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. kurume, 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. kurume 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. kurume, extracts of the pinnate blade had the highest inhibitory activity on bacterial growth. In cytotoxicity assays, methanol extracts of E. cava, E. kurume, 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. kurume, 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 compounds 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 pigmented 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.
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 H2, 5%, and N2, 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 × 10^6 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 × 10^6 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% NaHCO3, 1 mM sodium pyruvate, and 10% (v/v) heat-inactivated fetal bovine serum (FBS), in a humidified chamber with 5% CO2/95% 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% CO2 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 (NO2^-), in culture supernatants (Green et al., 1982). Briefly, RAW264.7 cells were seeded in 24-well culture plates at a density of 1 × 10^6 cells well^-1 and incubated for at least 2 hr to allow
Seaweed extracts activity against Propionibacterium acnes

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 NaNO₂ (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. latuscula methanol extract, none of the red algae exhibited antimicrobial activity. The methanol extracts of E. cava, E. kurume, I. sinicola, and S. latuscula 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. kurume (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 latuscula (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.](image-url)
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.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Collection site</th>
<th>Methanol extracts</th>
<th>Aqueous extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg disk⁻¹</td>
<td>5 mg disk⁻¹</td>
</tr>
<tr>
<td>Phaeophyta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dictyota dichotoma</td>
<td>Cheongsapo, Busan</td>
<td>-</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>Ecklonia cava</td>
<td>Unseong, Jeju</td>
<td>2.3±0.3</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>Ecklonia kurome</td>
<td>Bangeori, Pohang</td>
<td>3.0±0.0</td>
<td>5.7±0.3</td>
</tr>
<tr>
<td>Ecklonia stolonifera</td>
<td>Daebeyeon, Busan</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eisenia bicyclis</td>
<td>Sinyang, Jeju</td>
<td>-</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>Ishige okamurae</td>
<td>Sachon, Namhae</td>
<td>-</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>Ishige sinicola</td>
<td>Cheongsapo, Busan</td>
<td>2.2±0.3</td>
<td>6.3±0.8</td>
</tr>
<tr>
<td>Laminaria japonica</td>
<td>Cheongsapo, Busan</td>
<td>0.5±0.7</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>Pachydictyon coriaceum</td>
<td>Iho, Jeju</td>
<td>-</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>Sargassum horneri</td>
<td>Cheongsapo, Busan</td>
<td>-</td>
<td>1.3±0.4</td>
</tr>
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<td>Sargassum sagamianum</td>
<td>Cheongsapo, Busan</td>
<td>2.3±0.6</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>Sargassum thunbergii</td>
<td>Cheongsapo, Busan</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sargassum sp.</td>
<td>Iho, Jeju</td>
<td>2.5±0.7</td>
<td>3.5±0.7</td>
</tr>
<tr>
<td>Scytosiphon lomentaria</td>
<td>Cheongsapo, Busan</td>
<td>-</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>Rhodophyta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symphyocladia latiuscula</td>
<td>Cheongsapo, Busan</td>
<td>3.5±1.3</td>
<td>8.8±0.8</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Collection site</th>
<th>Methanol extracts</th>
<th>Aqueous extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg disk⁻¹</td>
<td>5 mg disk⁻¹</td>
</tr>
<tr>
<td>Ecklonia cava</td>
<td></td>
<td>1.8±0.5</td>
<td>5.7±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2±0.6</td>
<td>5.7±1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0±0.0</td>
<td>2.5±1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0±0.0</td>
<td>1.7±0.6</td>
</tr>
<tr>
<td>Ecklonia kurome</td>
<td></td>
<td>2.0±0.0</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3±0.4</td>
<td>4.0±0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1±0.1</td>
<td>3.2±0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0±0.0</td>
<td>2.5±0.5</td>
</tr>
</tbody>
</table>

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

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 (Burkhardt 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

<table>
<thead>
<tr>
<th></th>
<th>Saccharides</th>
<th>Lipids</th>
<th>Phenolics</th>
<th>Alkaloids</th>
<th>Nitrogen compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecklonia cava</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>12.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Ecklonia kurome</td>
<td>-</td>
<td>-</td>
<td>1.25</td>
<td>-</td>
<td>0.16</td>
</tr>
<tr>
<td>Ishige sinicola</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.31</td>
<td>-</td>
</tr>
</tbody>
</table>

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 µg ml⁻¹, 52% at 300 µg ml⁻¹ and 57% at 400 µg 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 µg 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 strongly 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 NH₃·H₂O and extracted with CHCl₃·MeOH (3:1, twice) and then with CHCl₃. 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. 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 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 phlorotannins, which have known antitumor, anticagulant, 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 h 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.

References


