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Effects of seaweed *Laminaria japonica* extracts on skin moisturizing activity *in vivo*

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Synopsis

Twelve species of edible seaweed from the coast of Korea were screened for skin moisturizing activity. We placed the lead of a Corneometer on an approximately 6-cm^2 test area of the forearm and measured both untreated skin (control) and skin treated with test moisturizing creams either containing or not containing 5% water:propylene glycol (50:50) extracts of seaweeds. Over the 8-h observation period, the strongest activity of the *Laminaria japonica* extracts occurred at the 2-h period. For the 10% extract, hydration with the *L. japonica* extract increased by 14.44% compared with a placebo. Transepidermal water loss (TEWL) was also measured using a test cream with 10% *L. japonica* extract. For up to 8 h after applying the creams, TEWL was decreased to 4.01 g/cm^2, which was approximately 20% of that seen with the control. We suggest that the *L. japonica* extract hydrates skin via the humectants and hydrocolloids that it contains. To confirm the safety of *L. japonica* extracts, we performed a patch test on human skin. The results suggested that at moderate doses humans can safely use the extracts. For commercial applications, we evaluated the physicochemical characteristics of the test cream products, including Hunter *L*, *a*, and *b* values; pH; refractive index; and coefficient of viscosity. *L. japonica* extract did not affect overall formulations of the test cream product in any of the tested aspects. These results suggest that *L. japonica* extract is a promising ingredient in moisturizing formulations.

INTRODUCTION

Skin is the most exposed organ of the human body and acts as the first line of defense against external insults. The appearance and function of skin are maintained by an important

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balance between the water content of the stratum corneum and skin surface. At least 10% water is needed in the skin to keep it soft and flexible. When the water content falls below this level, keratin becomes progressively less flexible. A variation in water content of only 1% can significantly change its elasticity and permeability (1,2).

Moisturizer prevents the skin from drying and chapping, thus slowing the aging process. A wide variety of moisturizing components are available in different formulations, such as vegetable glycerin, rose water, jojoba oil, vitamin E oils, and sorbitol. However, there remains a demand for a highly effective moisturizer with an excellent safety and efficacy profile. Thus, for many years, there have been numerous attempts to develop new agents capable of preventing and/or treating dry skin (2–4).

Interest in marine organisms as potential and promising sources of pharmaceutical agents has increased in recent years (5,6). Seaweeds can act as a source of bioactive compounds and are able to produce a great variety of secondary metabolites with different activities. Several cosmetic ingredients from seaweeds were recently shown to have whitening (7), antiaging (8), and antiwrinkle effects (3), and can protect against ultraviolet light (9).

However, few studies have measured the moisturizing effects of seaweed extracts (10). In addition, there has been no report on the skin moisturizing activity of indigenous seaweed species in Korea. Therefore, to develop a new moisturizer, we measured the moisturizing effects of Korean indigenous seaweed extracts using a Corneometer and a Tewameter. To confirm the safety of the extract, we conducted a patch test for human skin. For possible commercial application, we also measured the physiochemical characteristics of the test cream products such as Hunter *L*, *a*, and *b* values; pH; refractive index; and coefficient of viscosity.

MATERIALS AND METHODS

SEAWEED EXTRACTS

Seaweed thalli were collected from the coast of Korea from November 2005 to April 2006. Twelve edible seaweed species (four of Chlorophyta, six of Phaeophyta, and two of Rhodophyta) were used in this study. Seaweed tissues were dried for 1 day at room temperature using an electric fan and then ground into powder using a coffee grinder for 10 min. For the water/propylene glycol extraction, we used the methods described by Mekideche and Briand (10). To each 5 g of powder, 100 mL of 50% water/50% propylene glycol was added at room temperature for 1 day to extract the water/propylene glycol-soluble components. This procedure was repeated two times, and the combined extracts were stored at −20°C until use.

TEST CREAM WITH SEAWEED EXTRACT

To determine *in vivo* skin moisturizing activity, test creams were made with the 12 seaweed extracts. The test creams were kindly supplied by Silla B&H Co., Ltd. (Busan, Korea). The formulations studied (Table I) were prepared in a PRIMIX RM homomixer

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(PRIMIX Co., Ltd., Osaka, Japan) at 3000 rpm within 10 min and supplemented with different concentrations (%) (W/W) of seaweed extract. A placebo formulation was prepared without seaweed extract. The proximate compositions, free amino acid compositions and mineral contents of the selected seaweed water/propylene glycol extracts were also measured following the Korea Food and Drug Administration guidelines (KFDA) (11).

MEASUREMENT OF SKIN MOISTURIZING ACTIVITY USING A CORNEOMETER

We measured the skin moisturizing effects of the extracts using a Corneometer based on the methods of Dal'Belo *et al.* (4). The water content of the stratum corneum was measured with a skin capacitance meter (Corneometer CM 825; Courage & Khazaka Electronic GmbH, Cologne, Germany). This device determines the water content of the superficial epidermal layers down to a depth of approximately 0.1 mm and expresses the values in arbitrary units. Therefore, the moisture level of the treated skin was expressed as increased hydration (%) compared with untreated skin (10).

Ten women (median age, 27 years; range, 24–31 years) participated in the study after providing informed consent. Biophysical measurements were made on the inner forearm. We placed a Corneometer lead on a test area of approximately 6 cm^2 on the forearm. The dose in each case was 8 $\mu l/cm^2$. All data were obtained on the same day from each individual, and the average values of 10 measurements per site were used in subsequent calculations. Prior to all measurements, each subject washed her forearm with a liquid hand wash and then

Table I.
Formulation of the Test Cream Containing Seaweed Extract

INCI	Percentage of components (W/W)
Water (aqua), demineralized	as 100%
Seaweed extract	0–15.00
Disodium EDTA	0.10
Butylene glycol	6.00
Methylparaben	0.20
Triethanolamine	0.25
Sorbitan stearate, sucrose cocoate	4.50
Sorbitan sesquioleate	0.50
Polysorbate 60	0.50
Stearic acid	1.50
Cetearyl alcohol	1.50
Cetyl ethylhexanoate	8.00
Captylic/capric triglyceride	2.00
Gyclomethicone	4.00
Limnanthes alba seed oil	2.00
Dimethicone	0.50
Tocopheryl acetate	0.20
Propylparaben	0.10
Carbomer	0.25

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allowed at least 30 min for full skin acclimation to room temperature (20°C ± 2°C) and humidity (45%–60%) (12).

First, to screen for potential skin moisturizing activity, test creams with 5% of each of the 12 seaweed extracts were used for treatment. At 2 h after application of the creams, the increased hydration status of the treated skin compared with that of the untreated skin was measured, and the seaweed that exhibited the strongest moisturizing activity was selected. To find the best concentration, the moisturizing activity of the selected seaweed that showed the highest activity was determined at 0%, 1%, 3%, 5%, 7%, 10%, and 15% concentrations. At 2 h after application of the creams, the increased hydration compared with untreated skin was measured. To confirm the hydration-lasting effect of the test cream containing 10% of the selected seaweed extract, we measured skin moisturization at 0, 3, 5, 10, 15, and 30 min, and 1, 1.5, 2, 4, 6, and 8 h after application of the creams.

TEWL MEASUREMENTS USING THE TEWAMETER

To assess skin barrier function, the transepidermal water loss (TEWL) of the test cream with the selected seaweed extract was measured with an evaporimeter (Tewameter TM210; Courage & Khazaka Electronic GmbH, Cologne, Germany). TEWL measurements were performed according to the relevant guidelines (13,14), and the instrument was registered in g/m^2h for 2 min after probe equilibration on the skin for 30 s. To determine the best concentration, the TEWL of the selected seaweed was determined at 0%, 1%, 3%, 5%, 7%, 10%, and 15% concentrations 2 h after application of the creams. To confirm the hydration-lasting effect of the test cream containing 10% of the selected seaweed extract, we measured TEWL at 0, 3, 5, 10, 15, and 30 min, and 1, 1.5, 2, 4, 6, and 8 h after application of the creams.

PATCH TEST

The patch test procedure was conducted as previously described (15) to confirm safety of the extracts for human skin. The patch test was performed using the test cream containing 10% *L. japonica* extract on 25 human volunteers, of whom 20 were female and 5 were male. Subject age varied from 22 to 28 years, and the average age was 25.5 years. The quantity of test cream applied per test patch was 20 µl (20 mg for solid test materials). The test articles were dispensed onto Finn Chambers (Epitest, Ltd., Tuusula, Finland) on Scanpor tape (Alpharma, Oslo, Norway), and the patch was applied to normal skin on the forearm. The patch was removed up to 48 h after patch application. The treatment sites were assessed for the presence of irritation using a 5-point scale 6 h after patch removal. The degree of irritation was evaluated by visual scoring according to the following scale with grading defined as: 0 = no reaction; 0.5 = barely perceptible, very weak spotty erythema; 1 = slight erythema, either spotty or diffuse; 2 = moderate erythema; and 3 = intense erythema, infiltration, and possible vesicles (16).

PHYSIOCHEMICAL CHARACTERISTICS OF THE TEST CREAM PRODUCTS

For possible future application in moisturizing agents and cosmetic products for dry skin, the physiochemical characteristics of the test cream products containing 10% *L. japonica*

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were measured, including Hunter *L*, *a*, and *b* values; pH; refractive index; and coefficient of viscosity. Test cream color was measured by a color difference meter (Spectrophotometer CM-700d; Konica Minolta Sensing, Inc., Tokyo, Japan). The numerical value of the color was expressed by Hunter *L*, *a*, and *b* values. The Hunter values were monitored by a computerized system using SpectraMagic software (version 2.11; Minolta Cyberchrom Inc., Osaka, Japan). The overall color difference (ΔE) was calculated using the equation $\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{1/2}$. According to the Cosmetic Standard and Experimental Method (No. 2007-45) by the KFDA (17), after 10-fold dilution with distilled water, the pH of the cream was measured at 25°C using the pH meter SevenEasy pH S20K (Mettler-Toledo GmbH, Greifensee, Switzerland). The refractive index of the cream was measured at 25°C using an ABBE Refractometer DR-A1 (ATAGO Co., Ltd., Tokyo, Japan). The viscosity of the cream was measured in spindle No. SC4-25 at 1 rpm and 25°C using a Brookfield viscosity meter (Brookfield programmable DV-III+RHEOMETER, Stoughton, MA).

STATISTICAL ANALYSES

All experiments were performed at least three times independently. Differences between groups were calculated using a Student's *t*-test. Results were deemed statistically significant at $p < 0.05$.

RESULTS

SCREENING OF SKIN MOISTURIZING ACTIVITY

Of the 12 seaweed species screened for their potential skin moisturizing activity, the test creams containing 5% *Codium fragile*, *Ecklonia cava*, *Enteromorpha linza*, and *Undaria pinnatifida* extracts showed no skin moisturizing effect compared with the placebo cream (17.05%; Figure 1). Test creams containing 5% *Capsosiphon fulvescens*, *Hizikia fusiformis*, and *Sargassum horneri* exhibited 19.09%, 18.95%, and 18.16%, respectively, showing slight moisturizing effects. The test cream containing 5% *Gracilaria verrucosa*, *Laminaria japonica*, *Porphyra yezoensis*, *S. sagamianum*, and *Ulva pertusa* showed considerable moisturizing activity; the increased hydration percentage compared with untreated skin was 20.32%, 28.71%, 23.46%, 24.27%, and 19.99%, respectively. Among them, the strongest activity was exhibited by *L. japonica*. For this reason, we selected and tested this species for the next assay. Analysis of the *L. japonica* water/propylene glycol extracts revealed various nutritional components, such as carbohydrates, proteins, crude fat, free amino acids, sugars, minerals, etc. (Table II).

SKIN MOISTURIZING ACTIVITY OF L. JAPONICA EXTRACT

To identify the concentration that showed the highest activity, we measured the moisturizing activity of the cream containing *L. japonica* at different concentrations. These results are shown in Figure 2. In the test cream without the extract, the moisture level of skin was increased by 17.02%; when the same cream with 1%, 3%, 5%, 7%, 10%, and 15% extract was used for treatment, the moisture level of skin was increased by 21.31%, 24.21%, 27.31%, 29.16%, 31.46%, and 30.72%, respectively. In the *L. japonica* cream-applied group, the increased concentration of seaweed

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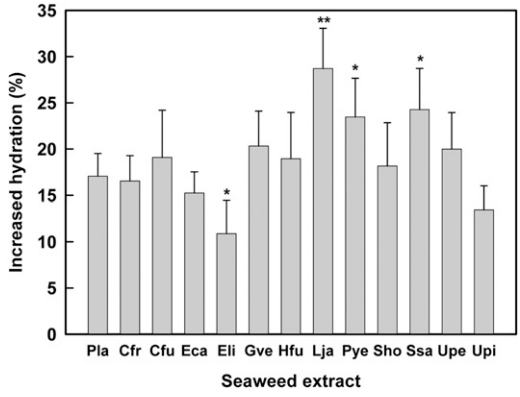


Figure 1. The 12 seaweed species from which extracts were obtained (Pla: Placebo control, Cfr: *Codium fragile*, Cfu: *Capsosiphon fulvescens*, Eca: *Ecklonia cava*, Eli: *Enteromorpha linza*, Gve: *Gracilaria verrucosa*, Hfu: *Hizikia fusiformis*, Lja: *Laminaria japonica*, Pye: *Porphyra yezoensis*, Sho: *Sargassum horneri*, Ssa: *S. sagamianum*, Upe: *Ulva pertusa*, and Upi: *Undaria pinnatifida*). The moisture content of skin was measured 2 h after applying the formulations containing 5% seaweed extract. All measurements were performed in triplicate, and values are an average of three replications. Differences were considered significant at $^{*}p < 0.05$, and $^{**}p < 0.01$ *versus* the placebo control.

extracts reasonably and dose-dependently increased the hydration compared with untreated skin from 0% to 10%. However, with the 15% concentration, the moisture level decreased.

To confirm the length of hydration from the test cream containing 10% *L. japonica* extract, we measured skin hydration at different times after application of the creams. Both with and without the extract, the hydration percentage was increased immediately and continued to decrease over time (Figure 3). The hydration percentage of skin was 73.78% with the test cream without the extract; when the same cream containing 10% extract was used for treatment, the hydration percentage of skin was 94.22%, which was 20.44% higher than that in the placebo. From 1 to 2 h, the moisturizing cream containing *L. japonica* extract increased skin hydration approximately 1.5 times more than did the same cream without the extract. At 8 h after application, the increased skin hydration percentage of skin treated with the test cream containing the extract was 18.70%, and that of the test cream without the extract was 15.81%. Namely, the hydration effect of the test cream containing 10% *L. japonica* extract lasted for 8 h, the time tested, showing the strongest activity at the 2-h period.

TEWL OF L. JAPONICA EXTRACT

To determine the best concentration, the TEWL of the treated skin was determined for 0%, 1%, 3%, 5%, 7%, 10%, and 15% concentration test creams with *L. japonica* at 2 h after application. These results are shown in Figure 4. In the test cream without the extract, the TEWL of skin was 8.67 g/cm^2; when the same cream containing 1%, 3%, 5%, 7%, 10%, and 15% extract was used for treatment, the TEWL of the skin was 8.25, 7.90, 7.06, 6.76, 6.54, and 6.70 g/cm^2, respectively (Figure 4). The TEWL of skin without any test cream was 12.22 g/cm^2. Among these test groups, the lowest TEWL was exhibited

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Table II.
Proximate Compositions, Free Amino Acid Compositions and Mineral Contents of the *L. japonica* Water/ Propylene Glycol Extracts

	Compositions	Unit	Contents
Proximate compositions	Carbohydrate	g/100g	0.6
	Sugars	g/100g	0.5
	Proteins	g/100g	0.2
	Crude fat	g/100g	0.2
	Saturated fatty acid	g/100g	0.1
	Unsaturated fatty acid	g/100g	-
Free amino acids	Alanine	mg/100mL	4.23
	Ammounium chloride	mg/100mL	1.62
	Arginine	mg/100mL	2.03
	Aspartic acid	mg/100mL	42.47
	Glutamic acid	mg/100mL	68.94
	Glycine	mg/100mL	0.17
	Hydroxyproline	mg/100mL	38.14
	Phosphoserine	mg/100mL	0.37
	Serine	mg/100mL	0.86
	Threonine	mg/100mL	0.42
Minerals	Na	mg/100g	40.4
	Ca	mg/100g	30.0
	K	mg/100g	102.2
	Mg	mg/100g	3.5
	Fe	mg/100g	0.05
	Zn	mg/100g	0.02

at a concentration of 10% *L. japonica*. However, at a concentration of 15%, the TEWL of skin was slightly increased.

To confirm the duration of TEWL-preventing effects, we measured TEWL at 0, 3, 5, 10, 15, and 30 min, and 1, 1.5, 2, 4, 6, and 8 h after application of the creams containing 10% *L. japonica* extracts. Within 5 min after application of the creams, the TEWL level was very large both with and without the cream. From 10 min to 2 h, the TEWL was dramatically reduced. Overall, in the testing group, the TEWL of skin treated with the test cream containing the extract was lower than that without cream (Figure 5). At 8 h after application, the TEWL of skin treated with the test cream containing the extract was 4.01 g/cm^2, and that of the test cream without the extract was 5.10 g/cm^2.

PATCH TEST

To confirm safety of the extracts for human skin, a patch test was performed on 25 human volunteers. When the 20 mg per test patch was applied with the test cream, none of the 25 subjects developed erythema, cellular infiltration, or vesicles. Based on these results, we suggest that the *L. japonica* extract is not irritating to normal human skin at moderate doses.

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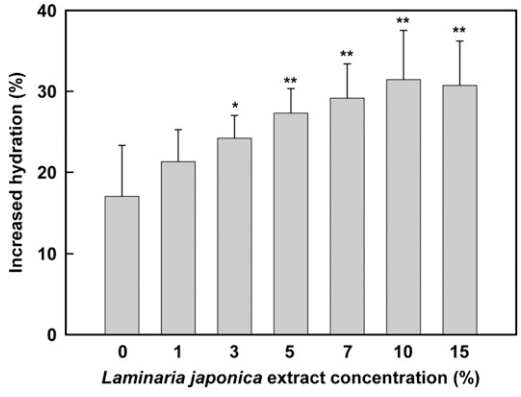


Figure 2. Effect of *Laminaria japonica* extract on skin moisturization at different concentrations. The moisture content of skin was measured 2 h after applying the formulations containing different concentrations of the seaweed extract. All measurements were performed in triplicate, and values are an average of three replications. Differences were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.01$ *versus* the control.

PHYSIOCHEMICAL CHARACTERISTICS OF THE TEST CREAM PRODUCTS CONTAINING 10% L. JAPONICA

For possible future application in cosmetic products and therapeutic agents for skin hydration, the physiochemical characteristics of the test cream products containing 10% *L. japonica* were measured, including chromaticity (as Hunter *L*, *a*, and *b* values), pH, refractive index, and coefficient of viscosity. These results are shown in Table III. Whereas the *L* value and Hunter color *a* value of the test cream without extract were 86.12 and −0.14, respectively, the *L* and *a* values of the test cream containing 10% *L. japonica* extract were 80.64 and −0.84, respectively, a notable decrease. Although the Hunter color *b* value of the creams increased from 1.01 to 4.91, adding 10% *L. japonica* extract showing *b* values

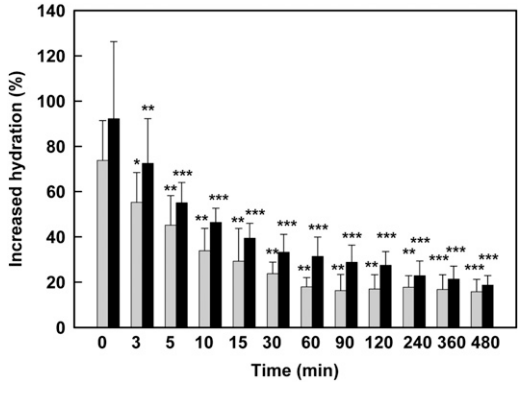


Figure 3. Moisturizing profile of creams without (▒) and with (■) 10% *Laminaria japonica* extract. The moisture content of skin was measured at different times after applying the formulations containing 10% seaweed extract. All measurements were performed in triplicate, and values are an average of three replications. Differences were considered significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ *versus* the control.

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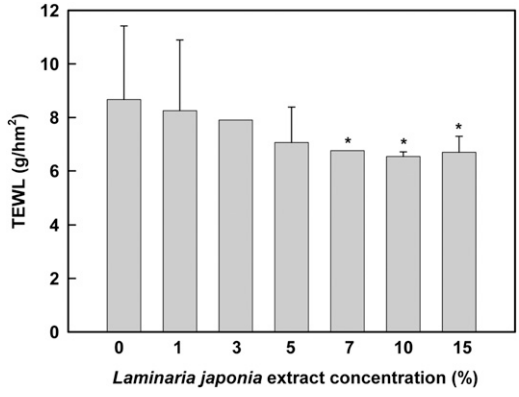


Figure 4. Effect of different concentrations of *Laminaria japonica* extract on TEWL. The TEWL of skin was measured 2 h after applying the formulations containing different concentrations of seaweed extract. All measurements were performed in triplicate, and values are an average of three replications. Differences were considered significant at $^{*}p < 0.05$ *versus* the control.

was notably increased. The overall color difference (ΔE) was 6.76, which was not a dramatic color change. With the addition of 10% *L. japonica* extract, the pH of the test cream changed from 6.35 to 5.91, perhaps as a result of the lower pH (5.60) of *L. japonica* extract itself. According to Notification No. 2009-158 of the KFDA, the normal pH range of cosmetics is between 3.0 and 9.0. Therefore, *L. japonica* extracts are suitable for cosmetic products. The refractive index of the cream with and without 10% *L. japonica* extract was 1.3543 and 1.3727, respectively. The coefficient of viscosity was 68, 945 Cp (centipoise) and 124,333 Cp, respectively, and this may have resulted from the lower viscosity of *L. japonica* extract itself. For all tested aspects, *L. japonica* extract did not affect the overall formulations of the test cream product.

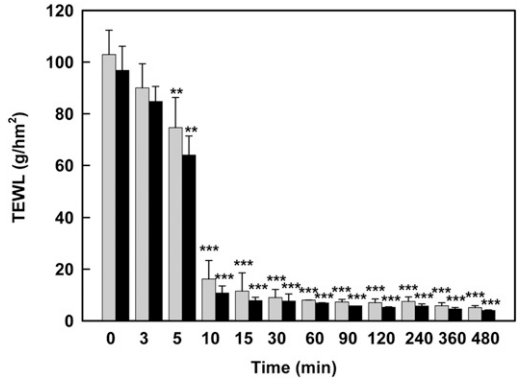


Figure 5. TEWL of cream without (▒) and with (■) 10% *Laminaria japonica* extract over time. The TEWL of skin was measured at different times after applying the formulations containing 10% seaweed extract. All measurements were performed in triplicate, and values are an average of three replications. Differences were considered significant at $^{**}p < 0.01$ and $^{***}p < 0.001$ *versus* the control.

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Table III.
Physiochemical Characteristics of Test Cream Without (a) and With (b) *Laminaria japonica* Extract. All Measurements Were Performed in Triplicate, and Values are an Average of Three Replications. Differences Were Considered Significant at $p < 0.05$

		Test cream products	
		a	b
pH		6.35	5.91
Refractive index(nD)		1.3543	1.3727
Viscosity (cps)		124,333	68,945
Color value	(Lightness, *L*)	86.12	80.64
	(Redness, *a*)	−0.14	−0.84
	(Yellowness, *b*)	1.01	4.91
	Δ*E* Value	—	6.76

DISCUSSION

Because skin is the most exposed organ of the human body, and because water determines the softness and smoothness of skin, moisturizers are most commonly used to treat dry skin. Skin hydration depends on the equilibrium between water transferred by osmosis from the dermis to the epidermis and water lost from the surface by evaporation. Dehydration of the upper layer of the skin results when water is lost from the stratum corneum more rapidly than it is received from the lower layer of the skin. Dry skin is also the result of loss of the horny layer of lipids. Dry skin is brittle and rough, although it is constantly supplied with water from inside the body (18).

Moisturizers are used to treat dry and irritated skin as well as atopic dermatitis and ichthyosis. A wide variety of moisturizers are currently available, but there remains a demand for a highly efficacious moisturizer with an excellent safety and efficacy profile. Thus, for many years, there have been numerous attempts to develop new agents capable of preventing and/or treating dry skin.

Intertidal seaweeds are periodically exposed to air and experience a variety of potentially stressful environmental conditions, including nutrient limitation, high light, high and low temperatures, desiccation, and osmotic stress (19). To overcome these situations, seaweeds produce a great variety of secondary metabolites with different activities. These biologically active secondary metabolites are valuable natural ingredients for cosmetics (7–9,20). In particular, seaweed polysaccharides such as fucoidan, carrageenan, alginates, and agar have been used as excipients in cosmetic formulae because of their high gelling, bonding, and viscosity-increasing properties (3). However, until now, few studies have measured the moisturizing effects of seaweed extracts (10). In addition, there has been no report on the skin moisturizing activity of indigenous seaweed species in Korea. For these reasons, we evaluated the skin moisturizing activities in easily collectible and edible intertidal seaweeds.

It is generally accepted that there are two major strategies for the measurement of skin moisturizing effects. The first is to assess skin hydration by measuring the water content of the stratum corneum using a Corneometer. The second is to assess skin barrier function by measuring the TEWL using a Tewameter.

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First, we compared the skin moisturizing activity of 12 seaweed species collected in Korea; *L. japonica* showed the highest activity and was chosen for this study. In a previous study (10), *Codium fragile* collected in France showed potent skin moisturizing activity; however, in our results, *Codium fragile* from Korea did not show any effects. These results may be caused by variations in cellular chemical compositions and biological activities according to season, habitat, and different thalli in seaweed (21).

When we were measured the increased skin hydration abilities of *L. japonica* creams at different concentrations, the highest activity was shown at 10%. When we measured the moisture level at different times after application of the 10% *L. japonica* extract cream, the moisturizing effect lasted up to 8 h, which was an increase of >15% compared with the placebo cream, showing the strongest activity at the 2-h period. It is generally accepted that when a new challenger agent is tested, if the skin moisturizing property is greater than 10%–20% compared with the placebo, the new agent is regarded as having moisturizing activity (22). In our results, 10% *L. japonica* extract showed favorable moisturizing capacity of >15% compared with the placebo.

When we evaluated the skin barrier function of *L. japonica* cream at different concentrations, the lowest TEWL was exhibited by the 10% extract. TEWL was dramatically reduced beginning immediately after the cream application to 10 min, and did not decrease until 2 h. After applying moisturizer, there are two types of TEWL measurements that can be done. One is when lipid formulations such as petrolatum are applied to the skin, evaporative water loss is immediately reduced due to closing of the stratum corneum by a specific lipid(s). Another is that within 15 min after applying a moisturizer, a large amount of water evaporation from the skin surface occurs in the oil-in-water (O/W) emulsion or an aqueous cosmetic solution (23,24). In this study, because the formulation of the test cream was an O/W emulsion, this tendency was observed.

Although the efficacy and mechanisms underlying the effects of moisturizers remains a topic of controversy, occlusives, humectants, and hydrocolloids can be used to improve skin moisturization. Occlusive moisturizers (e.g., petrolatum, lanolin, mineral oil, vegetable oil, acetyl alcohol, cholesterol, ceramides, and silicones) are oily substances that retard TEWL. Humectants (e.g., glycerin, propylene glycerol, polysorbate 30, polyglyceryl-3, sorbitol, gelatin, urea, sodium lactate, vitamins, and proteins) are hydroscopic substances that draw water from the viable epidermis/dermis to the stratum corneum. Hydrocolloids (e.g., colloidal oatmeal and proteins in moisturizers) are large molecular weight substances that form an artificial membrane (25).

L. japonica is commonly used as a foodstuff in Korea and Japan and is reported to contain various nutritional contents (26). In our study, the solids content of *L. japonica* water/ propylene glycol extracts was 25.2 mg/ml. We found that there are several different nutritional components, such as carbohydrates, proteins, free amino acids, sugars, and minerals, in the *L. japonica* water/ propylene glycol extracts (Table II). In our results, the test cream containing 10% *L. japonica* increased skin moisture immediately and over time. We suggest that the *L. japonica* extract addresses skin hydration via two mechanisms. First, hydroscopic substances such as free amino acids, sugars, and minerals that draw water from the viable epidermis/dermis to the stratum corneum, and the diffusion of these materials through the superficial epidermal layers, reinforces the activity of the natural moisturizing factors in skin that help to retain appropriate moisture levels in the epidermis. Second, phycocolloids such as alginate and protein in seaweed extracts attach

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to skin proteins to form a protective barrier for both immediate and long-term regulation of moisture loss.

To confirm the safety of the *L. japonica* extracts, we performed a patch test for human skin. The results suggest that the extracts can be safely used by humans at moderate doses. In addition, for commercial applications, we evaluated the physiochemical characteristics of the test cream products, including Hunter *L*, *a*, and *b* values; pH; refractive index; and the coefficient of viscosity. *L. japonica* extract did not affect overall formulations of the test cream product in any of the tested aspects. Regarding odor, we confirm that the extract added no objectionable odor to the cream, but the cream itself has a mild odor, which likely masks that of the seaweed.

In conclusion, *L. japonica* extract used at 5%–10% in cosmetic formulations may constitute an effective hydrating agent; it may become a promising and effective moisturizing ingredient in face- and body-care product formulations. In addition, it has been reported that *L. japonica* has additional benefits for the skin, such as antimicrobial (27), antioxidant (28), and anti-inflammatory activities (29). Therefore, it is expected that *L. japonica* will be used as a promising cosmetic ingredient that possesses various biological activities as well as skin moisturizing activity.

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