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Preparation and Evaluation of Cream Mask from Vietnamese Seaweeds

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Synopsis

Seaweed is rich source of natural bioactive compounds that could be exploited as functional ingredient for cosmetic applications. The aim of this study was to evaluate the biochemical compositions and bioactivities of 10 seaweeds collected from coastal waters of Vietnam. The present study also prepared and evaluated cream mask from mixture of seaweeds extracted with water. The results showed that *Caulerpa lentillifera*, *Sargassum crassifolium*, *Ulva reticulata*, and *Kappaphycus alvarezii* are potential rich sources of protein, polysaccharide, carotenoids, and vitamins with high antibacterial, cell proliferation, moisture retention, and tyrosinase inhibitory activities. Physicochemical analysis of cream mask from a mixture of these seaweed extracts indicated that it is yellowish brown in color with a specific odor of seaweed, stable, and homogeneous for up to 12 months of storage, with a pH of 6.1, and high spread and adhesive abilities. No total aerobic mesophilic microorganisms, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and heavy metals were detected in the seaweed mask cream. The seaweed cream mask was safe and caused no irritation to normal human skin, and it satisfied provisions of Circular No. 06/2011/TT-BYT dated January 25, 2011 of the Vietnam Ministry of Health, providing cosmetic management for the cosmetic products with anti-aging and moisturizing effects.

INTRODUCTION

Marine macroalgae or seaweed is taxonomically classified into three major groups: green (Chlorophyta), brown (Phaeophyta), and red algae (Rhodophyta) (1). They are rich sources of highly bioactive secondary metabolites for applications in foods, pharmaceuticals, and cosmetics (2). It has been recognized that seaweed contains significant amounts of mineral matter [1.1–2.5% dry weight (DW)], protein (1–30% DW), lipid (0.3–4% DW), polysaccharides (15–65% DW), phytohormones, and pigments (2,3), in which seaweed extracts such as agar, carrageenan, and fucoidan have been used for nutritional and nutraceutical

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benefits, as well as in successful cosmetic formulation (4). Most of the investigations on the metabolites derived from seaweed have revealed their potential antioxidant, anti-inflammatory, anti-wrinkle, anti-bacterial, and anti-aging properties, as well as their role in protection of the skin from ultraviolet rays, moisturizing, and whitening (5). Thus, seaweed is considered as low-costing, safe, and environmentally friendly raw materials for cosmetic industry.

Vietnam has a 3,200 km coastline, with great diversity in its algal flora. A total of 827 species comprising 88 Cyanophyta, 180 Chlorophyta, 147 Ochrophyta, and 412 Rhodophyta were compiled from various published sources (6,7). In addition, seaweed farming area in Vietnam has 10.000 ha with the productivity of more than 101.000 tons per year. However, there is no report of the cosmetic applications using Vietnamese seaweed. Until now, researches on seaweed in Vietnam have concentrated on only sampling surveys, taxonomy, exploitation of natural bioactive compounds (as fucoidan), and cultivation of economically important seaweed species such as *Gracilaria* spp., *Kappaphycus alvarezii*, and *K. striatum* (8). On the other hand, there still are few studies focusing on nutrition, biochemical composition of seaweed and their applications for functional food, traditional medicines, and biofertilizers (8,9). This study aimed to formulate and evaluate water extracts of four seaweeds, *C. lentillifera*, *S. crassifolium*, *U. reticulata*, and *K. alvarezii*, in cream mask with anti-aging and moisturizing effects. We first screened potential species from 10 seaweeds based on the values of some biochemical components as content of polysaccharide, carotenoid, and vitamins. The four selected seaweed species were extracted with water and performed various bioactivities such as antioxidant, antibacterial, cell proliferation, moisture retention, and tyrosinase inhibition *in vitro*. Finally, formulation of cream mask and evaluation of its physiochemical and microbiological characteristics from a mixture of four selected seaweeds were investigated.

MATERIALS AND METHODS

MATERIALS

Fresh seaweed species were collected from Nha Trang, Khanh Hoa, Vietnam (12°33′28.9″N; 109°17′55.1″E) from February to May, 2017, including *Caulerpa lentillifera* J. Agardh 1837, *Ulva lactuca* Linnaeus, 1753, and *Ulva reticulata* (Forssk) (Chlorophyceae); *Kappaphycus alvarezii* (Doty) Doty, and *K. striatum* (Schmitz) Doty (Pakaya); *Gracilaria tenuistipitata* C. F. Chang & B. M. Xia, 1976 and *Gracilariopsis bailiniae* J. Zhang & B. M. Xia, 1991 (Rhodophyceae); *Sargassum oligocystum* Montagne, 1845, *S. crassifolium* J. Agardh, 1848, and *S. denticarpum* T. Ajisaka, 1994 (Phaeophyceae). The identification of scientific names of these seaweed species was carried out by Tran Mai Duc (Nha Trang Institute of Technology Research and Application, Vietnam Academy of Science and Technology). All seaweed samples were cleaned, rinsed with seawater, dried under dim light, and stored at 2°–4°C until use.

METHODS

Biochemical composition analysis. The seaweed samples were washed in fresh water and subsequently dried at 60°C in an oven; the dried samples were ground to particle size <1 mm

and stored at room temperature in airtight plastic containers for biochemical analysis. Protein, lipid, and carbohydrate contents of all seaweed samples were analyzed as previously described (10). The contents of chlorophyll and carotenoid were identified based on the methods by Lichtenthale (11). The vitamin contents were estimated according to the report of Hong and Hien (10). The contents of cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) were analyzed by a atomic absorption spectrophotometer (8,10).

Preparation of seaweed extracts. 250 g *C. lentillifera* or 500 g *S. crassifolium* or 250 g *U. reticulata* or 100 g *K. alvarezii* were ground, extracted with 500 mL of distilled water at 60°C for 12 h, and centrifuged at 8,000 rpm/min for 5 min at room temperature. The suspensions were evaporated on a rotary vacuum evaporator to dryness and stored at −20°C until use.

Isolation of polysaccharide and fucoxanthin. Carrageenan from *K. alvarezii*, alginate from *S. crassifolium*, and ulvan from *C. lentillifera* and *U. reticulata* were isolated and determined as described by Aguilana et al. (12) and Cho et al. (13). Fucoxanthin from *S. crassifolium* was isolated and purified as described by Xia et al. (14).

1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. The antioxidation activity of the seaweed extracts was measured using DPPH radical scavenging assay as described by Harborne and Baxter (15). Ascorbic acid was used as standard control and evaluated for equivalent inhibition (16,17). The inhibition activity of free radicals was calculated in percentage (%) inhibition according to the following formula: % of inhibition = 100 − [(ODs)/(ODc) × 100] with ODs: average optical density of the sample and ODc: average optical density of the control samples (no sample, only DPPH, as 0% inhibitory value).

Antibacterial activity. For the antibacterial assay evaluation of seaweed extracts, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076, *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC 13245, and *Candida albicans* ATCC10231 obtained from the National Institute of Food Control, Hanoi, Vietnam, were using as evaluation/testing microorganisms/tools. The bacteria strains were grown on Luria-Bertani nutrient medium (18).

Stock solutions of the seaweed extracts, polysaccharide, fucoxanthin, and cream masks were prepared in dimethyl sulfoxide (DMSO), and the antibacterial assays were performed as described previously (19). The minimal inhibitory concentrations (MIC) were recorded as the lowest concentrations inhibiting bacterial and fungal growth. Streptomycin and Cycloheximide were used as positive controls.

Mushroom tyrosinase assay. Inhibitory of the seaweed extracts on cell-free mushroom tyrosinase activity was determined using spectrophotometry with 3,4-dihydroxyphenylalanine oxidase (DOPA; Sigma, St. Louis, MO) as a substrate. Fifty microliter of 0.03% tyrosine solution in distilled water and 75 µL of 0.1 M phosphate buffer (pH 6.8) with the different concentration of seaweed extracts, polysaccharides, fucoxanthin, and cream masks were added to a 96-well microplate. Finally, 25 µL of mushroom tyrosinase (400 U/mL 0.1 M phosphate buffers) were added, mixed, and incubated at 37°C for 20 min. The amount of DOPA chrome produced in the reaction mixture was determined at 475 nm. Inhibitory effects on the enzyme activity by tested samples were represented as % of inhibition, $[1 - (\text{sample } OD_{475}/\text{control } OD_{475})] \times 100$.

Evaluation of moisture retention. The *in vitro* moisture retention activity of seaweed extracts, polysaccharide, fucoxanthin, and mask creams was assayed gravimetrically as described in a report of Jiménez-Pérez et al. (20).

Activity on the proliferation of fibroblasts. Mouse fibroblasts NIH 3T3 were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 nutrient mixture (DMEM/F-12; 3:1 by volume, Sigma) supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin before treatment. All cells were grown in 5% CO_2 at 37°C. In the experiment, 2×10^5 cells were added to each well of a 24-well microtiter plate. After addition of seaweed extracts, polysaccharides, fucoxanthin, and mask creams into each well, the 24-well plate was maintained at 37°C in a CO_2 incubator for 2 d. After the cultivation was completed and DMEM removed, 60 mL of 0.5% MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) and 500 mL of fresh DMEM were added to each well. The plate was maintained in a CO_2 incubator for 2 h to allow formazan formation. The quantity of formazan produced can be regarded as an indicator of cell density or viability. After dissolving the formazan in DMSO, the absorbance at 565 nm was measured with a microplate reader (Multiskan Ex, Thermo Electron Co., Vantaa, Finland). The proliferation of fibroblasts was evaluated by comparing the absorbance with that of the untreated control.

Cream mask preparation. The formulation of cream mask was composed by demineralized water (49.95–64.95%), Blanose CMC 7HOF (0.5%), emulsifying wax (7%), propylene glycol (5%), Belsil DM 10 (4%), glycerin (1%), talc JA 24R (12%), lunamer 42 (0.5%), and preservative agent PE 9010 (0.05%), and supplemented with 5 mg/mL of mixture of *C. lentillifera*, *S. crassifolium*, *U. reticulata*, and *K. alvarezii* extracts with ratio of 1:1:1:1 (w:w:w:w). The control cream mask (CCM) was prepared without seaweed extracts.

Physiochemical characteristics of the test cream products. The physiochemical characteristics of the test cream products containing 5 mg/mL mixture of seaweed extract were analyzed, including color, coefficient of viscosity, pH, refractive index, heavy metals, and microorganism. The color, coefficient of viscosity, and refractive index of the test cream products were determined according to Vietnam standard 2627:1993, 2642-1993, and 2640:2007, respectively. The pH of the cream masks was measured at 25°C using the Horiba D-71 LAQUAact Portable pH Meter (Horiba Ltd., Kyoto, Japan). Homogeneity, spread ability, and adhesive tests were performed as described by Hanum and Laila (21). The heavy metals (arsenic, cadmium, chromium, cobalt, lead, mercury, and nickel) were identified as described by Hepp et al. (22). Microorganisms (such as *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli*) of the test cream products were determined according to Vietnam standard 4884-1:2015 (ISO 4833-1:2013) and TCVN 6972:2001.

Irritation test. The irritation test of cream masks was examined by patch test on 20 female volunteers with the age varied from 30 to 40 years. The quantity of test cream applied per test patch was 20 mg. The test articles were dispensed onto 8 mm Finn Chambers® on Scanpor® Tape (Dpro Scientific Sdn. Bhd., Petaling Jaya, Malaysia), and the patch was applied to normal skin on the forearm. The patch was removed after 48 h of 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 follows: 0 = no reaction; 0.5 = barely perceptible, very weak spotty erythema; 1 = slight erythematic, spotty or diffuse; 2 = moderate erythema; and 3 = intense erythema, infiltration, and possible vesicles (23).

Skin moisture analysis. The skin moisturizing effects of the cream masks on 20 female volunteers (with the age range of 30–40) were measured using a Skin detector SG-5E (Shenzhen, China) according to the protocol of the manufacturer. Before all measurements, each volunteer washed her forearm with a liquid hand wash and then allowed at least

30 min for full skin acclimation to room temperature. The forearm of the volunteer was applied test cream mask with the dose of 10 mg/cm^2 for 2 h. Two hours after application of the cream, we placed the skin detector lead on a test area of approximately 6 cm^2 on the forearm. All data were obtained on the same day from each individual, and the average values of five measurements per site were used in subsequent calculations. The moisture level of the treated skin was expressed as increased hydration (%) compared with untreated skin.

To determine the clinical efficacy of cream mask treatment, we studied three female volunteers (with the age range of 30–40), without current or prior skin disease. Subjects applied the cream mask to the full face every 2 d for 1 week. Part of face images were taken before and after cream mask treatment by using a KONG, Skin & Hair analysis System (Bomtech, Electronics, Co., LTD, Seoul, Korea).

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 $p < 0.05$ and $p < 0.01$.

RESULTS

BIOCHEMICAL COMPOSITION

The wide variety of the biochemical compositions in seaweeds provides excellent bioactive components for cosmetic product development. To select potential seaweed species for ingredients of cosmetic production, we screened 10 seaweed species which are *C. lentillifera*, *U. lactuca*, *U. reticulata*, *S. oligocystum*, *S. crassifolium*, *S. denticarpum*, *K. alvarezii*, *K. striatum* (Payaka), *G. tenuistipitata*, and *G. bailiniae* based on their biochemical compositions including protein, lipid, polysaccharides, total chlorophyll, and carotenoid, as well as vitamin contents. The parameters of these seaweed species are presented in Table I.

Proteins are considered useful ingredients for creating a suitable environment for healthy skin because they are able to bind water with the horny layer of skin and its annexes (24). The protein content in the 10 screened seaweed species ranged from 7.16% to 17.50% DW, with the highest value in *U. reticulata* (17.50% DW) and the lowest value in *G. bailiniae* (7.16%). Ten seaweed species possessed lipid content from 0.28% to 2.45% DW. Among them, the highest numbers of lipid content were observed in brown seaweed (ranged 1.32–2.45% DW), followed by green seaweed (0.95–1.95% DW), and the lowest numbers of lipid content were observed in red seaweed (0.28–1.3% DW). Some articles reported that among the group of steroids, phytosterols form an important group which may have particular biological activities such as anti-inflammatory and antioxidative effects (25).

It is widely recognized that moisturization is the first step in acting against aging of the skin helping to maintain its appearance and elasticity, while also strengthening its role as barrier to harmful environmental factor (26). Polysaccharides play an essential role in cosmetic formulations as humectants and moisturizers. These compounds have a high capacity for water storage and can be linked to keratin through hydrogen bonds. Thus, they improve skin moisturization (1,27). Table I presented that total polysaccharides content exhibited the highest value in *K. alvarezii* (53.08% DW) and the lowest value in *G. bailiniae* (43.78% DW). Further analysis of the main polysaccharide compositions is as follows: in green species, *C. lentillifera*, *U. lactuca*, *and U. reticulata* presented mainly ulvan,

Table I
Biochemical Composition of 10 Seaweed Species in Vietnam

Samples	Protein (% of DW)	Lipid (% of DW)	Polysaccharide (% of DW)	Carotenoid (μg/g of FW)	Total chlorophyll (μg/g of FW)	Vitamin content (μg/g of FW)		
						A	*E*	*C*
Caulerpa lentillifera	11.70 ± 1.15	0.95 ± 0.05	44.10 ± 0.52	86.20 ± 13.17	790.12 ± 50.21	2.87 ± 0.06	19.8 ± 0.06	140.75 ± 2.01
Ulva lactuca	16.79 ± 2.50	1.28 ± 0.07	47.20 ± 0.42	58.50 ± 4.97	551.41 ± 37.12	Nd	0.21 ± 0.02	142.96 ± 1.49
U. reticulata	17.50 ± 1.22	1.95 ± 0.12	49.70 ± 1.02	63.50 ± 6.32	653.27 ± 20.35	Nd	0.40 ± 0.01	150.12 ± 2.03
Sargassum oligocystum	10.45 ± 1.15	1.90 ± 0.12	50.14 ± 1.05	25.61 ± 5.23	251.36 ± 30.41	0.97 ± 0.31	2.54 ± 0.05	90.28 ± 2.19
S. crassifolium	12.57 ± 1.17	2.45 ± 0.72	52.30 ± 1.15	30.24 ± 2.75	281.86 ± 20.63	1.64 ± 0.07	4.50 ± 0.01	102.52 ± 1.17
S. denticarpum	10.50 ± 1.15	1.32 ± 0.35	49.87 ± 1.14	20.43 ± 1.42	215.12 ± 30.25	1.02 ± 0.13	0.23 ± 0.05	95.52 ± 1.46
Kappaphycus alvarezii	10.69 ± 1.11	0.85 ± 0.05	53.08 ± 1.97	37.63 ± 4.08	205.35 ± 29.85	1.23 ± 0.03	2.70 ± 0.02	85.68 ± 1.12
K. striatum	8.15 ± 1.16	0.28 ± 0.04	49.90 ± 2.53	26.52 ± 3.04	175.89 ± 24.02	0.56 ± 0.03	2.04 ± 0.07	80.23 ± 2.42
Gracilaria tenuistipitata	8.50 ± 1.20	1.30 ± 0.12	45.80 ± 1.75	22.61 ± 2.98	130.63 ± 15.11	1.97 ± 0.41	1.13 ± 0.02	82.45 ± 1.63
Gracilariopsis bailiniae	7.16 ± 1.10	1.17 ± 0.08	43.78 ± 1.50	19.20 ± 2.26	117.19 ± 10.38	1.45 ± 0.08	0.97 ± 0.01	74.17 ± 1.21

Nd: not detected.

respectively, 20%, 15.2%, and 16.6% of DW; red algae such as *K. alvarezii* and *K. striatum* were mainly rich in carrageenans (up to 45.8% of DW in *K. alvarezii*), whereas *G. tenuistipitata* and *G. bailiniae* presented mainly agar (up to 34.6% of DW); brown algae including *S. oligocystum*, *S. crassifolium*, and *S. denticarpum* were mainly rich in alginate (up to 30.9% of DW in *S. crassifolium*). These compounds have been reported that they have high antioxidant capacity (28).

Like sulfated polysaccharides, pigments such as chlorophyll and carotenoid also represent a safe alternative for the cosmetics industry (29,30). Notably, carotenoids have antioxidant and anti-inflammatory properties that contribute to skin photo-protection through inhibition of Ultraviolet A-induced reactive oxygen species toxicity and enter in the formulation of many sunscreens (31). For the major photosynthetic pigments, the total chlorophyll contents of 10 studied seaweed species ranged from 117.19 to 790.12 μg/g of fresh weight (FW) and the carotenoid content ranged from 19.2 to 86.20 μg/g of FW. The highest chlorophyll and carotenoid contents were observed in *C. lentillifera*, with values of 790.12 μg/g of FW and 86.20 μg/g of FW, respectively. *C. lentillifera* also possessed the highest vitamin A and vitamin E (2.87 and 19.8 μg/g of FW, respectively), whereas *U. reticulata* exhibited the highest value of vitamin C (150.12 μg/g of FW).

Antioxidants such as sulfated polysaccharides, pigments, and vitamins can help to maintain the organoleptic properties of cosmetic products by inhibiting lipid oxidation, thus avoiding changes in appearance, odor, and flavor (32).

According to defined standards of biochemical compositions as follows: protein content of 10% DW, lipid- 0.8% DW, polysaccharides- 44% DW; carotenoid, and chlorophyll contents-30, 205 μg/g of FW and vitamin E, C contents-0.4, 85.68 μg/g of FW, we selected four potential species including *C. lentillifera*, *S. crassifolium*, *U. reticulata*, and *K. alvarezii* to develop cosmetic products with ingredients from seaweeds. In addition, previously, we reported that *Sargassum swartzii* and *U. reticulata* collected in Vietnam have potent analgesic and anti-inflammatory effects, without any serious toxic effect at highest possible doses in animal model (9). Our previous study also showed that either dried powder of the *U. reticulata* or the methanol extract of *S. swartzii* has hypolipidemic effects in mice (8). They may be useful as food to prevent hyperlipidemia. Moreover, presently, *C. lentillifera* and *K. alvarezii* are cultivated at a commercial scale in Vietnam, whereas *S. crassifolium* and *U. reticulata* have high natural productivities. For these reasons, they are suitable as raw materials for cosmetics.

According to Burtin (33), seaweeds must meet safety regulations in terms of toxicological criteria. In France, the quality criteria applied to edible seaweeds revealed the standards as upper limit for arsenic less than 3 ppm, 5 ppm for lead, 0.5 ppm for cadmium, and 0.1 ppm for mercury. Thus, those species were further studied for heavy metal content. The contents of heavy metals in four selected seaweed species are shown in Table II. The obtained results indicated that the heavy metal concentration in the selected seaweeds was within the tolerable value reported as the quality criteria for cosmetic.

BIOACTIVITY OF SEAWEED EXTRACTS

Effect of seaweed extracts on cell proliferation activity. Aging results mainly in the loss of dermal collagen and the accumulation of unorganized collagen and elastin fibers in the dermis. Fibroblasts play the key role in wrinkle formation because they produce basic structural

Table II
Heavy Metal Content of Four Selected Seaweed Species

No	Samples	Pb (ppm)	Cd (ppm)	As (ppm)	Hg (ppm)
1	*Caulerpa lentillifera*	0.390	Nd	0.74	Nd
2	*Ulva reticulata*	0.455	0.045	2.008	0.017
3	*Sargassum crassifolium*	0.542	0.078	1.958	Nd
4	*Kappaphycus alvarezii*	0.425	0.098	1.002	0.020

Nd: not detected.

skin substances: collagen, elastin, and hyaluronic acid. During the aging process, the proliferative and metabolic activity of fibroblasts decreases, the fibers' functions are impaired, and their structure becomes modified and then destroyed (34). Therefore, the capability of seaweed extracts and their mixture to stimulate proliferation of skin cells was investigated at a concentration of 3 and 5 mg/mL. As shown in Figure 1A, each seaweed extract or their mixture significantly stimulated the proliferation of fibroblasts by approximately 10–57%, in a dose-dependent manner. Notably, the highest cell proliferation was observed in cells stimulated 5 mg/mL of the mixture. Similar but stronger trends were observed in cells stimulated sodium lauryl sulfate which have been reported for fibroblast proliferation (35). These findings suggest that water extract of seaweeds might be useful in anti-aging treatment.

Evaluation of moisture retention. Proper hydration of skin is crucial for healthy skin function, and moisturizers are essential components of basic skin care. The *in vitro* moisture retention property of seaweed extracts was examined gravimetrically and compared with

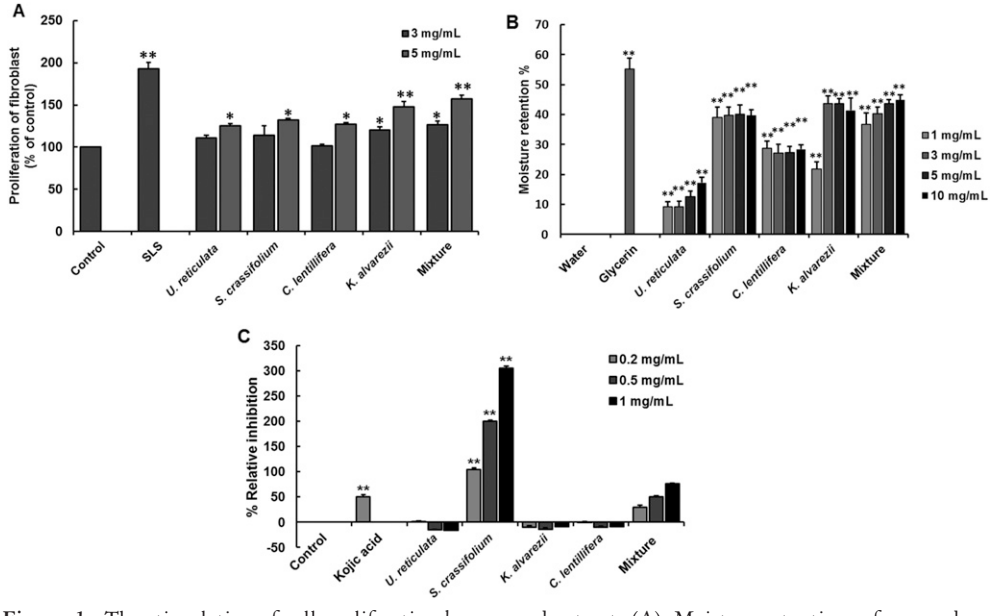

Figure 1. The stimulation of cell proliferation by seaweed extracts (A). Moisture retentions of seaweed extract (B). Inhibitor effects of seaweed extracts on the activity of mushroom tyrokinase (C). Results are expressed as mean ± SEM (the standard error of the mean) of three separate experiments. $*p < 0.05$, $**p < 0.01$, versus control of each group.

that of glycerin. Glycerin exists in the skin's outer layer, the stratum corneum, as a natural endogenous humectant and it is also a cosmetic ingredient regularly used as a hygroscopic and humectant agent (36). In this study, glycerin at 10% showed greater than 50% of moisture retention activities (Figure 1B). Similar but lower trends were observed in seaweed extracts, and these values were significantly different across species. Among tested seaweed extracts, *U. reticulata* showed the lowest moisture retention activity (Figure 1B). *S. crassifolium*, *K. alvarezii*, and a mixture of four seaweeds exhibited the strongest moisture retention capacities as compared with water. Ten percent solution of *S. crassifolium*, *K. alvarezii*, and a mixture of four seaweeds had values of moisture retention activities at 39.8%, 43.7%, and 40.2%, respectively (Figure 1B). It is clears that *S. crassifolium*, *K. alvarezii*, and the mixture of four seaweeds can be used for dry skin masks to deliver moisturizers to the skin.

Inhibitory effects of seaweed extracts on mushroom tyrosinase activity. Melanin is a major factor to determine skin color and plays important roles in the prevention of sun-induced skin injury (37). In this study, the inhibition of tyrosinase activity with various concentrations of seaweed extracts was quantified using arbutin as a control. As shown in Figure 1C, arbutin exhibited the best inhibitory effect on mushroom tyrosinase activity compared with control. Among tested seaweed extracts, *S. crassifolium*, *K. alvarezii*, and the mixture of extracts inhibited mushroom tyrosinase activity in dose-dependent manner. The extract of *S. crassifolium*, *K. alvarezii*, and the mixture showed stronger inhibitory effect on tyrosinase activity than those of *U. reticulata* and *C. lentillifera*. *S. crassifolium* and *K. alvarezii* at concentrations from 0.5 to 1 mg/mL reduced DOPA oxidase activity by 9.1–13.7% and 12.6–20.1%, respectively. At concentrations of 0.5 and 1 mg/mL, the mixture of seaweed extracts reduced DOPA oxidase activity by 4.7% and 9.6%. Our data showed similar results with Chang and Teo (38) and Chan et al. (39).

Antibacterial activity of seaweed extracts. Preservative-free cosmetics and antimicrobial plant/seaweed extracts have attracted attention because of its ability to reduce the risk of allergies connected to synthetic preservatives. In this study, four selected seaweed extracts were examined for their antibacterial activity against three Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *B. cereus*), three Gram-negative bacteria (*E. coli*, *P. aeruginosa*, and *S. enterica*), and one fungus (*Candida albicans*). As shown in Table III, the MIC of seaweed extracts was varied according to species. The MIC values ranged from 2.5 to 10 mg/mL. The *U. reticulata* extract was the most effective against *E. faecalis*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and *S. enterica* (MIC: 2.5–3 mg/mL), whereas it inhibited *E. coli* and *C. albicans* growths at MIC of 10 mg/mL. *K. alvarezii* was strongly inhibited *E. faecalis*, *B. cereus*, and *C. albicans* (MIC:

Table III
MIC (mg/mL) of Seaweed Extracts

	Gram-positive bacteria			Gram-negative bacteria			Yeast
Species	*E. faecalis*	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
U. reticulata	2.5	3	3	10	3	2.5	10
K. alvarezii	3	5	3	10	10	10	3
S. crassifolium	3	2.5	2.5	2.5	5	5	2.5
C. lentillifera	10	10	10	10	10	10	10
Mixture	10	5	10	5	10	5	10
Streptomycin (μg/mL)	256	256	128	32	256	128	—
Cycloheximide (μg/mL)	—	—	—	—	—	—	32

3 mg/mL), whereas *S. aureus* was highly inhibited by *K. alvarezii* with a MIC of 5 mg/mL. This result concurred with a previous report described by Prasad et al. (40) that Gram-positive bacteria were more susceptible to crude *Kappaphycus* sp. extract than Gram-negative bacteria. Both Gram-positive and Gram-negative bacteria were more inhibited with *S. crassifolium* extract, and the MIC values ranged from 2.5 to 5 mg/mL. This result was similar to the study of Tajbakhsh et al. (41), in which MIC value of *Sargassum oligocystum* extracts was ranging from 3 to 10 mg/mL. *C. lentillifera* and a mixture of seaweed extracts in our study also showed the inhibition of bacteria and fungus growths, and the MIC values were 5–10 mg/mL. Taken together, it can be concluded that the extracts of all tested seaweed species and mixture of extracts showed antibacterial activity against bacteria and fungus and can be used as ingredients for the development of cosmetic products.

Biological active components in seaweed extracts. It is reported that seaweed is widely used in numerous cosmetic products in treatment of skin problems, such as aging, dryness, tanning, and pigment disorders. Steroids, polysaccharides, polyphenols, carotenoids, and vitamins were considered as the components that contribute to the health beneficial properties of seaweeds (1). We, therefore, identified active compounds in the seaweed extracts. Results showed that water extracts of selected seaweeds were rich in polysaccharide including carrageenan in *K. alvarezii* (46% of extract), aginate in *S. crassifolium* (53% of extract), and ulvan in *U. reticulata* and *C. lentillifera* (46% and 18% of extracts). In addition, we identified higher amount of fucoxanthin in *S. crassifolium* (25 μg/g extract). Because selected seaweed extracts had antibacterial, cell proliferation, moisture retention, and tyrosinase inhibitory activities, we further identified active compounds responsible for these activations. Here, we demonstrated that carrageenan and alginate at concentration of 100 μg/mL had cell proliferation, moisture retention, and tyrosinase inhibitory activities, ulvan at a concentration of 100 μg/mL had moisture retention activity, and fucoxanthin at a concentration of 50 μM had tyrosinase inhibitory activities (Figure 2). Taken together, we suggested that carrageenan, aginate, ulvan, and fucoxanthin are responsible for seaweed extract bioactivities.

EVALUATION OF CREAM MASK FORMULATION CONTAINING MIXTURE OF *U. RETICULATA*, *S. CRASSIFOLIUM*, *K. ALVAREZII*, AND *C. LENTILLIFERA* EXTRACTS

It is reported that a mixture of ingredients in cosmetics usually increases beneficial activities, and together with bioactivity results aforementioned, we formulated seaweed cream mask (SCM) containing 5 mg/mL of mixture of four seaweed extracts, *U. reticulata*, *S. crassifolium*, *K. alvarezii*, and *C. lentillifera*.

According to Asian guidelines for the safety assessment of a cosmetic product, the determinant step in the development of a cosmetic formulation involves stability study, with the objective of predicting physicochemical and microbiological alterations that may occur since its manufacturing, until the end of its expiration date. Besides, this study allows the evaluation of the cosmetic product performance, safety, and efficacy and contributes for its development time reduction which is highly required by the market and the consumers. For the purpose of commercial applications, the physicochemical and microbiological properties of the cream mask product containing the mixture of seaweed extracts were evaluated (Table IV). As shown in Table IV, the color of SCM changed from white to yellowish brown with a specific odor of seaweed. All cream masks were stable and homogeneous for up to 12 mo of storage. With the addition of mixture of seaweed

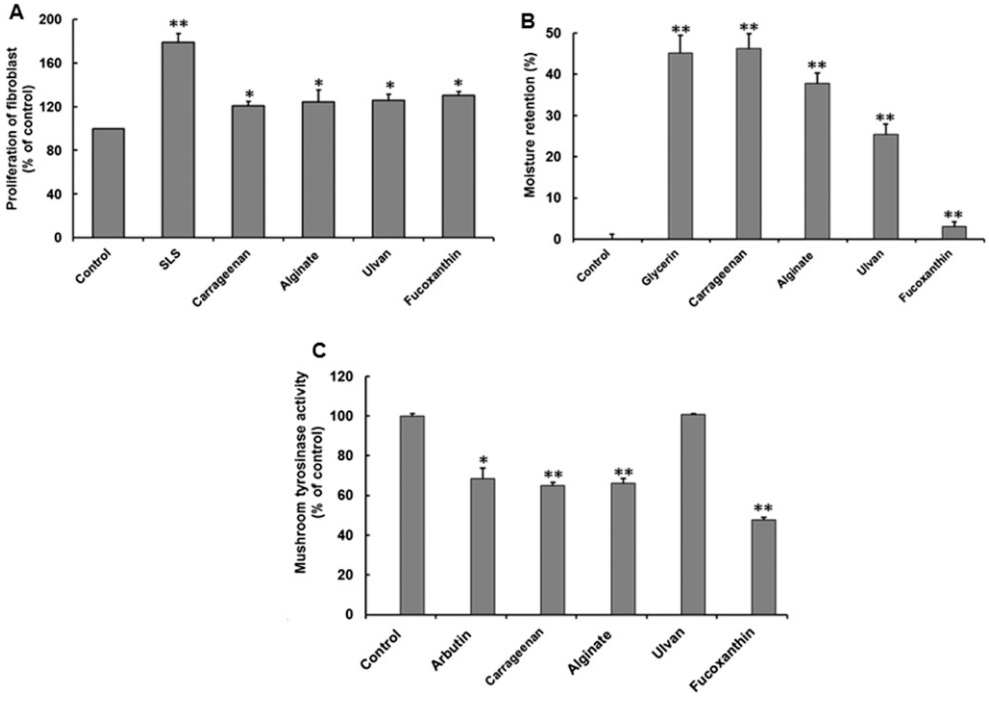

Figure 2. Cell proliferation (A), moisture retention (B), and tyrosinase inhibitory activities (C) of biological active components in seaweed extracts. Value represents mean ± SEM ($n = 5$). *$p < 0.05$, **$p < 0.01$ versus control of each group.

extracts, the pH of the SCM slightly decreased from 6.3 to 6.1. It was reported that the normal pH range of cosmetics is between 3.0 and 9.0 (42). Therefore, the mixture of seaweed extracts was suitable for cosmetic products. The refractive index of the cream mask with and without the mixture of seaweed extracts was 1.47 and 1.50, respectively. The viscosity of SCM [89.1 centipoises (Cp)] was lower than that of CCM (110.3 Cp), and this result was due to the lower viscosity of the mixture itself. Spread ability test is an evaluation to determine the ability of the cream to spread. The spread area of 25–49 cm^2 shows that semisolid textures are convenient in use (43). Spread ability of SCM was higher than that of control cream. It was suitable with suggestion of Hanum and Laila (21), in which the low spread ability was related to the high viscosity of the mask if the given pressure was applied equally in each gel formula. The adhesiveness test was conducted to investigate the cosmetic product ability to attach on the skin. If the formula attached extensively, then the therapeutic effect given by the product will be prolonged because of the long contact time on the skin. There is no specific requirement regarding the adhesiveness of the semisolid product. However, the adhesiveness of the semisolid product is better more than 1 s (21). There was no observed difference in the adhesive ability between control and SCM.

Because of the wide range of formulations, it is necessary to control microbiological growth in cosmetics. According to Asian guidelines on limits of contaminant for cosmetic, the total aerobic mesophilic microorganisms (bacteria, yeast, and molds) must be below 1,000 colony-forming unit/g. *S. aureus*, *P. aeruginosa*, and *C. albicans* must be absent in 0.1 g or 0.1 mL test

Table IV
Physical, Physicochemical, Chemical, and Microbiological Properties of CCM and SCM

Parameters	Test cream mask products	
	CCM	SCM
Physical properties		
Organoleptic	White	Yellowish brown with a specific odor
Homogeneity	Homogen	Homogen
Physical stability at 10°C ± 2°C and 45°C ± 2°C	Stable	Stable
pH	6.3	6.1
Viscosity	110.3	89.1
Refractive index	1.47	1.50
Spread ability (cm^3)	10.2	24.2
Adhesive ability (second)	10.0	10.5
Irritation test	Nonirritating	Nonirritating
Heavy metal		
Arsenic	<1	<1
Cadmium	Nd	Nd
Mercury	Nd	Nd
Lead	<5	<5
Microorganisms		
Total aerobic mesophilic microorganisms (bacteria, yeast, and molds)	<10	<10
S. aureus	Nd	Nd
C. albicans	Nd	Nd
P. aeruginosa	Nd	Nd

Nd: not detected.

sample. In this study, no specified microorganisms were detected in both control cream and SCM (Table IV).

In general, heavy metals are commonly found in seaweed because of the living habit. In our study, some heavy metals were found in the selected seaweeds. And according to Asian guidelines on limits of contaminant for cosmetic, the limit of arsenic, cadmium, lead, and mercury is 5, 20, and 1 ppm, respectively. We, therefore, detected the presence of those heavy metals in the cream masks. As shown in Table IV, there were no heavy metals in the cream mask products.

Contact sensitivity to cosmetics is a common phenomenon for human skin. Patch test is a method used to determine whether a specific substance causes allergic inflammation on a patient's skin. To confirm safety of the extracts for human skin, a patch test was evaluated on 20 female volunteers. When the 20 mg of mixture per test patch was applied, none of the 25 subjects developed erythema, cellular infiltration, or vesicles (Table IV). Based on these results, we suggest that the mixture of seaweed extracts is safe and not irritating to normal human skin at tested doses. Collectively, our obtained results suggested that the physicochemical and microbiological properties of the cream mask from the mixture of seaweed extracts meet provisions of Circular No. 06/2011/TT-BYT dated January 25, 2011 of the Vietnam Ministry of Health, providing cosmetic management.

A cream mask is one of the most gentle face masks made to remove dead cells while toning and lifting skin. They typically contain ingredients such as hyaluronic acid to provide

a surge of hydration, restoring natural radiance. After using a cream face mask, treated skin will feel soft, smooth, and refreshed. In this study, we identified cell proliferation, tyrosinase inhibitory activities of SCM in *in vitro*, and moisturizing property of SCM in human trial. As shown in Figure 3A and B, the addition of a mixture of seaweed extracts had a great influence to stimulate the proliferation of fibroblasts (22%) and inhibit mushroom tyrosinase activity (18%) in the cream mask. Compared with the CCM, percentage of hydration in volunteers was increased by 35% with SCM (Figure 3C). The length of hydration is an important factor for moisturizing property of the cream mask; therefore, we measured skin hydration at different times after application of the cream containing seaweed extracts. Both with and without the seaweed extracts, the hydration percentage was increased immediately and then decreased over time (Figure 3D). The hydration percentage of skin was 62.71% with the CCM; when the SCM was used for treatment, the hydration percentage of skin was 80.08%, which was 17.37% higher than that in the control. From 1 to 2 h, the SCM increased skin hydration approximately 1.5 times more than the control cream did. At 8 h (480 min) after application, the increased skin hydration percentage of skin treated with the SCM was 12.00% and that of the CCM was 10.90%.

The dry skin has a parched look caused by its inability to retain moisture. It usually feels "tight" and uncomfortable after washing unless some type of moisturizer or skin cream is applied. It looks dull. Skin seems to be with reduced wrinkles after the first week of moisturizer application. So it can be considered that all the moisturizers improved the skin

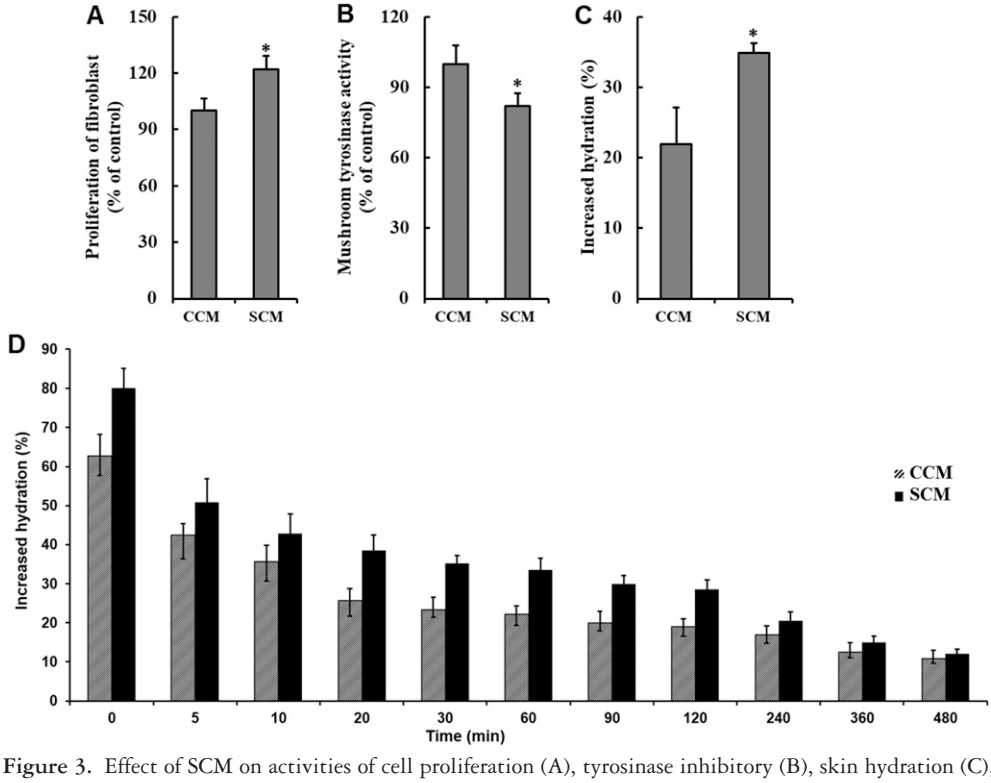

Figure 3. Effect of SCM on activities of cell proliferation (A), tyrosinase inhibitory (B), skin hydration (C), and (D) moisturizing profile of CCM and SCM in different times after applying the cream. *$p < 0.05$, **$p < 0.01$, versus controls. Data are mean ± SEM.

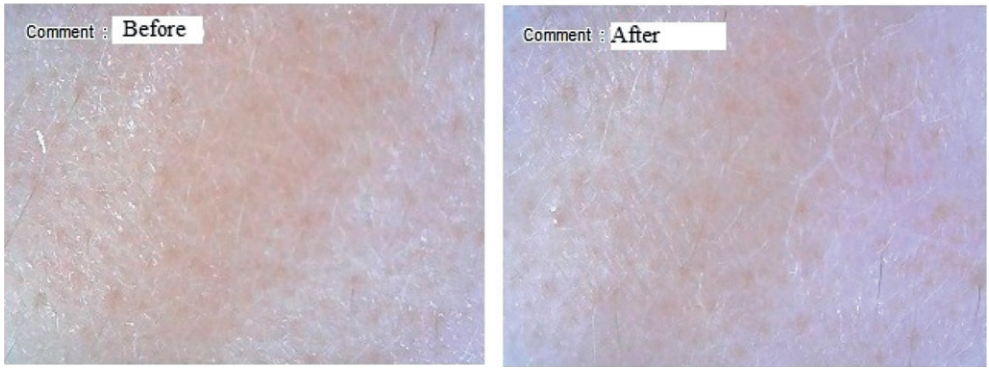

Figure 4. Comparative pictures showing change in skin appearance (picture taken initially, after first week).

appearance. Skin pictures taken at baseline and after the end of study period are shown in Figure 4. Improvement in the appearance of skin supports the data for the increase in hydration.

In summary, *C. lentillifera*, *S. crassifolium*, *U. reticulata*, and *K. alvarezii* are rich sources of polysaccharide and pigment components and exhibit antioxidant, antibacterial, moisture retention, and tyrosinase inhibitory properties. The mixture of these seaweed extracts is an effective and safety formulation for improving skin hydration and skin-whitening agent in cream mask application.

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