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In vitro study on the inhibitory effects of Korean brown, green, and red seaweed extracts on collagenase, elastase, and hyaluronidase

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Abstract

Skin aging is classified according to intrinsic factors, such as genetic and metabolic processes, and extrinsic factors, such as stress and exposure to ultraviolet radiation. These factors activate enzymes in the skin, such as collagenase, elastase, and hyaluronidase (HAse), thereby promoting skin aging. In this study, we investigated the effects of a Korean edible seaweed extract on collagenase, elastase, and HAse, three extracellular matrix enzymes involved in the aging process. Brown and green seaweed extracts showed high collagenase inhibitory activity. Among these, *Cladophora wrightiana* var. *minor* exhibited the highest inhibitory activity. In elastase inhibitory activities of seaweed extracts, the brown seaweed extract showed the highest elastase inhibitory activities compared to other seaweed extracts. *Padina gymnospora* showed the highest inhibitory activity among tested seaweed extracts. Green seaweed extracts of *C. wrightiana* var. *minor* showed the highest inhibitory activity, followed by brown seaweed extract. The results of this study suggest that seaweed extract strongly inhibits collagenase, elastase, and HAse, which are responsible for skin aging and antiwrinkle effects.

Keywords: Antiaging, Collagenase, Elastase, Hyaluronidase, Korean seaweeds

Introduction

Globally, seaweeds comprise approximately 6,000 species of which 150 are used as food (Devi et al., 2011; Meenakshi et al., 2011). They are classified into three groups based on their pigmentation and morphological characteristics: green, brown, and red seaweed (Deyab, 2016). Most seaweed grows more rapidly and efficiently than terrestrial plants (Kindleysides et al., 2012). Due to their markedly different living environments compared to terrestrial plants, seaweeds possess unique bioactive substances and can produce a wide range of primary and secondary metabolites (Holdt & Kraan, 2011; Kim et al., 2015). Additionally, they contain various natural compounds, such as polyphenols, polysaccharides, sterols, and peptides, which exhibit diverse physiological activities (Wang

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et al., 2018). South Korea ranks fourth globally in seaweed production, with Korean seaweeds known for their diverse biological activities (Cha & Kim, 2008; Ryu et al., 2023).

Skin aging is classified according to intrinsic factors, such as genetic and metabolic processes, and extrinsic factors, such as stress and UV exposure (Riani et al., 2018). Modern lifestyle habits such as irregular sleep schedules and a diet poor in quality accelerate the production of collagenase, elastase, and hyaluronidase (HAse), which degrade major components of the extracellular matrix in the dermis (Nema et al., 2011). Collagenase breaks down collagen networks, whereas elastase breaks down elastin fibers, resulting in the formation of wrinkles and loss of skin elasticity (Wanakhachornkrai et al., 2020). Hyaluronidase breaks down hyaluronic acid (HA), leading to skin dryness and sagging (Abd Razak et al., 2020; Fayad et al., 2017). These enzymes promote skin aging by producing symptoms such as wrinkles, decreased elasticity, dryness, and sagging. Although research is underway to discover alternative inhibitors of collagenase, elastase, and HAse, the aging-related enzyme-inhibitory activities of Korean seaweed extracts remain unknown. Therefore, we investigated the inhibitory activities of collagenase, elastase, and HAse in Korean edible seaweeds (14 species each of brown, green, and red seaweed).

Materials and Methods

Chemicals

Collagenase from *Clostridium histolyticum* (EC 3.4.24.3), elastase from porcine pancreas (EC 3.4.21.36), HAse from bovine testes (EC 3.2.1.35), phloroglucinol, sodium hyaluronate, N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA), and *n*-succinyl-Ala-Ala-Ala-*p*-nitroanilide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dimethylsulfoxide (DMSO), sodium carbonate (Na_2CO_3), potassium tetraborate, and acetic acid were purchased from the Junsei Chemicals (Tokyo, Japan). The tris was purchased from the National Diagnostics (Atlanta, GA, USA). The epigallocatechin gallate (EGCG) was purchased from the Tokyo Chemical Industry (TCI, Tokyo, Japan). The tricine was purchased from the Alfa Aesar (Ward Hill, MA, USA). The sodium acetate anhydrous was purchased from the Duksan Reagents (Seoul, Korea). The *p*-dimethylaminobenzaldehyde was purchased from the Samchun Chemicals (Seoul, Korea).

Sample preparation

Fourty-two seaweeds were selected for this study, as shown in Table 1. These seaweed samples were obtained from the Marine Biodiversity Institute of Korea in Seocheon, Korea. Upon col-

Table 1. Collagenase, elastase, and hyaluronidase inhibitory effects of brown seaweed extracts

Brown seaweeds	Total phenolic contents (PGE mg/g)	IC_{50} values for collagenase inhibition (μg/mL)	IC_{50} values for elastase inhibition (μg/mL)	IC_{50} values for hyaluronidase inhibition (μg/mL)
Carpomitra costata	9.66 ± 0.16^{i}	> 100	> 1,000	> 250
Desmarestia tabacoides Okamura	5.24 ± 0.06^{l}	> 100	> 1,000	> 250
Ecklonia cava	98.65 ± 0.40^{b}	95.08 ± 0.78	465.13 ± 15.63	219.16 ± 4.73
Eisenia bicyclis	96.81 ± 0.56^{b}	> 100	> 1,000	212.88 ± 4.10
Ishige foliacea	32.99 ± 0.53^{g}	> 100	> 1,000	> 250
Ishige okamurae	57.37 ± 0.94^{d}	> 100	> 1,000	> 250
Padina gymnospora	66.94 ± 0.30^{c}	> 100	260.05 ± 26.71	246.45 ± 3.59
Sargassum coreanum	96.73 ± 0.81^{b}	> 100	388.65 ± 18.64	225.17 ± 4.52
Sargassum horneri	6.85 ± 0.13^{k}	> 100	> 1,000	> 250
Saccharina japonica	3.11 ± 0.04^{m}	> 100	> 1,000	> 250
Sargassum macrocarpum	14.64 ± 0.23^{h}	> 100	> 1,000	> 250
Sargassum micracanthum	51.63 ± 0.43^{e}	> 100	> 1,000	> 250
Sporochnus radiciformis	8.53 ± 0.05^{j}	97.02 ± 1.95	> 1,000	> 250
Sargassum serratifolium	34.47 ± 0.20^{f}	87.58 ± 0.49	> 1,000	> 250
EGCG[1)] (mM)		0.17 ± 0.01	2.34 ± 0.07	0.25 ± 0.00

[1)] Positive control.
[a–m] Small letters presented differences between treatments in each column.
EGCG, epigallocatechin gallate; IC_{50}, half-maximal inhibitory concentration; PGE, phloroglucinol equivalent.

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lection, each sample (brown, green, and red) was immediately stored at –20℃ and then lyophilized at –40℃ using a vacuum freeze dryer (FDT-8650; Operon, Gimpo, Korea). The lyophilized samples were finely ground and subjected to three extractions using an ultrasonicator (WUC-N30H; Daihan Scientific, Seoul, Korea), with each extraction lasting for 1 hour using 70% ethanol. Following the extraction procedure, the samples were concentrated using a Büchi® Rotavapor® R-210 (Buchi, Flawil, Switzerland) at 50℃. Finally, the samples were dissolved in DMSO.

Quantification of the total phenolic content of the seaweed extracts

The total phenolic (TP) content of the samples was estimated using the Folin-Ciocalteu (FC) colorimetric method based on the procedure of Eom et al. (2011) using phloroglucinol as the standard phenolic compound. Diluted samples (0.1 mL) were mixed with 0.5 mL of 0.5 M FC reagent and 0.4 mL of 20% Na_2CO_3 in microcentrifuge tubes and allowed to stand for 3 min. After incubation at 22℃ for 45 min, the tubes were centrifuged (1,600×g, 8 min). The optical density (OD) of the supernatant was measured at 765 nm using a microplate reader (Synergy HTX Multi-Mode Microplate Reader; BioTek; Winooski, VT, USA). The phenolic compound contents were expressed as mg phloroglucinol equivalent (PGE).

Collagenase inhibition assay

The inhibition of collagenase was determined by a slightly modified method of Barrantes & Guinea (2003). First, 25 μL of 0.5 mM tricine buffer (with 10 mM $CaCl_2$ and 400 mM NaCl) was added to each well of the 96-well plate, 25 μL of a sample with various concentrations and 25 μL of collagenase (0.8 U/mL) were added, followed by incubation at 37℃ for 20 min. After 25 μL FALGPA (2 mM) was added, the change in OD at 345 nm was measured using a Synergy HTX Multi-Mode Microplate Reader (BioTek) at 37℃. EGCG was used as a positive control and 50 mM tricine buffer was used as a negative control. Collagenase inhibitory activity was calculated as follows.

$$\text{Inhibition of collagenase (\%)} = [(OD_{control} - OD_{sample}) / (OD_{control})] \times 100 \quad (1)$$

Elastase inhibition assay

The inhibition of elastase was determined by a slightly modified method of Landa-Cansigno et al. (2023). The method involves measuring p-nitroaniline produced by hydrolysis of *n*-succinyl-Ala-Ala-*p*-nitroanilide by elastase. First, 60 μL of Tris-HCl (0.2 M) was added to each well of a 96-well plate, followed by 10 μL of each sample with various concentrations, and 20 μL of 0.34 U/mL elastase from porcine pancreas, and incubated at 25℃ for 15 min. Subsequently, 10 μL of *n*-succinyl-Ala-Ala-Ala-*p*-nitroanilide was added and further incubated at 25℃ for 20 min. OD was measured at 410 nm using a Synergy HTX Multi-Mode Microplate Reader (BioTek). EGCG was used as a positive control and 0.2 M Tris-HCl was used as a negative control. Elastase inhibitory activity was calculated as follows.

$$\text{Inhibition of elastase (\%)} = [(OD_{control} - OD_{sample}) / (OD_{control})] \times 100 \quad (2)$$

Hyaluronidase inhibition assay

The inhibition of HAse was determined by a slightly modified method of Acıkara et al. (2019). The method involves colorimetric measurement of the amount of N-acetylglucosamine generated by the degradation of HA by HAse (Sahasrabudhe et al., 2010). First, HAse (7,900 U/mL) dissolved in 0.1 M acetate buffer (pH 3.6) was added to 100 μL, followed by 100 μL of the sample at varying concentrations, and incubated at 37℃ for 20 min. As a HAse activator, 100 μL of 12.5 mM calcium chloride was added to the mixture and further incubated at 37℃ for 20 min. Then, 500 μL of HA (1.2 mg/mL) was added and incubated under the same conditions for 40 min. To stop the reaction, 100 μL of NaOH (0.4 M) and 100 μL of potassium tetraborate (0.4 M) were added and incubated at 100℃ in a water bath for 3 min. After cooling, 3,000 μL of *p*-dimethylaminobenzaldehyde solution was added for color development and incubated at 37℃ for 20 min. The OD was measured at 585 nm using a Synergy HTX Multi-Mode Microplate Reader (BioTek). EGCG was used as a positive control and phosphate buffered saline was used as a negative control. Hyaluronidase inhibitory activity was calculated as follows.

$$\text{Inhibition of HAse (\%)} = [(OD_{control} - OD_{sample}) / (OD_{control})] \times 100 \quad (3)$$

Statistical analysis

Results are expressed as the mean ± SD values of triplicate. Three or more groups were statistically analyzed using Duncan's multiple-range test. $p < 0.05$ were considered statistically significant. The SPSS 26.0 software (SPSS, Chicago, IL, USA) was used

for data analysis.

Results and Discussion

The phenolic content of the seaweed extracts

Polyphenols are compounds found in seaweeds that exhibit various biological properties, such as antioxidant, anti-inflammatory, and anticancer effects (Nursid et al., 2020). Phlorotannins, marine-derived polyphenols in seaweeds, are oligomers of phloroglucinols that function as both primary and secondary metabolites (Santos et al., 2019). The TP content of seaweeds varies depending on the season, harvest time, geographical location, and species (Rajauria et al., 2016). The TP content of seaweed extracts was determined by constructing a standard curve using phloroglucinol. The TP content of brown seaweed ranged from 3.11 to 98.65 mg PGE/g (Table 1). The TP content of *Ecklonia cava* was the highest at 98.65 mg PGE/g, followed by *Eisenia bicyclis* (96.81 mg PGE/g) and *Sargassum coreanum* (96.73 mg PGE/g). The TP content of green seaweed ranged from 1.29 to 39.95 mg PGE/g (Table 2). The TP content of *C. wrightiana* var. *minor* was the highest at 39.95 mg PGE/g, followed by *Chaetomorpha moniligera* (29.59 mg PGE/g) and *Ulothrix flacca* (22.60 mg PGE/g). The TP content of red seaweed ranged from 4.93 to 39.58 mg PGE/g (Table 3). The TP content of *Gloiopeltis furcata* was the highest at 39.58 mg PGE/g, followed by *C. moniligera* (29.59 mg PGE/g) and *U. flacca* (22.60 mg PGE/g). Brown seaweed extracts showed higher TP content than green and red seaweed extracts. In particular, *E. cava* (98.65 mg PGE/g) had the highest TP content among all seaweed extracts. In another study comparing the TP content of Korean seaweed species, *E. cava* was found to have the highest TP content (Lee et al., 2021). The current findings of this study, using extracts from Korean seaweeds, reveal that marine-derived polyphenolics are present in the seaweed. This finding is consistent with the results of other researchers studying the same species of seaweed (Lee et al., 2021). It has been reported that brown seaweeds have the highest levels of tannins, with concentrations varying significantly among different species. Therefore, brown seaweeds, which have the highest TP content, are considered to have several beneficial biological effects.

Collagenase inhibitory effects of the seaweed extracts

Degradation of collagen by matrix metalloproteinase-1 is associated with skin aging (Chung et al., 2000). In this study, we evaluated the antiaging effects of collagenase by assessing its inhibitory effects. Among the brown seaweeds, *S. serratifoli-*

Table 2. Collagenase, elastase, and hyaluronidase inhibitory effects of green seaweed extracts

Green seaweeds	Total phenolic contents (PGE mg/g)	IC_{50} values for collagenase inhibition (μg/mL)	IC_{50} values for elastase inhibition (μg/mL)	IC_{50} values for hyaluronidase inhibition (μg/mL)
Chaetomorpha moniligera	29.59 ± 0.52[b]	> 100	> 1,000	> 250
Cladophora albida	9.12 ± 0.15[e]	> 100	> 1,000	> 250
Cladophora wrightiana var. *minor*	39.95 ± 0.50[a]	77.14 ± 0.31	> 1,000	93.24 ± 2.18
Codium fragile	3.12 ± 0.10[j]	> 100	> 1,000	> 250
Codium subtubulosum Okamura	1.29 ± 0.00[l]	> 100	> 1,000	> 250
Codium tenuifolium	2.50 ± 0.14[k]	> 100	> 1,000	> 250
Ulothrix flacca	22.60 ± 0.69[c]	> 100	> 1,000	> 250
Ulva australis	15.95 ± 0.42[d]	> 100	> 1,000	> 250
Ulva compressa	6.43 ± 0.18[h]	> 100	> 1,000	> 250
Ulva intestinalis	4.07 ± 0.02[i]	> 100	> 1,000	> 250
Ulva linza	7.47 ± 0.17[g]	> 100	> 1,000	> 250
Ulva ohnoi	8.57 ± 0.18[f]	> 100	> 1,000	> 250
Ulva prolifera	9.34 ± 0.25[e]	> 100	> 1,000	> 250
Umbraulva japonica	3.39 ± 0.07[j]	> 100	> 1,000	> 250
EGCG[1)] (mM)		0.17 ± 0.01	2.34 ± 0.07	0.25 ± 0.00

[1)] Positive control.
[a–m] Small letters presented differences between treatments in each column.
EGCG, epigallocatechin gallate; IC_{50}, half-maximal inhibitory concentration; PGE, phloroglucinol equivalent.

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Table 3. Collagenase, elastase, and hyaluronidase inhibitory effects of red seaweed extracts

Red seaweeds	Total phenolic contents (PGE mg/g)	IC_{50} values for collagenase inhibition (μg/mL)	IC_{50} values for elastase inhibition (μg/mL)	IC_{50} values for hyaluronidase inhibition (μg/mL)
Ceramium boydenii	15.86 ± 0.55[f]	> 100	> 1,000	> 250
Ceramium kondoi Yendo	25.73 ± 0.79[c]	> 100	> 1,000	> 250
Chondracanthus tenellus	30.59 ± 0.66[b]	> 100	> 1,000	> 250
Chondrus ocellatus	11.02 ± 0.23[h]	> 100	> 1,000	> 250
Fushitsunagia catenata	14.67 ± 0.42[g]	> 100	> 1,000	> 250
Gracilaria textorii	8.77 ± 0.08[j]	> 100	> 1,000	> 250
Gracilaria vermiculophylla	15.49 ± 0.53[f]	> 100	> 1,000	> 250
Grateloupia angusta	19.10 ± 0.15[e]	> 100	> 1,000	> 250
Grateloupia elliptica	10.09 ± 0.06[i]	> 100	> 1,000	> 250
Grateloupia turuturu	9.07 ± 0.14[j]	> 100	> 1,000	> 250
Gloiopeltis furcata	39.58 ± 0.34[a]	> 100	> 1,000	> 250
Gymnogongrus flabelliformis	21.44 ± 0.31[d]	> 100	> 1,000	> 250
Meristotheca papulosa	4.93 ± 0.08[l]	> 100	> 1,000	> 250
Plocamium telfairiae	6.42 ± 0.02[k]	> 100	> 1,000	> 250
EGCG[1)] (mM)		0.17 ± 0.01	2.34 ± 0.07	0.25 ± 0.00

[1)] Positive control.
[a–m] Small letters presented differences between treatments in each column.
EGCG, epigallocatechin gallate; IC_{50}, half-maximal inhibitory concentration; PGE, phloroglucinol equivalent.

um exhibited the highest collagenase inhibitory effect with a half-maximal inhibitory concentration (IC_{50}) of 87.58 μg/mL, followed by *E. cava* (95.08 μg/mL) and *Sporochnus radiciformis* (97.02 μg/mL) (Table 1). Among green seaweeds, *C. wrightiana* var. *minor* showed the highest collagenase inhibitory effect with an IC_{50} value of 77.14 μg/mL, while the other seaweeds did not inhibit collagenase activity (IC_{50} > 100 μg/mL) (Table 2). The red seaweed extracts did not exhibit notable collagenase inhibition (IC_{50} > 100 μg/mL) (Table 3). In our study, IC_{50} values of *S. micracanthum* were not measured above 100 μg/mL, but a report by Pak et al. (2016) found an IC_{50} value of 488.20 μg/mL. When brown algae are found to be more effective at inhibiting collagenase than green and red algae, it is because of their higher polyphenol content. The phenolics found in brown seaweeds showed moderate to significant inhibition of collagenase (Choi et al., 2012).

Elastase inhibitory effects of the seaweed extracts

Elastase degrades elastin and maintains skin elasticity (Shin et al., 1999). This study evaluated the antiaging effect of elastase inhibition in preventing wrinkle formation. Among the brown seaweeds, *P. gymnospora* exhibited the highest elastase inhibitory effect with an IC_{50} of 260.05 μg/mL, followed by *S. coreanum* (388.65 μg/mL), and *E. cava* (465.13 μg/mL) (Table 1). Green and red seaweed extracts did not inhibit the elastase activity (Tables 2 and 3). Brown seaweed has also been found to effectively inhibit elastase. According to Cho & Choi (2010), water extract from *E. cava* showed was reported 44% inhibition at a concentration of 1 mg/mL. Our study demonstrates an even better elastase inhibition effect of *E. cava* with an IC_{50} of 465.13 μg/mL. Furthermore, it has been confirmed that polyphenols are primarily extracted through acidified organic solvent extraction. Bu et al. (2006) have shown that these polyphenols act as enzyme coordination complexes, which results in an inhibitory effect.

Hyaluronidase inhibitory effects of the seaweed extracts

Degradation of HA by HAse leads to reduced skin elasticity and wrinkle formation, resulting in skin aging (Kim et al., 2005). In this study, we analyzed the antiaging activity of HAse by confirming its inhibitory effects. Among the 14 brown seaweeds, *E. bicyclis* exhibited the highest HAse inhibitory effect with an IC_{50} 212.88 μg/mL, followed by *E. cava* (219.16 μg/mL), *S. coreanum* (225.17 μg/mL), and *P. gymnospora* (246.45 μg/mL) (Table 1). Among green seaweeds, *C. wrightiana* var. *minor* exhibited the strongest HAse inhibitory effect, with an IC_{50} value of 93.24 μg/

mL (Table 2). The red seaweeds extracts did not exhibit notable HAse inhibition (IC_{50} > 250 μg/mL) (Table 3). Arunkumar et al. (2021) reported that brown seaweed (*S. tenerrimum*, *S. vulgare*, and *E. arborea*) exhibited the highest HAse inhibitory and reported that phlorotannin was associated with a higher level of anti-HAse effects. Phlorotannins effectively inhibit HAse by forming enzyme-inhibitor complexes (Bu et al., 2006). The content of polyphenols is likely to have an association with the inhibition of HAse.

In conclusion, this study evaluated the antiaging activity of edible Korean seaweed extracts. Inhibitory effects on collagenase, elastase, and HAse were analyzed. Brown seaweeds exhibited significantly higher collagenase inhibitory activity than other seaweeds. The extract of *S. serratifolium* showed high inhibitory activity with an IC_{50} value of 87.58 μg/mL, followed by *E. cava* (95.08 μg/mL) and *S. radiciformis* (97.02 μg/mL). In elastase inhibition, only brown seaweeds showed an effect, with *P. gymnospora* showing the highest inhibitory activity with an IC_{50} value of 260.05 μg/mL, followed by *S. coreanum* (388.65 μg/mL) and *E. cava* (465.13 μg/mL). Since *Padina* sp. has been reported to contain high levels of flavonoids and phenolics, the differences in their chemical compositions and structures in phenolic constituents, along with other molecules such as carbohydrates, terpenes, and inorganic compounds, could be attributed to these variations (Nazarudin et al., 2022). In HAse inhibition, brown seaweeds demonstrated high inhibitory activity, with the *E. bicyclis* extract showing the highest effect with an IC_{50} value of 212.88 μg/mL, followed by *E. cava* (219.16 μg/mL), *S. coreanum* (225.17 μg/mL), and *P. gymnospora* (246.45 μg/mL). Red seaweed extracts did not exhibit significant inhibitory activity against these enzymes. Upon evaluating the inhibitory activity of the 42 seaweed extracts on antiaging enzymes, brown seaweed showed high inhibitory activity against all enzymes. However, few studies have investigated the inhibitory effects of collagenase, elastase, and HAse on various seaweeds. Overall, these findings highlight their potential as foundational data for antiaging treatments. Among these, *E. cave* exhibited strong inhibitory activity against all three enzyme such as collagenase, elastase, and HAse, suggesting its potential use as a comprehensive antiaging agent.

Competing interests
No potential conflict of interest relevant to this article was reported.

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Availability of data and materials
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate
Not applicable.

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