



A MARINE-DERIVED FACIAL SERUM INCORPORATING *Caulerpa racemosa* EXTRACT: FORMULATION, STABILITY, AND MOISTURIZING EFFICACY

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Submitted: 23 February 2026/Accepted: 22 July 2026

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How to cite (APA Style 7th): Nurfitriyana, Putri, D. K., Lestari, S. M., Syarif, H., & Waluyo, D. A. (2026). A marine-derived facial serum incorporating *Caulerpa racemosa* extract: formulation, stability, and moisturizing efficacy. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 29(9), 794-810. <http://dx.doi.org/10.17844/0p2ks734>

Abstract

Marine-derived cosmeceuticals are increasingly being studied as sources of bioactive compounds with antioxidant potential and good safety profiles for topical application. This study aimed to develop a marine serum containing 96% ethanolic *C. racemosa* extract and evaluate its quality, efficacy, stability, and safety. *Caulerpa racemosa* was macerated with 96% ethanol and analyzed for extract quality standards, phytochemicals, and heavy metals (Pb, Hg, As, and Cd) using atomic absorption spectrophotometry (AAS). Antioxidant activity was measured using the DPPH method. Three extract-containing formulas (5%, 7.5%, and 10% extract) and a base formula (0% extract) were assessed for organoleptic properties, homogeneity, pH, spreadability, cycling-test stability, open patch safety in 30 volunteers, and 28-day skin hydration response, with signs of irritation (erythema, papules, vesicles, and edema). The moisturizing effectiveness was determined by changes in skin hydration. The *C. racemosa* extract met the quality standards and contained flavonoids, saponins, terpenoids, and steroids. Heavy metals (Pb, Hg, As, and Cd) were not detected or were below the stated limits. The extract exhibited strong antioxidant activity, with a DPPH value of 84.94 ± 0.42 ppm. All serum formulations met the quality requirements, and cycling tests confirmed the stability of all formulas. Cycling tests confirmed acceptable preliminary physical stability, especially in the 10% formulation, and patch tests showed no visible irritation. The 10% formulation achieved the highest hydration recovery after 28 days (60.82%) because its 10% extract supplied more flavonoids, saponins, terpenoids, and steroids, supporting antioxidant barrier protection, hydration, emollient effects, and safe topical serum performance..

Keywords: antioxidant, marine cosmeceutical, moisturizing efficacy, phytochemistry, sea grapes

Pengembangan, Efikasi, Stabilitas, dan Keamanan Serum Wajah Ekstrak Etanol Anggur Laut (*Caulerpa racemosa*)

Abstrak

Kosmetik medis berbasis sumber daya laut makin banyak diteliti sebagai sumber senyawa bioaktif yang berpotensi memiliki aktivitas antioksidan dan profil keamanan yang baik untuk aplikasi topikal. Penelitian ini bertujuan mengembangkan sediaan serum berbasis bahan laut yang mengandung ekstrak etanol 96% *C. racemosa*, serta mengevaluasi mutu, efektivitas, stabilitas, dan keamanannya. *Caulerpa racemosa* diekstraksi menggunakan metode maserasi dengan etanol 96%. Ekstrak yang diperoleh selanjutnya dievaluasi berdasarkan parameter mutu ekstrak, kandungan fitokimia, dan cemaran logam berat berupa timbal (Pb), merkuri (Hg), arsen (As), serta kadmium (Cd) menggunakan spektrofotometri serapan atom (SSA). Aktivitas antioksidan ekstrak ditentukan menggunakan metode DPPH. Tiga formula yang mengandung ekstrak, yaitu 5%, 7,5%, dan 10%, serta formula dasar tanpa ekstrak (0%), dievaluasi berdasarkan karakteristik organoleptik, homogenitas, pH, daya sebar, stabilitas melalui cycling test, tolerabilitas kulit melalui uji tempel terbuka pada 30 sukarelawan, dan peningkatan hidrasi kulit selama 28 hari. Parameter iritasi yang diamati meliputi eritema, papula, vesikel, dan edema. Efektivitas pelembap ditentukan berdasarkan perubahan tingkat hidrasi kulit. Hasil penelitian menunjukkan bahwa ekstrak *C. racemosa* memenuhi parameter mutu yang diuji serta mengandung flavonoid, saponin, terpenoid, dan steroid. Cemaran logam berat Pb, Hg, As, dan Cd tidak terdeteksi atau berada di bawah batas maksimum yang ditetapkan. Ekstrak menunjukkan aktivitas antioksidan kuat dengan nilai IC₅₀ DPPH sebesar 84,94±0,42 ppm. Seluruh formula serum memenuhi persyaratan mutu fisik yang diuji. Hasil *cycling test* menunjukkan stabilitas fisik awal yang dapat diterima, terutama pada formula yang mengandung ekstrak 10%, sedangkan uji tempel tidak menunjukkan adanya tanda iritasi secara visual. Formula dengan ekstrak 10% menghasilkan peningkatan hidrasi kulit tertinggi setelah 28 hari, yaitu sebesar 60,82%. Hasil tersebut menunjukkan bahwa serum yang mengandung 10% ekstrak *C. racemosa* berpotensi dikembangkan sebagai sediaan kosmetik topikal dengan aktivitas antioksidan dan efektivitas pelembap yang baik.

Kata kunci: aktivitas antioksidan, anggur laut, efektivitas pelembap, kosmetik bahari, serum wajah

INTRODUCTION

Marine macroalgae are renewable sources of pigments, phenolics, sterols, terpenoids, polysaccharides, and other metabolites relevant to food, pharmaceuticals, and cosmetics. *C. racemosa* was selected because it is an edible tropical green macroalga used as fresh sea grapes and explored as a functional food, natural colorant, and source of antioxidant and skin-protective compounds. Indonesian seaweed production increased from 9.62 million tons in 2020 to 10.81 million tons in 2024, although the national statistics are dominated by *Kappaphycus/Eucheuma* and *Gracilaria* and do not report *C. racemosa* separately. The same KKP dataset records only small, fluctuating volumes for other *Caulerpa* species, illustrating both the biological availability of the genus and its

underdeveloped value chains. Converting locally collected *C. racemosa* into a standardized topical ingredient may therefore broaden the utilization of Indonesian seaweed beyond conventional hydrocolloid commodities (KKP, 2025; Pangestuti *et al.*, 2025).

Marine-derived bioactive compounds have attracted significant interest in the cosmetics industry because of their unique chemical compositions and potential health benefits. Among marine resources, seaweeds are particularly valued for their abundance of secondary metabolites, including flavonoids, phenolics, carotenoids, and polysaccharides, which display potent antioxidant, anti-inflammatory, and photoprotective properties (Firda *et al.*, 2023). The rationale for cosmetic development is supported by the



phytochemical diversity of *C. racemosa*. *C. racemosa*, a green seaweed common in tropical coastal areas, has shown high levels of these bioactive compounds, making it a promising functional ingredient for cosmetic use (Hainil *et al.*, 2023; Damayanti *et al.*, 2024; Viruly *et al.*, 2024; Ferdinandus *et al.*, 2025). Solvent-dependent studies have reported the presence of phenolics, flavonoids, tannins, pigments, sterol-related metabolites, and antioxidant activity in the extracts. Additionally, a clionasterol-rich fraction protects human keratinocytes against particulate matter-associated oxidative injury (Liyanage *et al.*, 2022; Palaniyappan *et al.*, 2023; Satyantini *et al.*, 2025). Marine macroalgae are increasingly incorporated into topical systems because their antioxidant, moisturizing, anti-inflammatory, and film-forming constituents may complement conventional humectants (Bogdan *et al.*, 2025). Nevertheless, the chemical composition varies with species, collection site, season, environmental exposure, extraction solvent, and processing. Consequently, the activity reported for one extract cannot be directly extrapolated to another extract.

A facial serum was selected as a low-viscosity, water-rich vehicle that could uniformly distribute a concentrated extract, spread readily, and combine marine bioactives with humectants. The principal formulation challenge is that increasing the amount of a colored, chemically complex extract can alter the pH, viscosity, odor, appearance, and polymer hydration, potentially compromising acceptability and stability (Handayani *et al.*, 2023; Iskandar *et al.*, 2025). Earlier studies have examined algae-based anti-aging serums and *C. racemosa* lotion, but evidence linking graded *C. racemosa* concentrations to serum physicochemical behavior, tolerability, and instrumental hydration remains limited (Novika *et al.*, 2024)

Previous studies have reported the antioxidant and functional potential of *Caulerpa* species; however, the concentration-performance relationship of *C. racemosa* extract in facial serum systems remains insufficiently defined. Therefore, a precise formulation gap exists: limited data are available on whether

increasing *C. racemosa* extract concentration can improve moisturizing performance while maintaining acceptable preliminary stability, physicochemical quality, and skin tolerability in a serum vehicle. This study hypothesized that an optimized extract concentration would enhance hydration performance through the combined contribution of marine bioactives and humectant-based serum excipients, without causing unacceptable physical instability or irritation. Accordingly, this study aimed to formulate a marine-derived facial serum containing *C. racemosa* extract and evaluate its physicochemical characteristics, antioxidant activity, stability during cycling tests, safety, sensory acceptance, and moisturizing efficacy.

MATERIAL AND METHODS

Extract Preparation

C. racemosa was collected from Kurau Beach, Central Bangka Regency, Bangka Belitung, Indonesia, and identified at the Oceanography Laboratory of the National Research and Innovation Agency (BRIN). The samples were then washed, chopped, and blended. The prepared material was extracted by maceration using 96% ethanol at a sample-to-solvent ratio of 1:3 for three days at room temperature, protected from direct light. The filtrate was separated and concentrated using a rotary vacuum evaporator (Buchi R-100, Switzerland) at a water bath temperature of 70°C to obtain a thick ethanolic extract.

Phytochemicals of *C. racemosa* Extract

Phytochemical screening was performed to identify the secondary metabolite constituents in the *C. racemosa* extract (Nurfitriyana *et al.*, 2025; Stuthmann, 2021). The procedures, reagents, and positive indications used for screening are summarized in Table 1, and the screening results are reported in Table 5.

Heavy Metal Analysis of Extracts

Heavy metal content was measured using atomic absorption spectrophotometry

Table 1 Qualitative phytochemical screening procedures for *C. racemosa* extract

Phytochemical group	Procedure/reagents	Positive indication
Alkaloids	The extract solution was acidified and treated with Dragendorff's reagent.	The formation of an orange to reddish-orange precipitate indicated the presence of alkaloids.
Flavonoids	The extract solution was treated with magnesium powder followed by concentrated HCl (Shinoda test).	The development of a pink, red, or orange coloration indicated the presence of flavonoids.
Phenolics & Tannins	The extract solution was treated with a 1–5% ferric chloride solution.	Blue-black (indicative of hydrolyzable tannins) or greenish-black (indicative of condensed tannins).
Saponins	The extract solution in distilled water was vigorously shaken and observed for foam persistence.	Persistent froth (≥ 1 cm height, stable for >10 min).
Terpenoids	The extract solution was mixed with chloroform, and concentrated H_2SO_4 was carefully added along the side of the tube to form a lower layer (Salkowski test).	A reddish-brown interface.
Steroids	The extract solution was mixed with acetic anhydride, followed by the cautious addition of concentrated H_2SO_4 along the wall of the test tube (Liebermann–Burchard test).	A green-to-blue coloration.

Table 2 Reagents and wavelengths for heavy metal analysis of extracts

Heavy metal	Reagent	Wavelength (nm)
Lead (Pb)	HNO_3	283.3
Mercury (Hg)	$HClO_4 + HNO_3$	253.7
Arsenic (As)	HCl	193.7
Cadmium	$HClO_4 + HNO_3$	228.8

(AAS; APHA.322 B-2005) based on the principle of atomic absorption. The metals analyzed included lead (Pb), mercury (Hg), and arsenic (As), each of which was detected at its characteristic wavelength (Damayanti *et al.*, 2024). The analytical reagents and

wavelengths are summarized in Table 2, and the measured results are presented in Table 6. The results were interpreted using the heavy metal limits stated in BPOM Regulation No. 16 of 2024 and the ASEAN cosmetic heavy metal method framework (BPOM, 2024).



Characterization of Extract

The extract was characterized by determining the moisture content, total ash, loss on drying, and water- and ethanol-soluble extractive values using standard pharmacognostic methods (Koodkaew *et al.*, 2024).

Determination of Antioxidant Activity

The antioxidant activity of *C. racemosa* extract was evaluated using the DPPH assay. A DPPH solution was prepared under light-protected conditions, and the maximum absorbance was confirmed by UV-Vis spectrophotometry at approximately 515–517 nm. The extract solutions were prepared in ethanol at concentrations of 2, 4, 6, 8, 10, and 12 ppm. Each extract solution (1 mL) was mixed with 2 mL of DPPH solution, incubated in the dark for 40 min, and measured against an appropriate blank sample. Vitamin C served as the positive control. The percentage inhibition was calculated using the following equation:

$$\text{inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

A0 = control signal (no inhibitor)

A1 = test signal (with inhibitor/sample)

The IC₅₀ value was determined from the linear regression of concentration versus percentage inhibition, following the DPPH method (Novika *et al.*, 2024).

Formulation of *C. racemosa* Extract Facial Serum

The serum compositions are presented in Table 3. F0 served as the base formula without the extract, whereas F1, F2, and F3 contained 5%, 7.5%, and 10% *C. racemosa* extract, respectively. Carbomer was dispersed in purified water and neutralized with triethanolamine to form a gel network. Methyl paraben and BHT were dissolved separately, glycerin and propylene glycol were added as humectants, and the extract was pre-dissolved in propylene glycol before incorporation into the serum base. The mixture was stirred until a homogeneous serum was obtained (Aggnihotri, 2021).

Table 3 Formulations of sea grape (*C. racemosa*) extract facial serum

Ingredients	Formulas (%)			
	0	5	7.5	10
Sea grape extract	-	5	7.5	10
Carbomer	0.5	0.5	0.5	0.5
Glycerin	10	10	10	10
Propanediol	10	10	10	10
BHT	0.1	0.1	0.1	0.1
Propylene glycol	5	5	5	5
TEA	1	1	1	1
Methylparaben	0.5	0.5	0.5	0.5
Aquadest ad	50	50	50	50

Note: The facial serum base consisted of carbomer, glycerin, propanediol, BHT, propylene glycol, TEA, and methylparaben. *C. racemosa* extract (50%) was incorporated as an active ingredient at concentrations of 5%, 7.5%, and 10%.

Evaluation of Characteristic and Physical Stability of Facial Serum

The preliminary physical stability was evaluated using six temperature-stress cycles. One cycle consisted of storage at 4 ± 2 °C for 24 h, followed by 40 ± 2 °C for 24 h. The organoleptic properties, homogeneity, pH, spreadability, and viscosity were measured before and after the cycling test, which was used as a screening procedure and not as a substitute for real-time stability testing (Nurfitriyana *et al.*, 2020). The organoleptic characteristics were examined visually and by odor assessment for color, odor, appearance, and consistency of the samples. Taste was not evaluated because the product was intended for topical use (Kedar *et al.*, 2025). Homogeneity was assessed by spreading a thin layer between glass slides and observing them under adequate illumination for lumps, coarse particles, or phase separation (Malahayati *et al.*, 2024). The pH was measured in triplicate using a calibrated MW102 pH meter after the electrode readings stabilized. Spreadability was determined by placing 1 g of serum between two 10×10 cm glass plates, applying a 100 g load for 1 min, and recording the mean diameter in two perpendicular directions (Malahayati *et al.*, 2024). The viscosity was measured in triplicate at room temperature using a Brookfield viscometer with the spindle and rotational speed kept constant across samples. The values were recorded after the readings were stabilized (Rahmawati *et al.*, 2024).

Safety Evaluation (Irritation Test)

The safety of the formulation was evaluated in 30 human panelists using an open patch test. The facial serum was applied to a 2.5×2.5 cm area on the lower forearm three times daily for seven days. Skin reactions were assessed by a dermatologist and graded from no reaction (–) to edema (++++). (Pangestuti *et al.*, 2025).

Efficacy of Facial Serum

Preliminary topical tolerability was evaluated in 30 human volunteers using a repeated, open-patch application method. The

inclusion criteria were healthy adults aged 18-45 years with normal skin on the lower forearm and willingness to provide written informed consent. The exclusion criteria included a history of allergy or hypersensitivity to seaweed or cosmetic ingredients, active dermatitis or wounds at the test site, current use of topical corticosteroids or antihistamines, pregnancy or lactation, and participation in another skin test in the previous two weeks. Each formulation was applied to a 2.5×2.5 cm area on the lower forearm three times daily for seven days. Skin reactions were assessed at baseline (day 0) and after treatment (day 7) by a dermatologist using the following visible irritation parameters: erythema, papules, vesicles, and edema. Reactions were graded from no reaction (–) to severe reaction (++++). (Yeskar *et al.*, 2023; Pangestuti *et al.*, 2025).

Hedonic Test

A hedonic test was conducted with 30 panelists to assess the sensory acceptability of the extract-containing facial serum. The inclusion criteria were adults aged 18-45 years who were familiar with topical cosmetic products and had no known allergy to *Caulerpa* or the listed excipients. The exclusion criteria were active skin disease, recent irritation of the hands or forearms, strong aversion to marine odor, or inability to complete the assessment form. The sensory parameters included color, aroma, texture, and overall acceptance. Taste was excluded because the serum was not intended for oral administration. Participant preferences were evaluated using a five-point hedonic scale ranging from strongly dislike (1) to strongly like (5) (Septiyanti *et al.*, 2019).

Ethical Consideration

The irritation test and efficacy evaluation of the facial serum involving human participants were approved by the Institutional Ethics Committee of the Faculty of Medicine and Health, Universitas Muhammadiyah Jakarta, under approval no. 287/PE/KE/FKK-UM/XI/2024. Written informed consent was obtained from all participants prior to the study.



Data Analysis

This study used a completely randomized design with one factor: *C. racemosa* extract concentration (0%, 5%, 7.5%, and 10%). The physicochemical data from three replicate measurements are presented as mean $\pm$ standard deviation. Data normality and homogeneity were assessed using the Shapiro–Wilk and Levene’s tests, respectively. Normally distributed data were analyzed using one-way ANOVA followed by Tukey’s test, whereas non-normally distributed data were analyzed using the Kruskal–Wallis test followed by Bonferroni-adjusted Mann–Whitney U tests. Hedonic scores from 30 panelists were analyzed using non-parametric tests. Different superscript letters indicate significant differences ($p < 0.05$). The organoleptic properties, homogeneity, irritation, and skin hydration recovery were descriptively analyzed. Statistical analyses were performed using SPSS version 24.0 (Heryana, 2023).

RESULT AND DISCUSSION

C. racemosa Extract Preparation

Characterization showed that the extract met the quality requirements based on the Indonesian Herbal Pharmacopoeia, 2nd edition (2017), and phytochemical screening confirmed the presence of flavonoids, saponins, terpenoids, and steroids in the

C. racemosa extract. The results of the *C. racemosa* characterization are presented in Table 4.

Maceration extraction was chosen for its simplicity and minimal heat exposure (70°C). The concentration was carried out under reduced pressure to lower the effective boiling temperature of ethanol and reduce the duration of heat exposure. Because thermolabile marine bioactives may degrade under excessive heating, the extraction time was minimized, and the extract was protected from direct light (Sommer *et al.*, 2022). Table 4 shows that the moisture content should remain below 30% to prevent microbial growth and to maintain stability (Septiyanti *et al.*, 2019). Loss on drying indicates the water and other volatile compounds released during heating. Water- and ethanol-soluble extractive values represent the constituents extracted by polar and less polar solvents, respectively. The higher ethanol solubility suggests the predominance of semi-polar to non-polar compounds, and all parameters, including ash content ($< 5\%$), met the required standards (Damayanti *et al.*, 2024).

Screening of Phytochemical Extracts

The positive identification of flavonoids, tannins, saponins, and steroids in the ethanolic sea grape leaf extract supports its potential

Table 4 Results of the characterization of sea grape (*C. racemosa*) extract

Parameter	Results (mean $\pm$ SD)	Requirement (FHI, 2017)
Organoleptic		
Color	Greenish brown	-
Consistency	Viscous extract	-
Odor	Characteristic	-
Taste		-
Extraction yield (%)	23.36	-
Loss on drying (%)	5.56 $\pm$ 0.12	≤ 10
Moisture (%)	12.49 $\pm$ 0.89	≤ 30
Ash (%)	1.30 $\pm$ 0.02	≤ 5
Water-soluble extract (%)	6.39 $\pm$ 0.23	≤ 15
Ethanol-soluble extract (%)	3.42 $\pm$ 0.21	≤ 10

pharmacological activities. Flavonoids and terpenoid-related constituents may contribute to radical-scavenging activity by donating electrons or hydrogen atoms to neutralize free radicals (Swastini *et al.*, 2025). Saponins, owing to their amphiphilic properties, may also influence formulation behavior and skin-feel characteristics. Steroid/triterpenoid-like compounds may support barrier-related topical effects (Yusoff *et al.*, 2022). Therefore, the phytochemical profile provides a mechanistic link between the extract, antioxidant activity, and observed moisturizing performance, although compound-specific quantification is still needed to confirm the dominant active constituents. These findings suggest that ethanol is an effective solvent for extracting bioactive constituents from sea grape leaves and support the potential application of the extract in cosmetics or pharmaceutical formulations (Sommer *et al.*, 2022).

The ethanolic extract tested positive for flavonoids, saponins, terpenoids and steroids (Table 5). This profile is broadly consistent with solvent-comparison studies on *C. racemosa*, which detected phenolics, flavonoids, tannins, pigments, and diverse metabolites, although the presence and intensity of qualitative reactions differed by solvent (Palaniyappan *et al.*, 2023). Metabolomic profiling of *C. racemosa* has also identified multiple secondary metabolites and a clionasterol-rich fraction with antioxidant and cytoprotective properties. Differences among studies may arise from geography, season, light, salinity, thallus age, extraction polarity and assay specificity. Flavonoid- and terpenoid-related compounds can donate electrons or hydrogen

atoms in radical-scavenging reactions, whereas amphiphilic saponins may influence wetting and sensory behaviors. Because these tests are qualitative and prone to matrix interference, compound-specific chromatography and quantitative phenolic/flavonoid assays are required before assigning the moisturizing response to any individual class (Liyanage *et al.*, 2022). The results of the characterization of the *C. racemosa* extract are presented in Table 5.

Heavy Metal Analysis of Extract

Heavy metals (Pb, Hg, Cd, and As) in the *C. racemosa* extract were analyzed using Atomic Absorption Spectrophotometry (AAS) to determine whether the marine raw material complied with the relevant cosmetic contaminant limits. As seaweeds may accumulate metals from their aquatic environment, heavy metal screening is important before cosmetic formulation (Yuwanda *et al.*, 2023). The results of the heavy metal analysis are presented in Table 6.

The results showed that Pb was not detected, whereas Hg, As, and Cd were detected at levels below 0.001 ppm (Table 6). These values were below the stated limits and support the suitability of the extract as a cosmetic raw material under the analytical conditions used. This supports the suitability of this batch for formulation under the stated analytical conditions but does not establish that all *C. racemosa* raw materials have the same profile as this batch. Metal accumulation in macroalgae depends on species, binding sites in the cell wall, seawater concentration, sediment characteristics, pH, salinity, season,

Table 5 Phytochemical screening results of sea grape (*C. racemosa*) extract.

Phytochemical group	Method	Result
Alkaloids	Mayer's test	not detected
Flavonoids	Shinoda test	detected
Phenolics & Tannins	Ferric chloride test	not detected
Saponins	Froth test	detected
Terpenoids	Salkowski test.	detected
Steroids	Liebermann–Burchard test.	detected

Table 6 Results of heavy metal analysis of sea grape (*C. racemosa*) extract

Metals	Results (ppm)	Regulatory limit (BPOM No 16/2024)
Lead (Pb)	Not detected	≤20 ppm
Mercury (Hg)	<0.001 ^a	≤1 ppm
Arsenic (As)	<0.001 ^a	≤5 ppm
Cadmium (Cd)	<0.001 ^a	≤10 ppm

thallus age, and proximity to anthropogenic sources of pollution. Studies of marine macroalgae consequently show site- and species-dependent contaminant patterns, and recent work on *Ulva lactuca* has likewise treated heavy metal profiling as a necessary batch-specific safety assessment. Future sourcing should therefore include location-based monitoring and validated limits of detection and quantification. (Hainil *et al.*, 2023; Afifta, 2024).

Antioxidant Activity

The DPPH assay was chosen for its simplicity, sensitivity, and widespread use in estimating radical scavenging activity (Octora *et al.*, 2020). The *C. racemosa* extract showed a DPPH IC₅₀ of 84.94±0.42 ppm compared to 15.00±0.03 ppm for vitamin C. A lower IC₅₀ of vitamin C indicates greater radical-scavenging potency under the assay conditions. Solvent screening studies on *C. racemosa* have reported that antioxidant responses and total phenolic content vary markedly among methanol, ethanol, acetone, ethyl acetate, hexane, and petroleum ether extracts (Palaniyappan *et al.*, 2023). Such variations reflect differences in solvent polarity, extraction yield, pigment content, phenolic composition, collection site, and assay protocol. Therefore, the present value indicates measurable antioxidant potential but should not be compared numerically across studies without considering DPPH concentration, reaction time, wavelength, and expression units. This activity aligns with the presence of flavonoid-, terpenoid-, and steroid-related constituents. However, quantitative chemical profiling is necessary to determine their individual contributions (Wulandari *et al.*, 2022; Damayanti *et al.*, 2024).

Formulation of *C. racemosa* Extract for Facial Serum

The facial serum was formulated to balance the active extract loading, viscosity, spreadability, and skin compatibility. The *C. racemosa* extract served as the active marine antioxidant component. Carbomer and triethanolamine formed the gel network and contributed to viscosity, whereas glycerin, propanediol, and propylene glycol acted as humectants and solvent/co-solvent components of the gel. BHT was included to support oxidative stability, methyl paraben was used as a preservative, and purified water was used as the vehicle. Therefore, the formula design combined a marine extract with a humectant-rich serum base intended to improve skin hydration (Handayani *et al.*, 2023; Novika *et al.*, 2024).

Figure 1 shows the visual characteristics of the serum formulations after incorporating *C. racemosa* extract at different concentrations. F0, the base formula without extract, appeared white and opaque, whereas 5%, 7.5%, and 10% showed progressively darker brown coloration as the extract concentration increased from 5% to 10%. This concentration-dependent color intensification confirmed the successful incorporation of the ethanolic extract into serum. The darker appearance of the 7.5% and especially 10% formulation was expected, since higher extract levels contributed a greater amount of dissolved phytochemical constituents, including flavonoids, saponins, terpenoids, and steroids, which may influence the overall visual appearance of the preparation.

Despite these color differences, all formulations remained physically uniform and showed no visible phase separation, indicating adequate dispersion of the extract in

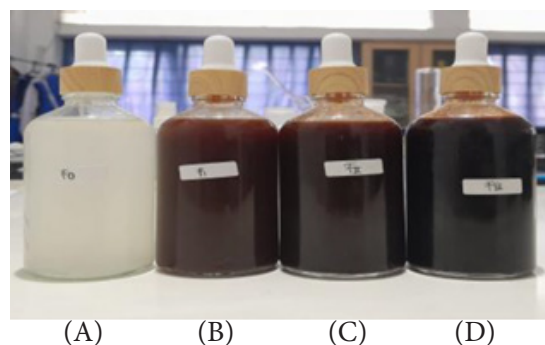


Figure 1 Physical appearance of facial serums containing *C. racemosa* extract at (A) 0%, (B) 5%, (C) 7.5%, and (D) 10%.

the carbomer-based serum matrix. The serum base was formed by carbomer neutralization with triethanolamine, glycerin and propylene glycol acted as humectants, and propanediol facilitated extract incorporation before mixing into the base. From a formulation perspective, the visual differences among 5%, 10%, and 10% mainly reflected the graded extract concentration rather than instability. Notably, 10% contained the highest extract concentration exhibited the darkest color, consistent with its superior moisturizing performance after 28 days. This finding suggests that the increased bioactive load in the 10% extract contributed not only to a more intense appearance but also to improved functional efficacy.

All freshly prepared formulations appeared uniform, without visible phase separation. Algae-based serum studies similarly report that extract concentration can modify color, odor, viscosity, and consumer acceptance, while compatibility depends on the extract composition and the gelling system. The darker 10% serum also achieved the highest hydration response, but the parallel trends did not show that the color-causing constituents produced this effect. This response may reflect the total extract load together with glycerin and glycols; controlled vehicle comparisons and chemical marker assays are required to clarify the mechanism (Septiyanti *et al.*, 2019).

Physical Stability of *C. racemosa* Extract

The results of the organoleptic, homogeneity, pH, and spreadability

evaluations before and after the cycling test are summarized in Table 7. The term “stable” should be interpreted cautiously because the study used only a cycling test. The extract-containing formulas maintained organoleptic appearance and homogeneity after cycling, whereas the 0% formulation became non-homogeneous. However, the pH values of several formulas increased after cycling. The 10% extract formula maintained the most acceptable pH profile among the extract-containing formulas, while the no extract, 5%, and 10% formulations showed post-cycling pH values above the commonly cited skin-compatible range of 4.5-6.5.

Therefore, the cycling test suggests acceptable preliminary physical stability, particularly for the 10% formulation, but long-term stability testing and pH optimization remain necessary (Agginihotri, 2021). The addition of the extract appeared to improve the physical integrity of the serum system, as the 5-10% formulations remained homogeneous after cycling, whereas the 0% formulation did not. This may be associated with the interactions among the extract constituents, humectants, and the carbomer gel network (Handayani *et al.*, 2023). Viscosity is a critical parameter for serum retention, spreadability, and consumer perceptions. If the extract concentration increases viscosity, the mechanism may involve additional dissolved solids and interaction with the polymeric network; if viscosity decreases, extract components may interfere with carbomer hydration or neutralization. Therefore, viscosity data are essential to support the formulation mechanism proposed in this study (Yeskar *et al.*, 2023).

Table 7 Physical stability results of facial serums formulated with sea grape (*C. racemosa*) extract.

Sea grape extract (%)	Organoleptic					
	Before the cycling test			After the cycling test		
	Color	Odor	Consistency	Color	Odor	Consistency
0	Cloudy white	Odorless	Liquid	Cloudy white	Odorless	Liquid
5	Red-maroon			Red-maroon		
7.5	Deep maroon	Characteristic of sea grape	Slightly vicious	Deep maroon	Characteristic of sea grape	Slightly vicious
10	Blackish maroon			Blackish maroon		
	Homogeneity					
	Before the cycling test			After the cycling test		
0		Homogeneous			Non-Homogeneous	
5		Homogeneous			Homogeneous	
7.5		Homogeneous			Homogeneous	
10		Homogeneous			Homogeneous	
	pH					
	Before the cycling test			After the cycling test		
0		5.92±0.10 ^a			7.12±0.21 ^b	
5		6.43±0.04 ^c			6.92±0.17 ^b	
7.5		6.52±0.02 ^{cd}			6.80±0.08 ^b	
10		6.10±0.03 ^{ab}			6.41±0.12 ^a	
	Spreadability test (cm)					
	Before the cycling test			After the cycling test		
0		5.01±0.21 ^a			7.43±0.10 ^{cd}	
5		5.38±0.14 ^{ab}			6.12±0.21 ^a	
7.5		5.72±0.23 ^c			6.82±0.31 ^c	
10		5.93±0.32 ^{cd}			6.42±0.27 ^{ab}	
	Viscosity (cP)					
	Before the cycling test			After the cycling test		
0		894±1.64 ^a			945±0.67 ^a	
5		1,213±3.96 ^b			1,102±1.87 ^{ab}	
7.5		1,326±4.09 ^b			1,304±3.24 ^c	
10		1,402±2.84 ^b			1,379±2.94 ^{cd}	

Note: Different letters within the same column indicate a significant difference ($p < 0.05$).

Safety Evaluation

A safety evaluation through an irritation test was performed on 30 volunteers using an open patch method, in which the facial serum was applied to a specific area on the lower arm for seven consecutive days. The observations focused on common irritation parameters, including erythema, papules, vesicles, and edema. None of the volunteers showed signs of irritation, indicating that the formulations were well tolerated. However, because only visible irritation was assessed and no comparator beyond the base formula was included, the claim should be limited to preliminary topical tolerability rather than broad clinical safety (Novika *et al.*, 2024). This finding indicates short-term topical tolerability under specified repeated open application conditions. This does not exclude delayed sensitization, subclinical barrier disturbance, or reactions in broader populations. The absence of irritation is biologically plausible given the low measured metal

concentrations and acceptable formulation profile; however, algal composition, residual solvent, preservative exposure, individual skin sensitivity, dose, contact time, and occlusion can modify irritation outcomes. Larger controlled patch studies with standardized dermatological scoring and longer follow-up periods are required before making general safety claims (Bogdan *et al.*, 2025).

Efficacy of Facial Serum

Before treatment, the mean skin moisture values across all volunteers were in the dehydrated range. After 28 days of application, all formulas increased skin hydration; however, the magnitude of improvement differed among the formulas (Table 8 and Figure 3). Recovery at day 28 was 13.61%, 52.20%, 56.60%, and 60.82% for the 0%, 5%, 7.5%, and 10% formulations, respectively. The response of the 0% serum confirmed that glycerin and glycols contributed substantially through humectancy. The larger responses with extract-

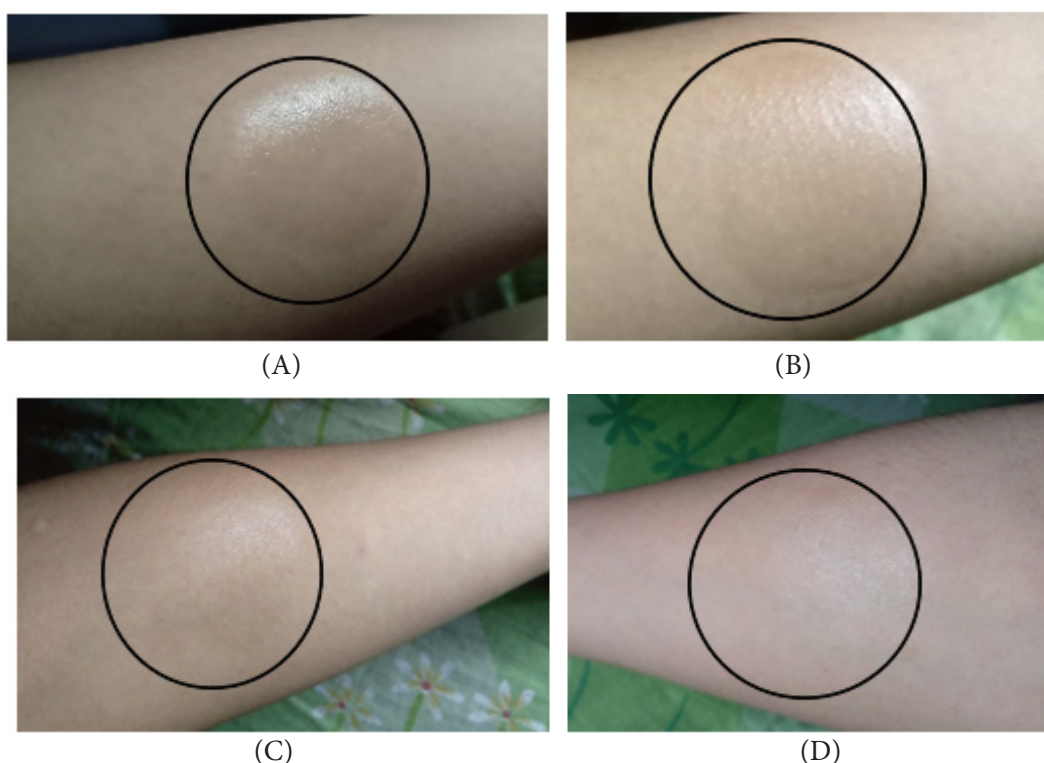


Figure 2 Representative Day-7 open-patch test observations following treatment with facial serum containing *C. racemosa* extract at (A) 0%, (B) 5%, (C) 7.5%, and (D) 10%. All test sites were clinically normal at baseline (Day 0) and showed no visible erythema, papules, vesicles, or edema after seven days of application.

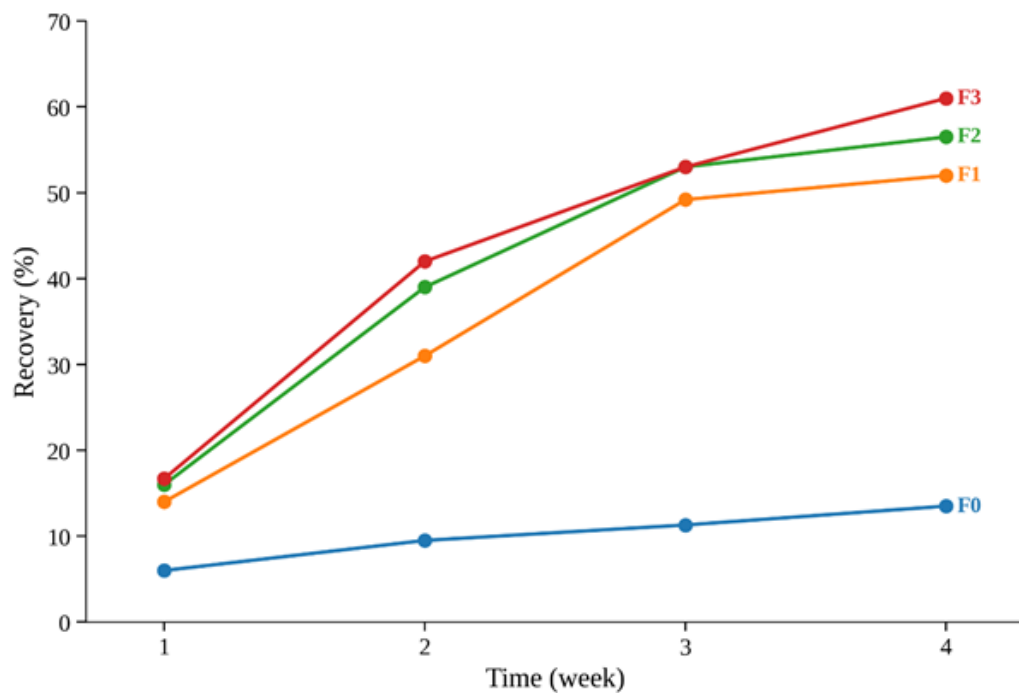


Figure 3 Skin hydration recovery during a 4-week treatment period with facial serums incorporating *C. racemosa* extract at concentrations of 0% (—), 5% (—), 7.5% (—), and 10% (—).

containing serums indicate an additional concentration-associated effect, potentially related to hydrophilic polysaccharides, film formation, and antioxidant support for barrier function.

Reviews of topical macroalgae describe similar moisturizing and antioxidant potentials, but also emphasize that species, extraction method, molecular composition, and vehicle strongly influence efficacy. Marine bioactives may indirectly support hydration by reducing oxidative stress and contributing to skin barrier maintenance, while humectants attract and retain water in the stratum corneum. Therefore, the results suggest a concentration-related improvement but should not be interpreted as proof that the extract alone caused the moisturizing effect without controlled mechanistic testing (Bogdan *et al.*, 2025). Because allocation, blinding, environmental conditioning, and between-treatment statistical comparisons were not fully controlled, the findings should be interpreted as preliminary efficacy rather than proof of a causal dose-response relationship.

Hedonic Test

The hedonic test was performed to evaluate the sensory acceptability of facial serum formulations with different concentrations of *C. racemosa* extract: Formula of 5%, 7.5%, and 0% among 30 panelists. The sensory parameters assessed included color, texture, aroma, taste, and overall preference. Statistical analysis using the Mann-Whitney U test ($p < 0.05$) was conducted to identify significant differences between the formulations.

The hedonic test evaluated the consumer-relevant sensory attributes (Table 8). The sensory evaluation focused on the extract-containing formulas) and included only parameters relevant to topical products. Formulas 10% showed the highest overall acceptance score (3.83 ± 0.03) and texture score (3.83 ± 0.09). In contrast, aroma scores decreased slightly as the extract concentration increased, suggesting that the characteristic sea grape odor may become more noticeable at higher concentrations. Overall, the 10% extract-containing formulas showed the best balance of texture, color, and acceptance

Table 8 Hedonic scores of facial serums containing *C. racemosa* extract (5%, 7.5%, and 10%)

Parameters	<i>C. racemosa</i> extract (%)		
	5	7.5	10
Color	2.96±0.03 ^a	3.01±0.02 ^b	3.10±0.11 ^b
Texture	3.67±0.16 ^a	3.72±0.14 ^a	3.83±0.09 ^a
Aroma	4.03±0.05 ^b	3.92±0.8 ^a	3.81±0.06 ^a
Overall	3.34±0.09 ^a	3.73±0.12 ^b	3.83±0.03 ^b

Note: Different superscript letters in the same row indicate significant differences ($p < 0.05$) based on the Mann–Whitney U test.

among the extract-containing formulas, with statistical grouping reported after confirming the final data. Normality testing was first performed using the Shapiro–Wilk test, with results of $p < 0.05$. Hedonic scores were derived from an ordinal scale, and the Mann–Whitney U test was used to compare the sensory acceptance of the formulations. A significance level of $p < 0.05$ was applied (Iskandar *et al.*, 2025).

CONCLUSION

A 10% (w/w) *C. racemosa* extract serum provided the best overall balance in this study, producing the highest 28-day hydration recovery (60.82%), remaining homogeneous after temperature cycling, and causing no visible irritation during the seven-day test. The extract exhibited DPPH antioxidant activity (IC_{50} 84.94±0.42 ppm) and heavy metal concentrations below the cited cosmetic limits. These results support the further development of *C. racemosa* as a moisturizing serum ingredient; however, pH optimization, verified inferential statistics, chemical standardization, and controlled long-term stability and efficacy studies remain necessary.

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