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Tyrosinase inhibition effects of Korean edible brown, green, and red seaweed extracts

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Abstract

The tyrosinase inhibition effects of 23 marine-derived seaweeds harvested in Korea were screened to determine their potential as skin-whitening agents. Of the 23 species initially screened, the total phenolic (TP) content of brown, green, and red seaweeds were 7.62–280.11, 5.24–62.37, and 0.63–28.76 phloroglucinol equivalents (PGE) mg/g, respectively. Brown seaweed extracts exhibited much stronger inhibitory activities than green and red seaweed extracts. Among the brown seaweeds, *Ecklonia cava* had the highest TP content (280.11 PGE mg/g) and the strongest tyrosinase inhibitory effect with a half maximal inhibitory concentration (IC_{50}) value of 4.38 μg/mL. The kinetics of tyrosinase inhibition, analyzed by Lineweaver–Burk plots, found *E. cava* extract to be a non-competitive inhibitor. This study's results indicated that *E. cava*'s inhibition of tyrosinase may have potential applications in the cosmetic industry.

Keywords: Brown seaweeds, Green seaweeds, Red seaweeds, Tyrosinase, Skin-whitening

Introduction

Globally, there are approximately 2,000 species of marine-derived seaweeds (Periaswamy Sivagnanam et al., 2015); these macroalgae are classified as blue-green, brown, green, and red algae (Widyaswari et al., 2021). About 900 seaweed species have been recorded in Korea, including 123, 193, and 592 green, brown, and red seaweed species, respectively (Kim et al., 2013). Some of these seaweeds have been used extensively as food and medicine (Mabeau & Fleurence, 1993). Seaweeds possess a diversity of components, including proteins, lipids, and fatty acids (Pirian et al., 2018). Apart from these major components, seaweeds produce various secondary metabolites, including polyphenols, peptides, sterols, and a range of other bioactive substances (Wang et al., 2018). In particular, seaweeds from Korea are recognized to have various biological activities (Ryu et al., 2023). However, there is limited information available about the skin-whitening effects of Korean seaweed extracts (Park et al., 2021).

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Melanin is a pigment that plays a role in skin, hair, and eye color and provides protection against ultraviolet (UV) radiation's harmful effects (Costin & Hearing, 2007). Melanocytes, found in the basal layer of the epidermis, are the cells responsible for melanin synthesis (Fitzpatrick & Breathnach, 1963). Melanocytes synthesize numerous secretory melanosomes containing melanin (Kondo & Hearing, 2011). The mature melanosomes in the melanocytic dendrites are subsequently transferred to the adjacent keratinocytes (Beaumont et al., 2011). Therefore, the skin's final tone is influenced by the amount and type of melanin pigment transferred to the keratinocytes (Wu et al., 2018).

Under standard conditions, melanin synthesis is vital for the protection of deoxyribonucleic acid (DNA) and the skin against UV radiation damage (Ko & Lee, 2021). However, the overproduction of melanin can contribute to the development of skin conditions, such as melasma, freckles, age spots, hyperpigmentation, and lentigo (Rao et al., 2013). These skin conditions can be controlled or inhibited by tyrosinase, a key enzyme in melanin production from tyrosine. Tyrosinase catalyzes the oxidation of L-tyrosine to 3,4-dihydroxyphenylalanine, and then to dopaquinone (Costin & Hearing, 2007). Ingredients known for promoting skin whitening, such as arbutin, azelaic acid, and kojic acid, have been reported to cause side effects, including cytotoxicity, skin cancer, and dermatitis, in long-term use (Huang et al., 2016). Therefore, the development of safe and effective depigmenting agents using Korean seaweeds is of interest. Consequently, this study analyzed the inhibitory effects of tyrosinase in Korean seaweed extracts on the production of melanin.

Materials and Methods

Chemicals

Phloroglucinol, tyrosinase from mushroom (EC 1.14.18.1), L-tyrosine, and Folin–Ciocalteu (FC) reagent were purchased from Merck KGaA (Darmstadt, Germany). The dimethylsulfoxide (DMSO) and sodium carbonate (Na_2CO_3) were purchased from the Junsei Chemicals (Tokyo, Japan). The kojic acid was purchased from the Tokyo Chemical Industry (TCI, Tokyo, Japan). All reagents and solvents used in this study were of analytical grade.

Sample preparation

Twenty-three seaweeds were selected for this study are shown in Table 1. The seaweed samples were obtained from the Marine Biodiversity Institute of Korea (Seocheon, Korea). Each of the provided seaweed samples (brown, green, and red) was stored at −20℃ immediately after collection and lyophilized at −40℃ with a vacuum freeze dryer (FDT-8650; Operon, Gimpo, Korea). The lyophilized samples were finely ground and extracted three times using an ultrasonicator (WUC-N30H; Daihan Scientific, Seoul, Korea); each extraction was for 1 hr with 70% ethanol. After the extraction step, the samples were concentrated using a Büchi® Rotavapor® R-210 (Merck KGaA) at 50℃. Finally, samples were dissolved in DMSO and stored at −70℃ until used.

Quantification of the total phenolic content of the seaweed extracts

The total phenolic (TP) contents of brown, green, and red seaweed extracts were determined by the modified FC method (Eom et al., 2011). Phloroglucinol was used as the standard. An aliquot (0.1 mL) of diluted sample was mixed with 0.5 mL of 0.5 M FC reagent in a microcentrifuge tube. After the addition of 0.4 mL of 20% Na_2CO_3, the mixture stood undisturbed for 3 min. The samples were then incubated at room temperature in the dark for 45 min and subsequently subjected to centrifugation (1,600×g, 8 min). The optical density (OD) of the supernatants was measured at 765 nm using a microplate reader (BioTek Synergy HTX Multi-Mode Reader; Agilent Technologies, Santa Clara, CA, USA). The concentration of TP contents was expressed as mg phloroglucinol equivalent (PGE).

Tyrosinase inhibition assay

The inhibition of tyrosinase was determined using the method of No et al. (1999), with slight modifications. First, 20 μL of tyrosinase and 10 μL of each seaweed extract (brown, green, and red) with different concentrations (ranging between 1–500 μg/mL) were incubated at 37.5℃ for 30 min. Then the assay mixture (170 μL) containing a 10:10:9 ratio of 1 mM L-tyrosine solution, 50 mM potassium phosphate buffer (pH 6.5), and distilled water was added. After incubation at 37℃ for 30 min, the OD was measured at 490 nm with the BioTek Synergy HTX Multi-Mode Microplate Reader. The tyrosinase inhibition activities were expressed as half-maximal inhibitory concentration (IC_{50}) values. The percentage inhibition of tyrosinase activity was calculated using the following equation:

$$\text{Relative inhibition (\%)} = [1 - (\text{Sample} - \text{Sample blank}) / (\text{Control} - \text{Blank})] \times 100$$

Table 1. Tyrosinase inhibitory effects of brown, green, and red seaweeds

	Scientific name	Total phenolic contents (PGE mg/g)	IC_{50} values for tyrosinase inhibition
Brown seaweeds	*Dictyopteris divaricata*	30.11 ± 1.72[d]	> 500 μg/mL
	Ecklonia cava	280.11 ± 9.93[a]	4.38 ± 0.08 μg/mL
	Eisenia bicyclis	72.94 ± 0.14 [b]	4.46 ± 0.52 μg/mL
	Ishige okamurae	76.84 ± 2.35[b]	8.97 ± 0.91 μg/mL
	Myagropsis myagroides	7.62 ± 0.77[g]	> 500 μg/mL
	Padina arborescens	8.81 ± 0.50[g]	> 500 μg/mL
	Padina crassa	16.60 ± 0.99[f]	> 500 μg/mL
	Sargassum horneri	39.49 ± 2.41[c]	6.20 ± 0.22 μg/mL
	Sargassum miyabei Yendo	42.66 ± 2.03[c]	> 500 μg/mL
	Sargassum serratifolium	25.34 ± 0.86[d,e]	> 500 μg/mL
	Sargassum thunbergii	20.73 ± 1.76[e,f]	> 500 μg/mL
	Sargassum *yendoi* Okamura & Yamada	30.51 ± 1.81[d]	7.15 ± 0.96 μg/mL
Green seaweeds	*Cladophora japonica*	5.24 ± 0.50[c]	> 500 μg/mL
	Cladophora wrightiana var. *minor*	62.37 ± 2.08[a]	> 500 μg/mL
	Codium fragile	56.65 ± 2.16[b]	> 500 μg/mL
	Salicornia europaea L.	ND [1)]	> 500 μg/mL
Red seaweeds	*Asparagopsis taxiformis*	11.36 ± 0.60[b]	> 500 μg/mL
	Champia parvula	ND[1)]	> 500 μg/mL
	Chondracanthus tenellus	5.40 ± 0.77[c]	> 500 μg/mL
	Gracilaria textorii	ND[1)]	> 500 μg/mL
	Grateloupia elliptica	28.76 ± 0.96[a]	281.77±3.49 μg/mL
	Meristotheca papulosa	ND[1)]	> 500 μg/mL
	Plocamium telfairiae	0.63 ± 0.56[d]	> 500 μg/mL
Kojic acid [2)]			58.75 ± 1.85 μM

[1)] ND, not detect.
[2)] Positive control.
[a–f] Values with different letters differ significantly.

The IC_{50} values were determined using linear regression analysis of the activity observed under the assay conditions. All analyses were performed in triplicate. Kojic acid was used as the positive control.

Kinetic analysis of the tyrosinase inhibition assay

The previously described reaction conditions were employed to generate a Lineweaver–Burk plot for the seaweed extracts' enzyme reactions at various concentrations. The identification of inhibition types was characterized by plotting the slope versus the inverse of the substrate concentration.

Statistical analysis

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

Results and Discussion

Total phenolic content of the seaweed extracts

Polyphenols exhibit various biological properties to different extents, including anticancer, antioxidant, and anti-inflammatory effects. The polyphenols of marine algae, called phlorotannins, are synthesized and produced by the polymerization of phloroglucinol (Hakim & Patel, 2020). To evaluate the biological properties of Korean seaweed extracts, their TP contents were determined. The TP contents of the brown seaweed extracts are shown in Table 1. The TP contents of the 12 brown seaweed

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extracts were between 7.62 and 280.11 mg PGE/g (dry weight); among these, the TP content of *Ecklonia cava* was the highest at 280.11 mg PGE/g.

The TP contents of the four green seaweed extracts included in the study ranged between 5.24 and 62.37 mg PGE/g (Table 1). The TP content of *Cladophora wrightiana* var. *minor* was the highest at 62.37 mg PGE/g, followed by *Codium fragile* (56.65 mg PGE/g) and *Cladophora japonica* (5.24 mg PGE/g). The TP content of the *Salicornia europaea* L. extract was below the limit of detection.

The TP content of the seven red seaweed extracts included in the study ranged between 0.63 and 28.76 mg PGE/g (Table 1). The highest TP content was observed in *Grateloupia elliptica* (28.76 mg PGE/g), followed by *Asparagopsis taxiformis* (11.3 mg PGE/g), *Chondracanthus tenellus* (5.40 mg PGE/g), and *Plocamium telfairiae* (0.63 mg PGE/g). However, the TP content was below the limit of detection for the *Champia parvula*, *Gracilaria textorii*, and *Meristotheca papulose* extracts. Overall, the brown seaweed extracts exhibited the highest TP contents compared with the green and red seaweed extracts.

Tyrosinase inhibitory effects of the seaweed extracts

To determine the skin-whitening properties of Korean seaweeds *in vitro*, the tyrosinase inhibition activity of several seaweeds was subjected to comparative analysis. The 70% ethanol extracts of brown, green, and red seaweeds were evaluated for tyrosinase inhibitory effects; the commercial inhibitor kojic acid was included as the control (Table 1). In the 12 brown seaweeds included in the study, *E. cava* had the highest tyrosinase inhibitory effect with a IC_{50} of 4.38 μg/mL, followed by *Eisenia bicyclis* (4.46 μg/mL), *Sargassum horneri* (6.20 μg/mL), *Sargassum yendoi* Okamura & Yamada (7.15 μg/mL), and *Ishige okamurae* (8.97 μg/mL). In comparison, Kim et al. (2005) reported that *E. cava* exhibited an IC_{50} of 13.3 μg/mL. Shim & Yoon (2018) reported that the tyrosinase inhibition activity of an *E. bicyclis* extract had an IC_{50} of 499.0 ± 6.6 μg/mL. Therefore, the tyrosinase inhibition activity observed in this study was substantially higher than previously reported values.

The four green seaweed extracts screened, namely, *C. japonica*, *C. wrightiana* var. *minor*, *C. fragile*, and *S. europaea* L., did not inhibit tyrosinase activity (IC_{50} > 500 μg/mL). Table 1 shows that the tyrosinase inhibition effect of the green seaweed extracts was relatively lower compared with that of the brown and red seaweed extracts. Therefore, further studies are suggested to identify green seaweeds with IC_{50} values of over 500 μg/mL.

Table 1 shows that, among red seaweeds, *G. elliptica* had a higher tyrosinase inhibitory function (IC_{50} = 281.77 μg/mL) compared with *A. taxiformis*, *C. parvula*, *C. tenellus*, *G. textorii*, *Meristotheca papulosa*, and *P. telfairiae*, which all had lower inhibition against tyrosinase (IC_{50} > 500 μg/mL). Therefore, there is a need to investigate additional concentrations to identify those with an IC_{50} of over 500 μg/mL.

Various studies have reported that polyphenolic compounds, which are principal bioactive constituents, demonstrate compelling functional and bioactive attributes. For example, recent studies have identified that seaweed polyphenols have significant benefits, such as anti-viral, anti-bacterial, anti-cancer, anti-diabetes, and anti-inflammatory properties, and are associated with the reduced risk of several diseases (Gómez-Guzmán et al., 2018). Table 1 shows that the TP contents of the seaweed extracts determined in this study were proportionally correlated with their inhibitory effects on tyrosinase. Previous studies have shown that the number and positioning of phenolic hydroxyl groups in polyphenols play a major role in influencing the inhibition of tyrosinase activity (Panzella & Napolitano, 2019). However, the existence of a hydroxyl and electron-donating group within the phenol ring of polyphenols can inhibit tyrosinase activity as a tyrosinase substrate. Therefore, the increased tyrosinase inhibitory effects evident in the brown seaweed extracts, especially *E. cava*, *S. horneri*, *S. yendoi* Okamura & Yamada, and *I. okamurae*, are associated with their higher polyphenol contents.

Tyrosinase inhibition pattern of the *Ecklonia cava* extracts

A Lineweaver–Burk plot analysis of the *E. cava* extract, which demonstrated the strongest tyrosinase inhibition among the seaweeds screened, was carried out to determine the type of inhibition. Fig. 1 shows that the *E. cava* and *E. bicyclis* extracts were non-competitive inhibitors; this implies that the extracts' tyrosinase binds at tyrosinase protein sites other than active sites and does not interact with the substrate at the active site decreasing the enzyme's efficacy (Blat, 2010). In addition, Lee et al. (2015) have reported that eckol, a polyphenol from *E. cava*, is a non-competitive tyrosinase inhibitor.

Conclusion

Twenty-three seaweed extracts' tyrosinase inhibition activity and IC_{50} values were determined in the present study. Among the brown seaweeds, *E. cava* extract had the most inhibitory ef-

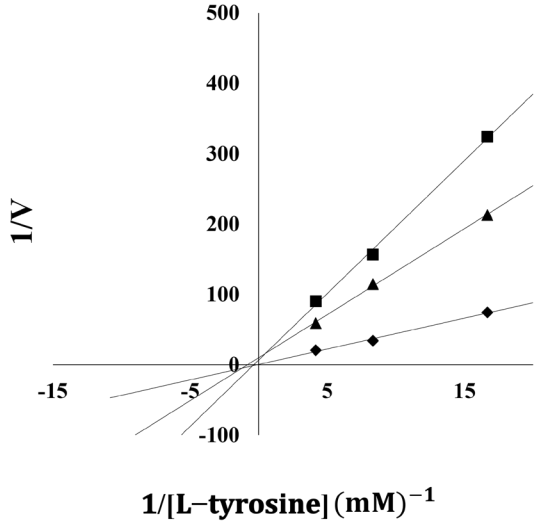

Fig. 1. Lineweaver–Burk plots of tyrosinase activity in the presence of *Ecklonia cava* extracts (◆, control; ▲, 4 μg/mL; ■, 5 μg/mL) using L-tyrosine as enzyme substrate. Each value is presented as the mean of three independent experiments.

fect on mushroom tyrosinase and was identified as a non-competitive inhibitor in the kinetic study. Furthermore, *E. bicyclis* (4.46 μg/mL), *S. horneri* (6.20 μg/mL), *S. yendoi* Okamura & Yamada (7.15 μg/mL), and *I. okamurae* (8.97 μg/mL) had higher tyrosinase inhibitory activities compared with the other brown seaweed extracts analyzed. Among the red seaweed extracts, only *G. elliptica* exhibited a tyrosinase inhibitory effect (IC_{50} = 281.77 μg/mL). The green seaweed extracts did not exhibit any notable tyrosinase inhibition. The *E. cava* extract appeared to have a complex composition that warrants further analysis to identify and purify the main components.

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
This article does not require IRB/IACUC approval because there are no human and animal participants.

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