for further study and possible commercial applications.

To determine the main active compound(s) in the seaweed extracts, *E. cava*, *E. kurome,* and *I. sinicola* powders were successively fractionated according to polarity into five classes of constituents: saccharides, lipids, phenolics, alkaloids, and nitrogen compounds (Table 4). *Ecklonia cava* powder (20 g) was extracted three times with 1 lit. methanol-water (4:1), and the crude extract was evaporated, yielding a dark brown gummy residue. The fraction acidified to pH 2 and extracted with chloroform was discarded, and the remaining aqueous acid layer was basified to pH 10 with $NH_4OH$ and extracted with $CHCl_3$-MeOH (3:1, twice) and then with $CHCl_3$. Then, the aqueous basic layer was evaporated and extracted with MeOH to produce a dark brown nitrogen compound extract (98.8 mg), which contained the major antimicrobial activity. Using *E. kurome* powder (20 g), the same procedure was conducted and dark brown nitrogen compounds were recovered (72.2 mg); these compounds also contained significant antimicrobial activity. For constituent separation, *I. sinicola* powder (20 g) was extracted three times with 1 lit. of methanol-water (4:1), and the crude extract was evaporated, yielding a dark brown gummy residue. The fraction that was acidified to pH 2 and extracted with chloroform, yielding a moderately polar extract of phenolic compounds (1.6 mg), contained the major antimicrobial activity. The primary active compounds are now being isolated.

Acne in young people develops due to several factors, including hormonal imbalance, stress, diet, cosmetic application, and bacterial infection. Normal skin commensals, including *P. acnes*, *P. granulosum*, *S. epidermidis,* and *Malassezia furfur*, proliferate rapidly during puberty and are often involved in the development of acne (Hamnerius, 1996). In particular, the anaerobic *P. acnes* bacterium, commonly found on the skin surface, induces inflammation in the sebaceous gland or hair pore (Webster *et al*., 1978). *S. epidermidis* is an aerobic organism usually involved in superficial infections within the sebaceous unit (Burkhart *et al*., 1999). Therapeutic agents for acne, including the antibiotics triclosan, azelaic acid, tetracycline, erythromycin, and clindamycin, are typically used to inhibit inflammation and/or kill bacteria. However, antibiotics also may induce side effects, and resistance against them has increased in the dermatologic setting (Swanson, 2003). Thus, many researchers have sought to develop new therapeutic agents for acne with reduced side effects and high antibacterial activity (Park *et al*., 2004; Chomnawang *et al*., 2005).

Seaweeds are known to produce many secondary metabolites, including bioactive compounds with various activities (Newman *et al*., 2003; Del Val *et al*., 2001). In the present study, 57 species of seaweeds were examined for their antimicrobial activities against *P. acnes* and *S. epidermidis*. The present results showed that 13 methanol and 2 aqueous extracts effectively inhibited the growth of *P. acnes* (Table 1). Among them, the methanol extracts of *E. cava*, *E. kurome*, *I. sinicola,* and *S. latiuscula* showed strong inhibitory effects against *P. acnes*; however, no positive effects of these extracts were observed against *S. epidermidis*. It has been reported that some plant extracts exhibited more antibacterial effects against anaerobic bacteria than against aerobic bacteria (Weckesser *et al*., 2007; Baik *et al*., 2008). In this study, we also found remarkable differences between anaerobic and aerobic bacteria in susceptibility to the seaweed extracts.

*Ecklonia cava* Kjellman and *E. kurome* Okamura are large, perennial members of the brown alga (Laminariaceae) that are widely distributed in the subtidal regions of South Korea. Because of the economic and ecological importance of these species, they have been studied extensively (Bolton and Anderson, 1994). *Ecklonia cava* and *E. kurome* have been used widely as sources for fucoidan, fucan sulfate, and phlorotanins, which have known antitumor, anticoagulant, antioxidant, and antithrombin properties (Takashi *et al*., 2007; Heo *et al*., 2005; Nagayama *et al*., 2002). In Korea and China, *E. cava* and *E. kurome* have been used in traditional herbal medicines for the treatment of cancer and inflammation (Xu *et al*., 2002). Additionally, *E. kurome* is used in Chinese herbal medicine in the treatment of goiter and scrofula, urinary diseases and dropsy, stomach ailments, hemorrhoids and anal fistulas, boils, and as a laxative and tonic for lying-in women (Zeng and Zhang, 1984).

We evaluated the cytotoxicity of methanol extract of *E. cava* and *E. kurome* in the murine macrophage-derived RAW264.7 cells. The extracts did not significantly affect cell viability after up to 24 h of incubation. The methanol extracts from these two species potently inhibited nitric oxide (NO) production at 50-400 μg $ml^{-1}$, dose dependently. The active compounds are now being isolated from dark brown nitrogen compounds extracted from *E. cava* and *E. kurome* for further study.

The brown seaweed *I. sinicola* is commonly used as a foodstuff in Korea and China (Oh *et al.*, 1990). This seaweed is widespread on rocks in the upper and middle intertidal zone on rough open coasts. Cytotoxicity analysis of the *I. sinicola* methanol extract in RAW 264.7 cells incubated for up to 24 hr showed no significant effects on cell viability. The methanol extract of *I. sinicola* strongly inhibited NO production. A yellowish phenolic fraction showing antimicrobial activity from *I. sinicola* is also being further characterized.

*Symphyocladia latiuscula* extract reduced cell viability by 55% at 200 μg $ml^{-1}$. *S. latiuscula* is known to contain high concentrations of bromophenols (Wang *et al.*, 2005). It has been reported that some bromophenols are toxic to bacteria and other living organisms (Hetu *et al.*, 1983; Calza *et al.*, 2008). Although methanol extracts of *S. latiuscula* clearly were the most effective antibacterial and anti-inflammatory agents, cytotoxic effects against eukaryotic cells were observed. For this reason, we selected the three other species, but not *S. latiuscula,* for further study.

In conclusion, the present investigation demonstrated that extracts from three seaweeds, *E. cava*, *E. kurome,* and *I. sinicola,* possessed strong anti - *P. acnes* and anti - inflammatory activity, without any serious toxic effect at the moderate doses assessed. Accordingly, these three seaweed extracts are promising sources of antibacterial agents that may be useful for cosmetics and in the development of new treatments for acne vulgaris.

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