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Inhibition of growth and urease of *Helicobacter pylori* by Korean edible seaweed extracts

Bo-Bae Lee[1,5], Jae-Suk Choi[1,5], Hye Eun Moon[1], Yu-Mi Ha[1], Myung Sook Kim[2], Kwang Keun Cho[3], and In Soon Choi[1,4,6]

[1]RIS Center, Industry-Academic Cooperation Foundation, Silla University, Busan, Republic of Korea
[2]Department of Biology, Jeju National University, Jeju-si, Jeju-do, Republic of Korea
[3]Department of Animal Resources Technology, Gyeongnam National University of Science and Technology, Jinju, Gyeongnam, Republic of Korea
[4]Department of Biological Science, Silla University, Busan, Republic of Korea
[5]Contributed equally
[6]Corresponding author: ischoi@silla.ac.kr

Abstract: Of 27 Korean seaweed species screened for potential anti-*H. pylori* activity, seven (25.9%) showed strong inhibitory activity based on the agar diffusion method. The strongest activity was observed for ethanol extracts from *Ishige okamurae*. At 1 mg/disk, the inhibition zone of *I. okamurae* extract was 9.0 mm, and the minimum inhibitory concentration was 12 μg/ml based on the broth microdilution assay. Based on the free urease assay system, the 80% methanol extracts from *I. okamura*e had 75.4% inhibition at 0.1 mg/ml. To identify the primary active compounds, I. okamurae powders were successively fractionated according to polarity into five classes of constituents including saccharides, lipids, phenolics, alkaloids, and nitrogen compounds. The *I. okamurae* phenolic compounds had significant antimicrobial activity (12 μg/ml minimum inhibitory concentration), while the nitrogen compound extract significantly inhibited *H. pylori* urease activity (80.84% at 1 mg/ml). We evaluated the *I. okamurae* ethanol and 80% methanol extract for acute toxicity in BALB/c mice. Over the 2-week observation period, no death occurred in any mouse administered a dose of 5 g/kg body weight. These results suggest that *I. okamurae* extract can be used to develop therapeutic agents for chronic gastritis and peptic ulceration.

Key words: antimicrobial activity, *Helicobacter pylori*, *Ishige okamurae*, seaweed, urease.

Helicobacter pylori is a Gram-negative, micro-aerophilic, spiral-shaped bacterium that infects up to 50% of the global population. Several investigations have shown that *H. pylori* cause many gastroduodenal diseases, such as gastritis, gastric and duodenal ulcers, and cancer (Marshall and Warren, 1984; Parsonnet *et al*., 1991). *Helicobacter pylori* can survive in the stomach by releasing urease, which is an extracellular, cell-bound enzyme with a molecular weight of approximately 580 kDa that accounts for up to 5-10% of the total cell protein. This enzyme converts urea to ammonia, which counteracts and neutralizes the stomach acid, creating an environment that protects *H. pylori* (Hassani *et al*., 2009). *Helicobacter pylori* is sensitive to various antibiotics such as macrolides, clarthromycin, metronidazole, amoxicillin, and tetracycline (Nagata *et al*., 1995). However, clinical trials with these antibacterial agents alone have predominately failed to eradicate *H. pylori* (Chiba *et al*., 1992). For long-term eradication, triple therapy consisting of two antimicrobial agents and a proton pump inhibitor, such as lansoprazole, is considered highly efficient, with 80% of patients showing clearance of the pathogen (Graham *et al*., 1992), albeit there are side-effects such as diarrhea. Recently, there has been an increase in the number of cases of metronidazole- and/or clarithromycin-resistant strains of *H. pylori* (Midolo *et al*., 1996). Thus, it is important to identify novel therapeutic agents, other than antibiotics, that are both highly effective and safe.

Seaweeds produce various secondary metabolites with different activities, making them a good source of bioactive compounds. Numerous studies have examined the biological activities of compounds from seaweeds against human pathogens, but few reports have explored their effects against *Helicobacter pylori*, an important etiological agent of chronic gastritis, peptic ulcers, and gastric cancer. The-

refore, this study examined the anti-*H. pylori* activity, and urease inhibition activity of seaweed extracts from 27 species of edible seaweed found along the coast of Korea.

Materials and methods

Seaweed extracts. A total of 27 seaweed species were collected from various locations in South Korea between June 2000 and April 2006. 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 ethanol-soluble components, 1 L ethanol was added to 20 g of each powder and extracted for one day. This was repeated three times, and the combined extracts were evaporated to dryness. To prepare the 80% methanol extract, the same procedure was performed. Stock solutions were prepared by adding 1 ml ethanol or 80% methanol 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. *Helicobacter pylori* (KCTC 12083) obtained from the Korean Collection for Type Cultures (KCTC; Daejeon, Korea) was maintained in Brucella broth base agar (Sigma B3051) with 5% horse serum (GIBCO) at 37 °C under microaerobic conditions (10% CO_2) in a CO_2 incubator (Heal Force, Shanghai, China).

Disk diffusion method. Antimicrobial activity was determined using the disk diffusion method, following the guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS) for M2-A8 (NCCLS, 2003a). The microbe was incubated in Brucella medium with 10% horse serum for 24 h, under microaerobic conditions, and then adjusted to approximately 2.0×10^8 CFU/ml. The solution (1 ml) was spread onto the Brucella 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 seeded with the organism to be tested. The plates were incubated at 37 °C for 72 h under microaerobic conditions. Antimicrobial activity was defined by measuring the diameter of the growth inhibition zone (mm). Positive controls were also used simultaneously. All disk diffusion tests were performed in triplicate, independently.

Determination of MIC values. The antimicrobial activity was determined using the broth microdilution assay, following the guidelines NCCLS for aerobic bacteria M7-A6 (NCCLS, 2003b) in 96-well U-shaped microplates. *Helicobacter pylori* inocula were prepared from 24 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard solution turbidity. The seaweed extracts were first diluted to the highest concentration (12.5 mg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 0.19 μg to 12.5 mg/ml. The 96-well plates were prepared by dispensing 100 μl inoculum and 100 μl of each sample into wells. The first well, containing 100 μl Brucella broth with no compound and 100 μl inoculum on each strip, was used as a negative control. The second well, containing 90 μl Brucella broth, 10 μl ethanol, and 100 μl inoculum on each strip was used as a vehicle control. The final volume in each well was 200 μl. The plates were incubated at 37 °C for 48 h under microaerobic conditions. The MIC value was defined as the lowest concentration that yielded no bacterial cell growth. Positive controls were also used simultaneously. All MIC tests were performed in triplicate, independently.

Urease preparation. Urease was obtained by collecting whole-plate-dense *Helicobacter pylori* colonies using a sterile plastic loop, which were resuspended vigorously by vortexing in 5 ml 20 mM sodium phosphate buffer (pH 7) for 30-40 s. Thereafter, the suspension was washed twice with the same buffer. The resuspended cells were centrifuged at 9,000 × g for 5 min at 4 °C. The supernatant was used for the urease inhibition assay (Tabak *et al*., 1999). The protein content of the enzyme solution was measured using the Bradford method (Bradford, 1976). Dye reagent (40 μl) (Bio-Rad protein assay kit II; Bio-Rad Laboratory, Hercules, CA) was added to 160 μl supernatant. After incubation at 25 °C for 30 min, the absorbance at 595 nm was measured.

Urease inhibition assay. Urease inhibition was measured using 100 μl enzyme solution and 300 μl 20 mM sodium phosphate buffer (pH 7) containing 100 mM urea, which was incubated at 37 °C for 7 min. Next, 100 μl 1 N sulfuric acid was added to stop the reaction. For ammonia determination, the indophenol method was used. Phenol-nitroprusside reagent and alkali reagents (2.5 ml each) were added to the reaction mixture. After incubation at 65 °C for 20 min, the absorbance at 630 nm was measured (Woo *et al*., 1998). Percent inhibition was determined using the following equation:

% inhibition = (activity without inhibitors - activity with inhibitors/activity without inhibitors) × 100.

Positive controls were also used simultaneously. All urease inhibition assays were performed in triplicate, independently.

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

Determination of total phenolic acid and nitrogen compounds. Total phenolic and nitrogen compound contents of the samples were determined with Folin-Ciocalteau reagent

and ninhydrin reagent, respectively. Total phenolic content was expressed as milligrams of gallic acid equivalents per one gram of sample, and total nitrogen compound content was expressed as milligrams of glycine equivalents per one gram of sample.

Acute toxicity test. To confirm the safety of the ethanol and 80% methanol extracts of *Ishige okamurae* for the development of therapeutic agents, BALB/c mice (8-10 weeks old; 20-25 g body weight) were used for acute toxicity tests (Cho *et al*., 2007). The animals were kept at room temperature (24 ± 1 °C) on a 12 h light/dark cycle with free access to food and water. For the acute toxicity test, mice were fasted for 6 h with water provided *ad libidum*. Both of the seaweed extracts were evaporated under vacuum at 35 °C using a rotary evaporator and then extracts (5 g/10 ml 5% Tween 80/kg body weight) were administered orally to mice (n = 5). The animals were observed for any abnormal behavior for 3 h, and mortality was noted for up to two weeks. A group of animals treated with Tween-80 alone served as the control. Animal experiments were performed in accordance with the US NIH Guidelines for the Care and Use of Laboratory Animals.

Statistical analysis. All experiments were performed at least three times independently. The significance of the results was calculated using Student t-test, and results were taken to be statistically significant at [a] $P < 0.01$ and [b] $P < 0.05$ as compared control.

Results

Screening for antimicrobial activity. Of the 27 seaweed species screened for potential antimicrobial activity against *Helicobacter pylori*, only seven species (25.9%) showed activity based on the disk diffusion method (Table 1). Among these, *Capsosiphon fulvescens*, *Ishige okamurae*, *I. sinicola*, *Meristotheca papulosa*, and *Ulva pertusa* exhibited considerable activity against bacterial growth (>10 mm at 3 mg/disk).

Ethanol extracts from the Chlorophyta *Codium fragile*, *Enteromorpha compressa*, and *E. linza*; the Phaeophyta *Ecklonia kurome*, *E. stolonifera*, *Eisenia bicyclis*, *Hizikia fusiformis*, *Laminaria japonica*, *Petalonia binghamiae*, *Sargassum thunbergii*, *Sargassum* sp., and *Undaria pinnatifida*; and the Rhodophyta *Bangia atropurpurea*, *Chondaria crassicaulis*, *Chondracanthus intermedia*, *Chondrus ocellatus*, *Gracilaria verrucosa*, *Grateloupia filicina*, *Hypnea charoides*, and *Porphyra yezoensis* exhibited low antimicrobial activity based on the disk diffusion method. As a positive control, the antimicrobial activity of amoxicillin and tetracycline against *Helicobacter pylori* at 0.5 μg/disk produced a 25.7 ± 1.5 and 12.7 ± 2.1 mm inhibition zone, respectively.

The antimicrobial activities of the seven selected species were evaluated by measuring the MIC values against *Helicobacter pylori* (Table 1). The MIC for *Ishige okamurae* was 12 μg/ml, showing the highest activity, followed by *I. sinicola*, showing 49 μg/ml. For the remainder of the samples, the MICs were above 100 μg/ml. As a positive control, the MICs of amoxicillin and tetracycline against *H. pylori* were 0.0060 and 0.047 μg/ml, respectively.

Inhibition of Helicobacter pylori urease activity. Because urease has a critical role in the pathogenesis of gastric diseases by *H. pylori*, we evaluated the inhibition activity of ethanol and 80% methanol extracts from the seven selected species against *H. pylori* urease (Table 2). Based on the *H. pylori* urease system, the inhibitory effect of 80% metha-

Table 1. Antimicrobial activity of ethanol extracts from various seaweeds against *Helicobacter pylori*. All measurements were performed in triplicate, and values are the mean of three replicates.

Scientific name	Collection site	Yield (%)	Disk diffusion method (mg/disk)		MIC (μg/ml)
			1	3	
Chlorophyta					
Capsosiphon fulvescens	Jangheung, Namhae	1.1 ± 0.1	5.5 ± 0.1	10.0 ± 0.1	195
Monostroma nitidum	Galmoonri, Wando	5.9 ± 0.6	3.25 ± 1.0	7.5 ± 2.1	391
Ulva pertusa	Cheongsapo, Busan	1.2 ± 0.2	6.0 ± 0.1	10.0 ± 1.4	391
Phaeophyta					
Ishige okamurae	Sachon, Namhae	5.5 ± 0.5	9.0 ± 0.1	10.5 ± 0.7	12
Ishige sinicola	Cheongsapo, Busan	14.1 ± 1.2	5.0 ± 0.1	10.5 ± 3.5	49
Scytosiphon lomentaria	Cheongsapo, Busan	5.7 ± 0.3	6.0 ± 1.4	8.0 ± 1.4	195
Rhodophyta					
Meristotheca papulosa	Cheongsapo, Busan	6.3 ± 0.4	5.5 ± 0.7	10.3 ± 2.5	195

Table 2. *Helicobacter pylori* urease inhibition activity by various seaweed extracts. All measurements were performed in triplicate, and values are the mean of three replicates. Statistical significance is [a] $P < 0.01$ and [b] $P < 0.05$ as compared control.

Scientific name	Ethanol extract (%) (μg/ml)			80% methanol extract (%) (μg/ml)		
	10	100	1000	10	100	1000
Chlorophyta						
Capsosiphon fulvescens	2.11 ± 0.61	2.17 ± 0.94	8.13 ± 1.78	7.26 ± 2.94	10.46 ± 2.05	15.40 ± 2.52
Monostroma nitidum	0.63 ± 1.50	3.32 ± 0.88	18.92 ± 3.77	9.00 ± 2.82	12.50 ± 2.34	18.68 ± 2.93
Ulva pertusa	3.40 ± 0.73	4.98 ± 1.81	10.35 ± 2.02	11.10 ± 1.90	12.32 ± 2.09	16.09 ± 2.54
Phaeophyta						
Ishige okamurae	1.42 ± 1.21	5.60 ± 1.98	25.81 ± 0.54	14.97 ± 2.07	75.41 ± 6.11[b]	92.82 ± 5.04[a]
Ishige sinicola	0.11 ± 0.56	2.53 ± 2.74	15.07 ± 1.59	12.69 ± 2.14	61.93 ± 6.54[b]	91.88 ± 5.13[a]
Scytosiphon lomentaria	0.35 ± 0.62	2.77 ± 1.43	16.24 ± 2.48	-0.02 ± 0.35	3.27 ± 2.69	12.58 ± 1.96
Rhodophyta						
Meristotheca papulosa	1.26 ± 0.46	2.06 ± 0.57	7.27 ± 1.02	7.87 ± 1.76	9.58 ± 1.83	13.62 ± 2.47

nol extracts were greater than ethanol extract, excluding 1 mg/ml *Monostroma nitidum*, which had a similar inhibitory effect. Among the seven selected species, 80% methanol extracts from *Ishige okamurae* showed the highest inhibitory activity of 75.41% and 92.82% at 100 μg/ml and 1 mg/ml, respectively, followed by *I. sinicola* with inhibitory activities of 61.93% and 91.88%, respectively. For the remainder of the samples, the inhibitory effect was below 20%. Thus, *I. okamurae* was selected and used for further studies. As a positive control using a reference drug, the inhibitory effect of the urease inhibitor, acetohydroxamic acid was 83.55% at 1 mg/ml.

Fractionation of Ishige okamurae extracts. To identify the primary active compounds, *I. okamurae* powders were successively fractionated according to polarity into five classes of constituents including saccharides, lipids, phenolics, alkaloids, and nitrogen compounds (Tables 3, 4). The dried powder (20 g) of *I. okamurae* that was collected in Sacheon, Namhae, was extracted three times with 1 L methanol-water (4:1), and the crude extract was evaporated, which yielded a dark brown gummy residue. The fraction acidified to pH 2 and extracted with chloroform yielded a moderately polar mixture of phenolic compounds (425.2 mg) with significant antimicrobial activity (Table 3). The remaining aqueous acid layer was basified to pH of 10 with ammonium hydroxide, and extracted with chloroform-methanol (3:1, twice) followed by chloroform. Next, the aqueous basic layer was evaporated and extracted with methanol to produce a dark brown nitrogen compound extract (2,144.7 mg), which had an inhibitory effect on *Helicobacter pylori* urease activity (Table 4).

MIC values of phenolics of Ishige okamurae collected from various sites. Antimicrobial activities may vary by season and habitat (Moreau *et al*., 1988; Stirk *et al*., 2007; Vidyavathi and Sridhar, 1991). To investigate variation in the inhibitory effect on *Helicobacter pylori* growth, we determined the MIC values of phenolics of *I. okamurae* that were collected at different sampling sites and sampling times, such as Sachon, Namhae (June 2000), Sinyang, Jeju (February 2006, April 2012), and U-do, Jeju (July 2009) (Figure 1, Table 3). Of these phenolics, the phenolic extract of *I. okamurae* collected from U-do, Jeju, in July 2009 had the highest MIC (12 μg/ml); followed by Sachon, Namhae, in June

Table 3. The MIC values of five different fractions of *Ishige okamurae* collected from different sites and at different times against *Helicobacter pylori* based on the broth microdilution assay. All measurements were performed in triplicate, and values are the mean of three replicates.

Collection Site	Period	MIC (μg/ml)				
		Saccharides	Lipids	Phenolics	Alkaloids	Nitrogen compound
Sachon, Namhae	June 2000	3,125	195	24	195	391
Sinyang, Jeju	February 2006	1,563	1,563	391	391	781
Sinyang, Jeju	April 2012	195	391	98	195	1,563
U-do, Jeju	July 2009	781	391	12	98	781

Table 4. Inhibition of *Helicobacter pylori* urease activity by five different fractions of *Ishige okamurae* collected at different sites and at different times (1,000 µg/ml). All measurements were performed in triplicate, and values are the mean of three replicates. Statistical significance is [a] $P < 0.01$, [b] $P < 0.05$ as compared control.

Collection Site	Period	Urease inhibition activity (%)				
		Saccharides	Lipids	Phenolics	Alkaloids	Nitrogen compound
Sachon, Namhae	June 2000	8.32 ± 1.93	8.95 ± 1.16	5.11 ± 0.74	12.44 ± 1.23	38.08 ± 5.91
Sinyang, Jeju	February 2006	12.01 ± 0.28	26.75 ± 1.48[b]	6.74 ± 0.17	10.25 ± 2.06	80.84 ± 3.74[a]
Sinyang, Jeju	April 2012	10.13 ± 1.49	20.90 ± 2.0[b]	16.30 ± 2.11	1.91 ± 0.48	23.62 ± 3.15
U-do, Jeju	July 2009	16.88 ± 2.17	-0.63 ± 1.55	8.19 ± 2.07	17.78 ± 1.53	30.05 ± 0.93

2000 (24 µg/ml); Sinyang, Jeju, in April 2012 (98 µg/ml); and Sinyang, Jeju, in February 2006 (391 µg/ml).

Inhibition of Helicobacter pylori urease activity by nitrogen compounds of Ishige okamurae collected from different sites. To investigate variation in the inhibition of *H. pylori* urease activity, we examined the inhibitory effect of nitrogen compounds on the urease activity of *I. okamurae* collected from different sampling sites and sampling times (Table 4). Of these nitrogen compounds, samples from Sinyang, Jeju, in February 2006 had the highest inhibitory effect (80.84%);, followed by Sachon, Namhae, in June 2000 (38.08%); U-do, Jeju, in July 2009 (30.05%); and Sinyang, Jeju, in April 2012 (23.62%).

Total phenolic acid and nitrogen compounds in Ishige okamurae fractions. To confirm the content of phenolics and nitrogen compounds fractionated according to polarity, we used the Folin-Ciocalteau and ninhydrin methods to evaluate the total phenolic and total nitrogen contents, respectively, in *I. okamurae* fractions collected at different sites and times. As seen in Table 5, *I. okamurae* samples collected from Sinyang, Jeju, in April, 2012 had the highest total phenolic acid content (117.97 mg/g); followed by those collected from U-do, Jeju in July, 2009 (112.36 mg/g); Sachon, Namhae in June, 2000 (86.21 mg/g); and Sinyang, Jeju in February, 2006 (83.10 mg/g). Samples collected from Sinyang, Jeju in February 2006 had the highest nitrogen compound content (32.98 mg/g); followed by those from Sachon, Namhae in June, 2000 (23.92 mg/g); U-do, Jeju in July, 2009 (23.36 mg/g); and Sinyang, Jeju in April, 2012 (18.74 mg/g).

Table 5. Total phenolic acid and nitrogen compound concentrations of *Ishige okamurae* fractions collected at different sites and times, estimated by the Folin–Ciocalteau and ninhydrin methods, respectively. All measurements were performed in triplicate, and values are the mean of three replicates.

Collection Site	Period	Total phenolic content (mg/g gallic acid equivalent)	Total nitrogen compound content (mg/g glycine equivalent)
Sachon, Namhae	June 2000	86.21 ± 3.57	23.92 ± 0.01
Sinyang, Jeju	February 2006	83.10 ± 2.48	32.98 ± 0.75
Sinyang, Jeju	April 2012	117.97 ± 5.57	18.74 ± 0.78
U-do, Jeju	July 2009	112.36 ± 3.23	23.36 ± 0.46

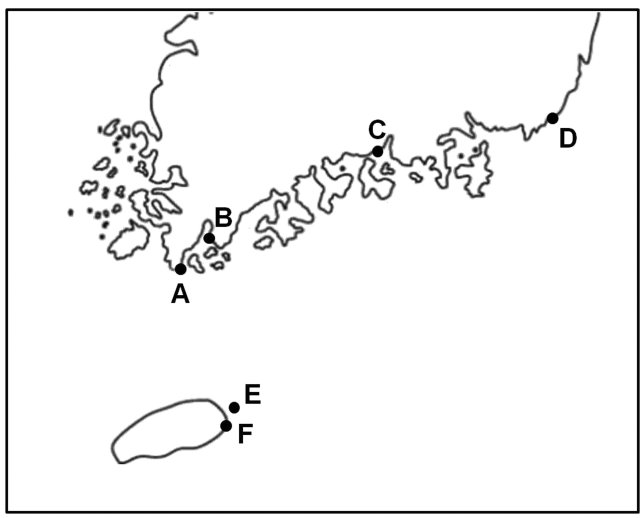

Figure 1. Sampling sites of the seaweed used in this study. **A.** Galmoonri, Wando (34° 22′ 11.13″ N, 126° 38′ 37.15″ E); **B.** Jangheung, Namhae (34° 25′ 58.46″ N, 126° 48′ 24.19″ E); **C.** Sachon, Namhae (34° 57′ 56.28″ N, 127° 58′ 27.43″ E); **D.** Cheongsapo, Busan (35° 09′ 42.28″ N, 129° 11′ 40.01″ E); **E.** U-do, Jeju (33° 30′ 49.73″ N, 126° 57′ 47.30″ E); **F.** Sinyang, Jeju (33° 26′ 00.27″ N, 126° 55′ 47.13″ E).

Acute toxicity. Although, *Ishige okamurae* is commonly used as a foodstuff in Korea and China (Oh *et al*., 1990), we evaluated the acute toxicity of ethanol and 80% methanol extracts of *I. okamurae* in mice. Over the 2-week observation period, no death occurred in any mice administered a dose of 5 g/kg body weight. Mice administered seaweed extract reacted by wandering briefly and returned to normal behavior after ~10 min. According to the World Health Organization (1992), a herbal medicine is considered toxic if the LD_{50} is lower than 5 g/kg body weight. Thus, these ex-

tracts would be classified as non-toxic. Therefore, our data suggest that these extracts can be safely used by humans at moderate doses.

Discusssion

Helicobacter pylori is classified by the World Health Organization and the International Agency for Research on Cancer as a class 1 carcinogen. *Helicobacter pylori* is a Gram-negative microaerophilic helical bacillus that inhabits various areas of the human stomach. Infection with this organism is strongly associated with chronic gastritis, peptic ulcer, and gastric carcinoma. The bacterium colonizes the gastric epithelial surface, and withstands the stomach's hostile ambience by microaerophilic growth capability and high urease activity, which accounts for approximately 6% of the soluble protein. Unlike urea-positive bacteria, *H. pylori* urease is located both in the cytoplasm and on the surface of *H. pylori* cells, and is reportedly one of the major surface proteins (Bode *et al*., 1989; Hu and Mobley, 1990). Urease is critical for *H. pylori* colonization of the gastric mucosa; namely, urease hydrolyzes urea and releases ammonia, which neutralizes acid and allows for survival of the bacterium and initial colonization. Therefore, if urease activity is inhibited, *H. pylori* could not survive in the presence of gastric acid.

Eradication of the organism from the stomach results in significant remission from the above diseases. Current eradication regimens involve the use of combination therapies (a proton pump inhibitor such as lansoprazole and two antibiotics, most commonly macrolides, clarthromycin, metronidazole, amoxicillin, and tetracycline) with an expected success rate between 80% and 90%. However, increasing resistance to these drugs is a growing global concern. Therefore, it is important to develop an effective method for reducing the level of resistant *Helicobacter pylori* strains. Previous studies have attempted to develop a novel antimicrobial agent capable of preventing and/or treating *H. pylori*. Until now, most studies have mainly focused on terrestrial plants (Boyanova and Neshev, 1999; Tabak *et al*., 1999; Takabayashi *et al*., 2004; Robles-Zepeda, *et al*., 2011).

In this study, ethanol extracts of 27 edible seaweed species were prepared and evaluated for their antimicrobial activity against *Helicobacter pylori* using the disk diffusion method. Among these, seven extracts showed high levels of antimicrobial activity and were further evaluated. Among them, the brown seaweed *Ishige okamurae* showed the strongest anti-*H. pylori* activity and urease inhibition activity. This species is widespread on rocks in the upper and middle intertidal zone of rough open coasts and is commonly used as soil fertilizer and a foodstuff in Korea and China (Oh *et al*., 1990). This seaweed has various beneficial biological activities, such as antioxidant (Heo and Jeon, 2009), anti-inflammatory (Kim *et al*., 2010), anti-diabetes activities (Min *et al*., 2011), and can protect against ultraviolet light (Heo *et al*., 2010).

To identify the active compounds in *Ishige okamurae*, the seaweed powders were successively fractionated according to polarity into five classes of constituents. Of these fractionates, phenolics and nitrogen compounds had the lowest MIC values and highest inhibitory effect on urease, respectively, showing that different compounds may have different bioactivities. Generally, the seaweed showed variation in cellular chemical composition and biological activity according to season, habitat, and different thalli in seaweed (Lobban and Harrison, 1994). Antimicrobial activities may show seasonal and habitat variation (Moreau *et al*., 1988; Vidyavathi and Sridhar 1991; Stirk *et al*., 2007). When we investigated inhibition of *I. okamurae* growth and urease activity collected from different sites and times, phenolics from the seaweed collected at U-do, Jeju, in July 2009 showed the highest MIC values (12 μg/ml). Otherwise, the highest inhibitory effect (80.84%) was found for nitrogen compounds of *I. okamurae* collected from Sinyang, Jeju in February 2006. In this study, we also found variation in the antimicrobial activity in the seaweed collected at different sites and times.

We found that *Ishige okamurae* phenolic compounds possessed antimicrobial activity and that nitrogen compounds inhibited urease, which have a critical role in the survival of *Heliobacter pylori* in the gastric mucosa and in the pathogenesis of this organism. We compared the MIC values and total phenolic acid contents of the phenolic fractions of *I. okamurae* collected at different sites and times. The highest MIC value (12 μg/ml) was identified in phenolics from seaweed collected at U-do, Jeju in July, 2009. The highest total phenolic acid content (117.97 mg/g) was identified in phenolics from seaweed collected at Sinyang, Jeju in April, 2012. In the remainder of the samples, the MIC values were proportional to the total phenolic acid content.

The mismatch between the MIC values and total phenolic acid contents probably results from the difference between the content of each phenolic compound in the phenolics fraction and its antimicrobial activity. Further research is needed to elucidate the profiles, contents, and antimicrobial activities of phenolic compounds in the phenolics fraction of *Ishige okamurae* collected at different sites and times.

We compared the urease inhibitory effect and total nitrogen compound contents of the nitrogen-compound fractions of *Ishige okamurae* collected at different sites and times. The greatest inhibitory effect (80.84%) and the highest total nitrogen compound content (32.98 mg/g) was found in nitrogen compounds from *I. okamurae* collected from Sinyang, Jeju in February 2006. For the remainder of the samples, the inhibitory effects were proportional to the total nitrogen compound content.

In conclusion, our results suggest that the seaweed *Ishige okamurae* may be useful as a treatment for gastrointestinal

disorders caused by *Helicobacter pylori*. These results can be used as a basis for further characterization and elucidation of the active compounds responsible for this antimicrobial activity.

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