Antiaging effect of brown macroalgae *Sargassum cristaefolium* gel formulation by inhibition of collagen degradation

E S Prasedya¹, N W R Martyasari¹, B T K Ilhami¹, H Padmi¹, S Widyastuti², A L Sunarwidhi³*, and H Sunarpi¹

¹Department of Bioscience and Biotechnology, Faculty of Mathematics and Natural Sciences, University of Mataram, Mataram, Indonesia  
²Faculty of Food Technology and Agrobusiness, University of Mataram, Indonesia  
³Department of Pharmacy, Medical Faculty, University of Mataram, Indonesia  

*Corresponding author: anggit.sunarwidhi@unram.ac.id*

**Abstract.** Recent strategies for skin protection against UV radiation have included the incorporation of natural photoprotective agents, such as gel formulations. Our previous study demonstrated the UV photoprotective activity of brown macroalgae *Sargassum cristaefolium* ethanol extract (SCE). In this study, we aim to determine the optimum gel formulation for the incorporation of SCE. In addition, the UV antiaging activity was evaluated *in vivo* with mice as animal models. Gel formulation with various concentrations of Glycerin, Carbopol, and TEA was evaluated with the simple lattice design (SLD) method in Design-Expert Software. The optimum gel formula containing 0.1% SCE inhibited collagen degradation in mice skin irradiated by UV-A. It can be concluded that the gel formula successfully incorporates SCE and possibly provides antiaging activity *in vivo* by inhibition of collagen degradation. However, further investigations need to be done to conclude the photoprotective activity of SCE gel formulation (SCEg). In addition, exploration of the *Sargassum* brown macroalgae based gel formula should be considered for cosmeceutical applications.

**Keywords:** design-expert, hydrogel formulation, macroalgae, photoprotective, Sargassum

1. **Introduction**

Solar Ultraviolet Radiation (UVR) reaching the earth’s surface can be classified as UVA (320-380 nm) and UVB (290-320 nm) according to its wavelength [1]. UVR provides various life benefits such as the synthesis of vitamin D [2]. However, chronic exposure to UVR could also result in various negative health effects. Chronic UVR exposure has many effects on skin pathologies, including erythema, inflammation, cancer, and especially premature skin aging. Therefore, there are numerous efforts to investigate active ingredients from various natural resources for the development of photoprotective agents.

Phytoconstituents are gaining interest as ingredients in cosmetic formulations because have less potential toxicity in humans compared to synthetic agents [3]. Marine algae or seaweeds largely inhabit the intertidal coastal areas which are often exposed to extreme conditions such as UV radiation. To survive such conditions, macroalgae are well known to exhibit various bioactive substances with broad pharmaceutical and cosmeceutical potentials [4]. Based on previous studies, brown algae contributes the most to photoprotective activity. The brown macroalgae *Sargassum*

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*muticum* has demonstrated anti-wrinkle potential in both *in vitro* and *in vivo* studies [5]. Several algae-based skin products have also been marketed, such as Helionori by Gelyma and Helioguard365 and Protulines by Exysmol S.A.M., Monaco [6]. Therefore, algae could be considered a potential cosmeceutical agent for the development of natural-based skin care products.

Gels are cross-linked polymers with hydrophilic groups that enable them to absorb large amounts of water. Gel formulations provide advantages such as low toxicity potential and sustained drug release which is considered suitable carriers for cosmeceutical applications [7]. Furthermore, gels have the advantage of biocompatibility, softness, and high water content which could mimic natural tissue properties and because of their swelling and hydrating capability could avoid irritation to enclosed tissues.

In a previous study, we demonstrated the photoprotective activity of *Sargassum cristaefolium* in *vitro* and *in vivo*. Currently, there are limited reports of the gel formulations containing macroalgae extract as bioactive ingredients. Hence in this study, we aim to investigate the optimum hydrogel formulation for topical application of *Sargassum cristaefolium*, as well as to evaluate its potential photoprotective activity.

2. **Materials and Methods**

2.1. **Macroalgae collection and extract preparation**

Brown macroalgae *Sargassum cristaefolium* was collected from the west coastal area of Lombok (8°29′54.251″ S and 116°4′36.664″ E). Identification was carried out with determination assisted by algae electronic database. Samples were rinsed with surface seawater before the further process in the laboratorium. Samples were then dried under room temperature conditions for 3-4 days without direct exposure to sunlight. Dry samples were ground and dissolved in absolute ethanol solvent 5x volume of the sample biomass weight (w/v). Mixed solutions were incubated at room temperature for 48h maceration process followed by stirring every 6 hours. After 24 hours, the mixed solutions were filtered with Whatman grade 1 filter papers. Finally, the ethanol solvent in the macroalgae extracts was evaporated with a rotary evaporator (Sanjing,China). Obtained filtrates in paste form were then used as the macroalgae ethanol extract for further experiments.

2.2. **Optimization of gel formulation**

Determination of the optimum hydrogel formulation was conducted using simplex lattice design (SLD) by Design-Expert software version 11. Simplex lattice design optimized the mixture of three components which are Glycerin, Carbopol, and TEA from 13 formulations in various compositions. From these predicted formulations, manual measurements were carried to obtain spreadability, adhesive capability, and pH value.

2.3. **Animals**

For this experiment, a total of 30 female BALBL/c mice, 6–8-week-old and weighing 25–30 g were used. The mice had free access to food and water at a temperature (24–25 °C). Animals were also subjected to a 12 h light/dark cycle. All experimental procedures complied with the Health guidelines for Care and Use of Laboratory Animals and were approved on 15 January 2020 by the Bioethics Committee of Medical Faculty, University of Mataram.

2.4. **In vivo UV-A Irradiation**

Mice were randomly divided into 4 groups (n = 3 per group) that represented treatments and controls. These were: normal healthy controls with no UV treatment, normal healthy controls treated with UV after administration of gel formulation (Vg), group treated with UV irradiation after administered with *Sargassum cristaefolium* gel formulation (SCEg). Before experimentation, mice were anesthetized by intraperitoneal injection of 1% sodium pentobarbital, and hair was removed from the back skin of mice using hair removal wax. Parallel UV lamps Reptile UV150 PT2188 13W (Exo Terra, Winchester, MA, USA) were used for UV-A irradiation treatment. The distance of the UV lamp to mice was 20 cm to obtain UVA spectral radiance of 300 μW/cm². Animals were irradiated for 7 days, 2 h/day, with a daily dose of 2.16 J/cm²[5].

2.5. **Histological examination**
For histological studies, the skin specimens were fixed in 10% formaldehyde and embedded in paraffin. The sections were then deparaffinized in xylene, rehydrated through a graded alcohol series, and stained with Masson's Trichrome staining kit according to the manufacturer’s protocol. Pathological changes were observed under an inverted microscope (Zeiss Axio Observer Z1, Germany).

2.6. Statistical analyses
Statistical analyses were conducted using Graphpad Prism 8.0 (GraphPad Software, Inc., San Diego, CA). Statistical comparisons were performed using two-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. The data are presented as means ± standard deviation (SD). Differences among comparisons were considered statistically significant for $p$-values less than 0.05. Highly significant values for $p$-values less than 0.01.

3. Results and Discussion
3.1. Gel formulation of SCE
Crude extracts are sensitive to light and temperature which could reduce their activity [8]. Hence, for cosmeceutical applications, a vehicle is needed to effectively deliver the bioactive substance to target application areas. This study aims to investigate optimum gel formulation to incorporate *Sargassum cristaefolium* extract and evaluate its photoprotective activity.

Optimization and gel formulation were done using the simplex lattice design method available in Design-Expert software version 1.1. Based on variations in the concentration of three ingredients (Glycerin, Carbopol, and TEA), the software recommended 13 gel-based formulations. These suggested formulations were prepared manually and evaluated for their physical properties (Spreadability, Adhesive capability, and pH) to obtain the optimum formula. The gel formulation was prepared based on the obtained optimum formula to incorporate 0.1% SCE into hydrogel base (Table 1). The selection of this concentration is based on our previous work [5].

**Table 1.** Gel formulation of SCEg.

| Ingredients             | Concentration |
|-------------------------|---------------|
| Carbopol                | 0.75 %        |
| Glycerin                | 1.90 %        |
| Triethanolamine (TEA)   | 2.90 %        |
| Methyl paraben          | 0.2%          |
| Propyl paraben          | 0.1%          |
| Fragrance               | 5-7 drops     |
| Sargassum extract       | 0.1%          |
| Aquadest                | Add to 100 mL |

3.2. SCEg effects on collagen density
Gel formulation containing SCE (SCEg) was evaluated for its effects on maintaining collagen. Collagen is the most abundant protein in the dermal connective tissue [9]. Hence, preventing collagen degradation is crucial for preventing physiological aging effects due to UV radiation. Exposure to UV radiation is the primary factor for extrinsic skin aging which leads to collagen degradation. Collagen density was measured with Masson’s Trichrome staining (Figure 2). In the control group with no UV irradiation, collagen fibers stained with blue dye were abundantly found in the dermis of the dorsal skin. However, pre-treatment with SCEg showed reduced collagen degradation compared to control irradiated with UV (Vg). The previous study also shows that pre-treatment with *Sargassum muticum* before UV radiation show lower levels of damage in collagen fibers [10].
Figure 1. Masson’s Trichrome staining of dorsal skin sections treated with UVA for 7 days. The blue parts represent collagen fibers. Collagen fibers density was measured as with ImageJ Color deconvolution plugin (n=6). * is considered significantly different compared to control with no UV treatment (p<0.005). ** is considered highly significant compared to control treated with no UV treatment (p<0.001).

4. Conclusion
In conclusion, the optimum hydrogel formulation obtained from Design Expert Software successfully incorporated SCE (SCEg). SCEg significantly inhibited collagen degradation in mice compared to control group without SCEg treatment before UV radiation. Further applications of hydrogel formulation for delivering biological activity of bioactive substance should be considered.

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