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Evaluation of the Potential of *Gracilaria* sp. Extract from Gunungkidul, Daerah Istimewa Yogyakarta, as a Natural Sunscreen Agent in BALB/c Mice

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ABSTRACT

Indonesia's equatorial location, tropical climate, and decreasing ozone layer permeability result in high UV-B radiation exposure, increasing the risk of erythema, burns, edema, and skin cancer. Although sunscreens offer essential protection, many commercial products rely on synthetic ingredients linked to adverse effects such as dermatitis, premature aging, DNA damage, and potential carcinogenicity. *Gracilaria* sp., a red macroalga abundant in Krakal Beach, Yogyakarta, Indonesia, is a promising natural alternative due to its phytochemical compounds with known antioxidant and UV-B protective properties. This study aimed to identify the bioactive compounds in *Gracilaria* sp. and to evaluate their antioxidant capacity, sun protection efficacy, physical characteristics in sunscreen spray formulations, and *in vivo* effects using BALB/c mice. Extraction was conducted via maceration with 70% ethanol. Sunscreen sprays were formulated with extract concentrations of 4000, 6000, and 8000ppm. Analyses included phytochemical screening, total phenolic and flavonoid content, antioxidant activity (percentage inhibition value 80,4%), SPF measurement, physical stability, and *in vivo* testing. Results confirmed the presence of phenols, flavonoids, alkaloids, and saponins, with total phenolic and flavonoid contents of 11.23 mgGAE/g and 3.95 mgQE/g, respectively. The 8000ppm formulation showed the highest SPF (37,15), meeting Indonesian National Standard (SNI) criteria, while the 6000 pm product achieved SPF 26.75 and demonstrated 0 erythema *in vivo*, indicating effective UV-B protection. These findings highlight the potential of *Gracilaria* sp. ethanol extract as a safe, natural UV filter for sunscreen formulations.

INTRODUCTION

In recent decades, ozone depletion has drastically increased ultraviolet (UV) radiation, especially UV-B radiation that reaches the Earth's surface **(Chrapusta *et al..*, 2017)**. UV-B exposure is important for human health as vitamin D in the skin. However, excessive and repeated exposure to UV-B light can cause cell damage **(Kageyama & Waditee-Sirisattha, 2019)**. A frequent effect of UV-B exposure is erythema, which is abnormally red skin **(Chrapusta *et al..*, 2017)**. Based on **Punchard *et al.* (2004)**, the appearance of erythema starts with heat and pain, which then causes inflammation or an inflammatory reaction. Higher UV-B radiation can cause burns, edema, and stimulate

cancer growth **(Chrapusta *et al.*., 2017)**, because of the dangers of UV-B exposure for human health, additional skin protection is needed to avoid and prevent skin damage.

Sunscreen is a skin protection product that contains a UV protector that can protect the skin from direct exposure to UV-B rays **(Daud *et al.*, 2018)**. Sunscreen spray lotion is a spray lotion dosage form that is applied by spraying. This dosage form is a development of lotion preparations that are more practical in use, lightweight and comfortable to use **(Ariyanti *et al.*, 2022)**. Most commercial sunscreens have been using synthetic UV blocking agents **(Lumantow *et al.*, 2023)**. The most used UV filter materials are chemical filters or organic filters **(Mansuri *et al.*, 2021; Ballestin & Bartolome, 2023)**. Chemical filter types such as PABA, OC, avobenzone, oxybenzone, octiboxate, and BP-3 can be harmful to the human body such as causing dermatitis, endocrine disruption, premature aging, damage to genetic material, and skin cancer. Therefore, efforts need to be made to replace harmful synthetic-based sunscreens with natural ingredients that are safe for health. Natural ingredients that can be used as UV protectors in sunscreen can come from plants that contain antioxidant and anti-radiation compounds **(Dampati & Veronica, 2020)**. Compounds in plants that are known to have potential as antioxidants and UV protectors are phenolic compounds, flavonoids, terpenoids, and saponins **(Daud *et al.*, 2018; El-Sheekh *et al.*, 2024)**. Ethanol extract of *Gracilaria* sp. from Banten-West Java, found alkaloids, phenols, saponins, flavonoids, triterpenoids **(Purwaningsih & Deskawati, 2020)**. Flavonoid, terpenoid, and phenolic compounds in *Gracilaria* are expected to play a significant role as natural UV protectors.

Indonesia is an archipelago rich in macroalgae biodiversity, about 325 identified species consisting of 103 Chlorophyceae (green algae), 167 Rhodophyceae (red algae), and 55 Phaeophyceae (brown algae). Macroalgae are mostly found in mangrove forests and coral reefs on the islands of Sumatra, Java, Kalimantan, and Sulawesi **(Basyuni *et al.*, 2024)**. One of the macroalgae that is widely found in Indonesian waters and has high economic value is *Gracilaria* sp. that belongs to the family of Rhodophyceae **(Annisaqois *et al.*, 2018)**. *Gracilaria* is also one of the three species of Rhodophyceace (besides *Eucheuma, Kappaphycus*) that are commercially cultivated in most of coastal Indonesia **(Basyuni *et al.*, 2024)**. Several research mentioned that *Gracilaria* sp. contains secondary metabolites such as Phycoerithrin, polysaccharides, flavonoids, saponins, alkaloids and phenolics that can counteract UV radiation **(Bhernama *et al.*, 2020; Lu *et al.*, 2022; Setyorini, 2022; Amin, 2024)**. The deterrence mechanism is carried out by absorbing UV rays and acting as an antioxidant that counteracts free radicals so that it can overcome photoaging and skin cancer.

Krakal Beach in Gunungkidul Regency, Yogyakarta Special Region, Indonesia has sandy corals and dead coral substrates along the shore, which are natural growing places for various types of macroalgae, including Gracilaria **(Stephani *et al.*, 2014; Prasetyaningsih & Rahardjo, 2016; Wiyanto & Dwi, 2020)**. Macroalgae that live in a complex marine environment will produce various kinds of secondary metabolites in their

cells and are used as a defense against unfavorable environmental conditions including epiphytes, epibionts, bacteria and oxidative stress **(de Oliveira *et al*., 2017; Messina *et al*., 2019)**. Efforts to utilize this natural macroalgae are still limited to being used only as food ingredients for agar, while the potential compounds contained therein can be utilized in the fields of cosmetics and health. Therefore, it is necessary to conduct research to optimize the potential of Gracilaria as a UV-B protector.

Despite its richness in red algae diversity, this biotic potency of Indonesia has not been explored and utilized optimally for human interests **(Kurnia & Kasanah, 2025)**. Studies on the chemical diversity and potential bioactivity of red algae that grow naturally in Indonesia need to be carried out, especially to become natural ingredients in cosmetics and health. Therefore, this study aimed to identify bioactive compounds in red algae *Gracilaria* sp. from Krakal Beach, Yogyakarta, and evaluate their antioxidant activity, SPF efficacy, physical properties in sunscreen spray formulations, and *in vivo* performance using BALB/c mice.

MATERIALS AND METHODS

1. Sample preparation

Red algae, *Gracilaria* sp. (Fig. 1) was collected in February 2024 from Krakal Beach (8° 8' 48.2172" S, 110° 35' 49.9704" E) Gunungkidul Regency, Yogyakarta, Indonesia. Fresh samples were sorted, removed from dirt and corals and washed under running water **(Assaw & Mazlan, 2018)**. Clean samples were cut into smaller sizes, then wind-dried before being oven-dried at 40°C, until the moisture content was <10%. This dried alga was then ground until it became powder **(Tias *et al*, 2023)**. The powder was then stored in a dark room in food grade plastic at room temperature.

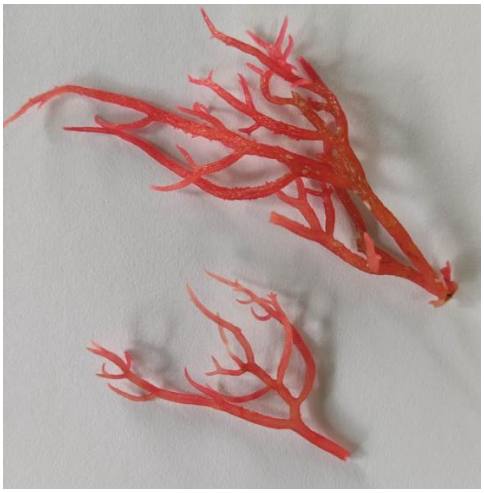

Fig. 1. Habitus of *Gracilaria* sp. from coast Gunungkidul, Daerah Istimewa Yogyakarta

2. Metabolite extraction

Gracilaria sp. powder of 130g (triplicate) was subjected to exhaustive extraction by macerating in 70% ethanol (Grade A/food grade) with a ratio of 1:7 at room temperature (27ºC) for 3 x 24 hours with ethanol replacement every 24 hours. Ethanol was evaporated using a rotary evaporator (brand) at 50°C, 50rpm to produce crude extract **(Susanto *et al*, 2021)**. The extract was stored in a refrigerator at 4°C, which was then used for experimental procedures.

3. Qualitative phytochemical analysis

3.1. Alkaloid test

A 3g sample of crude extract was added to 5mL of 2N HCl, then heated for 2–3 minutes. Subsequently, 0.3g of NaCl was added to 5mL of 2N HCl. The mixture was divided into three portions: the first served as a blank; the second was treated with 3mL of Dragendorff's reagent; and the third was treated with 2–3 drops of Mayer's reagent. The sample is considered positive for alkaloids if an orange precipitate forms in the second tube and a yellowish precipitate forms in the third tube **(Setyaningrum & Susanti, 2022)**

3.2. Flavonoid test

A 3g sample of crude extract was mixed with 10mL of water, boiled for 5 minutes, and then filtered. To 5mL of the filtrate, 0.05g of magnesium powder and 3 drops of concentrated HCl were added, followed by shaking. A positive result for alkaloids is indicated by the appearance of a red or orange-colored solution **(Nadzifah *et al.*, 2024)**

3.3. Terpenoid and steroid test

A 3g sample of crude extract was dissolved in 5mL of chloroform, and 5mL of anhydrous acetic acid was added. Subsequently, 1- 2mL of concentrated sulfuric acid was added through the tube wall. Tripenoid positive samples are characterized by the presence of brownish or violet rings on the border. Positive samples containing steroids are characterized by the formation of a bluish green color **(Susanto *et al*, 2021)**.

3.4. Tannin test

A 3g sample of crude extract was added to 10mL of hot water and 2-3 drops of FeCl3 1%. Positive samples contain tannins if they experience dark green or dark blue color changes **(Susanto *et al*., 2021)**.

3.5. Saponin test

A 3g sample of crude extract was added to 10mL of hot water, cooled and shaken vigorously for 10 seconds. The sample is positive for saponins if there is a consistent froth as high as 1-10cm for 10 minutes **(Susanto *et al.*, 2021)**.

3.6. Phenol test

A 3 g sample of crude extract was added to 10mL of hot distilled water and 3–4 drops of 10% NaCl. The mixture was stirred and filtered, then divided into three test tubes. The first tube served as the blank; to the second tube, 2–3 drops of $FeCl_3$ were

added, and to the third, 3 drops of 1% gelatin were added. The sample was considered positive for phenols if a green, red, purple, blue, or black color developed **(Sari, 2020; Majid, 2022)**.

3.7. Determination of total flavonoid content

A total of 0.019g (19mg) of quercetin was dissolved in 10mL of ethanol, and standard solutions with concentrations of 10–100ppm were prepared in 10ppm increments. From each concentration, 1mL was taken and mixed with 1mL of 10% $AlCl_3$ and 1mL of 1 M potassium acetate. The mixtures were incubated for 30 minutes at room temperature, and absorbance was measured using a spectrophotometer at a wavelength of 431nm.

For the extract sample, 3g (or 0.020g/ 20mg) was dissolved in 5mL of ethanol. Then, 1mL of this solution was mixed with 2mL of reagents (10% $AlCl_3$ and 1 M potassium acetate). After incubation for 30 minutes at room temperature, the absorbance was measured at 431nm **(Kandarpa *et al.*, 2021)**.

3.8. Determination of total phenolic content

A 200ppm gallic acid stock solution (6mg/mL) was diluted with distilled water to a final volume of 30mL. Standard solutions of gallic acid were prepared at concentrations of 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200ppm. From each concentration, 100 µL was taken and mixed with 1mL of Folin–Ciocalteu reagent and 2mL of distilled water. The mixtures were vortexed and left to stand for 5 minutes at room temperature. Then, 1mL of 7% w/v Na_2CO_3 solution was added and incubated for 30 minutes. Absorbance was measured at 75nm.

The total phenolic content of the *Gracilaria* sp. extract sample was determined similarly. A 1000ppm stock solution of the extract was prepared, and the procedure followed the same steps as for the gallic acid standards **(Djapiala & Montoalu, 2013)**. The absorbance value of the blank was subtracted from that of the sample to obtain the net absorbance, which was then used to calculate the gallic acid equivalent concentration (mg/mL) using the linear regression equation from the standard curve. Total phenolic was calculated using the formula:

$$\text{KTFe} = \frac{V \times C \times FP}{g}$$

Where, KTFe = Total phenol content (mg GAE/g); g = Weight of extract used (g); C = Sample concentration (mg/mL); FP = Dilution factor; V = Volume of sample solution **(**mL**)**.

4. Semi-quantitative phytochemical analysis

4.1. Gas chromatography-mass spectrometry (GC-MS)

The detection of small molecules, including terpenoids with small molecular sizes, from *S. ilicifolium* extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Analysis was conducted at the Forensic Laboratory Center of the Indonesian National Police (abbreviated as POLRI in Indonesian) in Sentul, Bogor, West Java.) The GC-MS apparatus used was Agilent Type 5977B which was equipped with 19091S-433 capillary column and 93.92873 HP-5MS 5% Phenyl Methyl Silox which measures 30 m x 250 μm x 0.25 μm. The injector and detector temperatures were set to 325°C, and the initial column temperature was set at 60°C with a pressure of 8.2317 psi. One microliter of extract was injected in split mode using an autosampler. The helium carrier gas was programmed to maintain a constant flow rate of 1 ml min-1. The mass spectrum was operated at 70 ev and mass range from 35 to 650 m z-1, with threshold value 150 and solvent delay 2 minutes.

4.2. Liquid chromatography-mass spectrometry (LC-MS)

Liquid Chromatography-Mass Spectrometry (LCMS) analysis was conducted at the Forensic Laboratory Center of the Indonesian National Police (abbreviated as POLRI in Indonesian) in Sentul, Bogor, West Java. Prior to shipping, samples were prepared by weighing 15mg of sample extract, then placed into a falcon bottle, and homogenized using a vortex. Each falcon bottle was labeled with a sample code. After the LC-MS test, the chromatogram display was obtained. The LC-MS test method used was the MS ES + method, which means that in the injection process the sample was shot with 1 H atom, so that in the identification of compounds, the stage of reducing 1 H element in the chemical component was carried out.

5. Antioxidant assay

5.1. Stock solution and DPPH absorbance

DPPH (0.75mg) was dissolved in methanol to a final volume of 15mL in a dark bottle, resulting in a concentration of 50ppm. A total of 1mL of the 50ppm DPPH solution was mixed with 4mL of methanol (p.a.), vortexed, and incubated in dark conditions for 30 minutes. The absorbance was then measured using a UV-Vis spectrophotometer at the maximum wavelength of 517nm.

5.2. Measurement of antioxidant activity of vitamin C and samples

A total of 0.5 mg of vitamin C was dissolved in methanol (p.a.) to a final volume of 5mL, resulting in a concentration of 100ppm. Sample solutions at concentrations of 4, 5, 6, 7, and 8ppm were also prepared in methanol to a final volume of 5mL. Then, 1mL of DPPH solution was added to each, the mixtures were vortexed, and incubated for 30 minutes in dark conditions. Absorbance was measured using a UV-Vis spectrophotometer at 517nm **(Gusungi *et al.*, 2020)**.

The antioxidant activity of the extract samples was assessed using the same procedure as that of vitamin C. Extract concentrations of 500, 750, 1000, 1250, 1500, and 1750ppm were prepared. Absorbance was measured, and the percentage of inhibition was calculated using the formula described by **Sami *et al.* (2021)**:

$$\% \text{ inhibition} = \frac{\text{Abs DPPH Blanko} - \text{Abs sampel}}{\text{Abs DPPH Blanko}} \text{ X } 100\%$$

6. Sun protection factor (SPF) test of extract and product

6.1. Extract SPF test

The effectiveness of sunscreen ingredients was measured using the Sun Protection Factor (SPF) value. A high SPF value indicates better sunscreen protection against UV exposure **(Wiraningtyas *et al.*, 2019)**. The test concentrations used for the SPF test of the extracts were 2000, 4000, 6000, and 8000ppm. Oxybenzone 4000ppm was used as positive control.

6.2. Product SPF test

With the addition of *Gracilaria* extract to sunscreen lotion products, 3 concentrations with the best SPF values were selected, namely: 4000, 6000, and 8000ppm. As a positive control, 4000ppm of commercial sunscreen products, SPF 50 and SPF 15, were used. The SPF absorbance of both extracts and products was measured using a spectrophotometer and a blank (ethanol) of the solvent used. Measurement of absorbance values was carried out at a wavelength of 290- 320nm using a UV-Vis spectrophotometer and the formula according to **Dharmawan *et al.* (2023)**:

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}\ (\lambda) \times 1\ (\lambda) \times \text{abs}\ (\lambda)$$

Where, CF = Correlation factor (10), EE = Erythema efficiency, I = Sunlight simulation spectrum, Abs= Readable absorption value

7. Product manufacturing and quality test of sunscreen spray lotion

Formulations for the manufacture of the sunscreen spray lotion are listed in Table (1). The production process involves two main phases: the oil phase and the water phase. In the oil phase, cetyl alcohol, dimethicone, propylparaben, and Span 80 are combined. The water phase consists of one-third of the total volume of distilled water, glycerin, nipagin, and Tween 80. Both phases are heated separately at low temperature.

To prepare the acrylate copolymer mixture, 30mL of distilled water is mixed with triethanolamine (TEA), followed by the addition of the acrylate copolymer. The mixture is vortexed until homogeneous. Once the oil phase has fully liquefied, butylated hydroxytoluene (BHT) is added and mixed thoroughly.

Next, the water phase and acrylate copolymer mixture are gradually added to the oil phase using a mixer at medium speed for one minute. The remaining distilled water is then added and stirred gently for an additional two minutes. Once the mixture reaches

room temperature, the extract is added, and the final product is homogenized and transferred into spray bottles.

This formulation process was repeated in triplicate, as shown in Table (1).

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Table 1. Composition of sunscreen spray lotion formula with the addition of *Gracilaria* sp. extract **(**modified from **Ariyanti *et al.*, 2022)**

Material	**Basic (g)**	**F1 4000 ppm (g)**	**F2 6000 ppm (g)**	**F3 8000 ppm (g)**
Extract	0	0.4	0.6	0.8
Dimetikon	3	3	3	3
Cetyl alcohol	1	1	1	1
Propyl paraben	0.25	0.25	0.25	0.25
Span 80	0.7	0.7	0.7	0.7
BHT **(**Butylated Hydroxytoluene**)**	0.1	0.1	0.1	0.1
Glycerin	4	4	4	4
Nipagin	0.31	0.31	0.31	0.31
Tween 80	0.3	0.3	0.3	0.3
TEA **(**Triethanolamine**)**	0.6	0.6	0.6	0.6
Acrylates	0.9	0.9	0.9	0.9
Aquades	88.14	88.51	87.14	86.14

Note: F1 = Formulation 4000 ppm, F2 = Formulation 6000 ppm, F3 = Formulation 8000 ppm.

8. Product quality test

8.1. pH test

The purpose of pH testing is to ensure that the pH value of all formulations meets the specified quality requirements, which are standards with range limits based on SNI 16- 4399-1996, namely 4.5 to 7.5. **(Lumantow *et al.*, 2023)**. The pH value in all formulations was measured using a pH meter.

8.2. Homogeneity test

This test is performed using an object glass. Each sample was spread on the preparation glass and observed using a magnifier. Homogeneous samples indicate that the particle size visually shows uniformity **(Ariyanti *et al.*, 2022)**.

9. In vivo efficacy test of sunscreen spray lotion

The study employed male BALB/c mice (*Mus musculus*) aged two months, with a body weight of 20- 25g. The use of these mice has already been approved by the Ethics Committee for Health Research, Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta with ethical approval number 1654/C.16/FK/2024. Before testing, the mice underwent an acclimatization period of 14 days to allow them to adjust to their new environmental conditions. The mice's back hair was shaved to form an area of 15cm^2 (3x5 cm) for the UV-B exposure test. The treatment was divided into 6 groups with each treatment consisting of 4 mice. The first treatment is the negative control which is

applied with basic sunscreen only. The second and third treatments were positive controls applied with commercial sunscreen SPF 50 and SPF 15, and the fourth, fifth, and sixth treatments were applied with *Gracilaria* sp. ethanol extract lotion spray 4000, 6000, and 8000ppm. Each treatment was exposed to UV-B light for 24 hours for 24 hours **(Amini *et al.*, 2020)**. Analysis of the ability of sunscreen to protect the skin is done by visually observing the presence of red spots (erythema response) with a score of 0- 4 on the shaved skin area **(Kandarpa *et al.*, 2021)**. In scoring erythema, the provisions of Score 0 (No erythema), Score 1 (slight erythema), Score 2 (clearly demarcated erythema), Score 3 (moderate erythema), Score 4 (severe erythema) were used **(Okumura *et al.*, 2005)**.

RESULTS

1. *Red algae extraction*

Red algae *Gracilaria* sp. samples were extracted by maceration method with the aim of obtaining both polar and non polar compounds. The yield of *Gracilaria* sp. extract from 3 repetitions amounted to 12.9 ± 0.35 (Table 2).

Table 2. Yield of *Gracilaria* sp. extraction

Repetition	Extract weight (g)	Yield (g)	Yield Average ± SD
1	17	13.2	12.9 ± 0.35
2	16	12.9	
3	16	12.5	

2. *Phytochemical assay*

The results of qualitative identification of compounds in *Gracilaria* sp. extracts found only 5 groups, namely tannin, alkaloids, flavonoids, saponins and phenolics (Table 3), while steroids and terpenoids were not found in this extract. These results are different from two other studies that used the same species.

Table 3. Phytochemical content of *Gracilaria* sp. extract

No.	Active Component	Result
1	Alkaloids	+
2	Phenolic	+
3	Saponins	+
4	Steroids	-
5	Tannins	+
6	Flavonoids	+
7	Terpenoids	-

Description: (+) = the sample contains compounds, (-) = the sample does not contain the compound.

3. *Total flavonoid content*

The total flavonoid content in the ethanol extract of *Gracilaria* with quercetin as

the standard solution, reached 3.95 ± 0.04, mgQE/g (Table 4).

Table 4. Total flavonoid content in ethanol extract of *Gracilaria* sp.

Repetition	mgQE/g	mgQE/g ± SD
1	3.90	3.95 ± 0.04
2	3.90	
3	3.96	

4. *Total phenolic content*

The total phenolic content of *Gracilaria* extract in the study was 11.23 ± 0.41 KTFe ± SD (mgGAE/g) (Table 5).

Table 5. Total phenolic content in ethanol extract of *Gracilaria* sp.

Repetition	KTFe (mgGAE/g)	KTFe ± SD (mgGAE/g)
1	10.90	11.23 ± 0.41
2	11.70	
3	11.10	

5. *Semi-quantitative phytochemical analysis*

5.1. Gas chromatography-mass spectrometry (GC-MS)

The results of GC-MS analysis of the samples showed 11 peaks detected with an area of >2%. Based on the analysis, 3 compounds were found to have the most significant percentage area values, indicating the high relative abundance of compounds, namely the compound n-Hexadecanoic acid at 13.08%; the compound Hexadecanoic acid, ethyl ester at 20.32% and the compound Cholesteryl alcohol at 21.20% (Table 6).

Table 6. Identified phytochemicals in *Gracilaria* sp. extract using GC-MS

Compound	Molecular formula	Molecular Weight g/mol	Retention time	Area (%)	Bioactivity
Cholesteryl alcohol	C27H46O	386.7	31.743	21.20	anticancer activity, anti- inflammatory activity, antimicrobial activity, antioxidant activity, drug- loaded activity **(Zhang *et al.*, 2021**; **NCBI, 2025a)**
Hexadecanoic acid, ethyl ester **(**Ethyl palmitate**)**	C18H36O2	284.52	20.048	20.32	hepatoprotective bioactive, antimicrobial effect **(George *et al.*, 2012;**

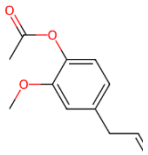
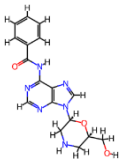
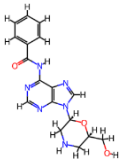
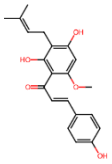
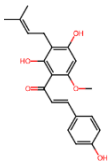
					Shaaban *et al.*, 2021; Gupta *et al.*, 2023; NCBI, 2025b)
n-Hexadecanoic acid **(**Palmitic acid**)**	C16H32O2	256.4241	19.664	13.08	activity against *Vibrio* spp **(Kurnia and Kasanah, 2025)**
Ethyl myristate **(**tetradecanoic acid, ethyl ester**)**	C16H32O2	256.42	17.527	12.3	strong cytotoxic activity against HL-60 and MCF-7 cell lines **(Andriani *et al.*, 2016; NCBI, 2025c)**
9-Octadecenoic	C6H6O2S	282.5	21.725	7.86	antifungal activity **(Sampaio *et al.*, 2022; NCBI, 2025d)**
Ethyl Oleate	C20H38O2	310.5	22.055	5.20	Anti-bacterial, anti-oxidant, anti-cancer **(Kumar *et al.*, 2024; NCBI, 2025e)**
Tetradecanoic Acid **(**Myristic Acid**)**	C14H28O2.	228.37	17.085	4.56	antioxidant activity, anti-microbial, anti-virus, anti- cancerous cells, and antiparasitic microbes **(Hasanudin, 2023; Javeed *et al.*, 2020; NCBI, 2025f)**
p-Dimethylaminobenzalrhodanine	C12H12N2OS2	264.4	25.600	2.44	high antiradical and cytoprotective activity **(Stalinskaya *et al.*, 2022; NCBI, 2025g)**
2-Heptanone	C7H14O	114.19	29.126	2.25	a Volatile Organic Compound **(Saccà *et al.*, 2021; NCBI, 2025h)**
Cholestane-3 6-dione	C27H44O2	400.6	36.452	2.15	cytotoxicity against KB tumor cells **(Dunaway *et al.*, 2018)**, antimicrobial **(Elena *et al.*, 2018; NCBI, 2025i)**

5.2. Liquid chromatography-mass spectrometry (LC-MS)

The results of compound identification in *Gracilaria edulis* extract using LC-MS found 14 peaks, which indicated the presence of 5 compounds, while 4 peaks were not

identified (Table 7).

Table 7. Dereplication results of compounds in *Gracilaria* sp. extract analyzed using LC-MS (based on comprehensive marine natural products database (CMPD) and Chem)

No	RT	Parent Ion (M/Z)	Ion Formula Molecule $(M-H)_+$	Compound Name	Compound Structure	Reference
1	6.153	167.0714	$C_9H_{10}O_3$	Ethylparaben *Gracilaria folifera*		**Blunt *et al.*, 2012; ChemSpider ID: 13846749**
2	6.379	167.0712	C9H10O3	Ethylparaben *Gracilaria folifera*		**Blunt *et al.*, 2012; ChemSpider ID: 13846749**
3	7.735	207.1030	C12H14O3	Acetyleugenol *Gracilaria asiatica*		**Blunt *et al.*, 2008 ChemSpider ID: 6869**
4	9.957	355.1550	C17H18N6O 3	Benzamide, N-[9- [(2R,6S)-6-(hydroxymethyl)-2- morpholinyl]-9H- purin-6-yl]- *Gracilaria vermiculophylla*		**Carroll *et al.*, 2021; ChemSpider ID: 17595192**
5	9.844	355.1553	C17H18N6O3	N-phenyl-1-[(E)-(2,3,4-trimethoxyphenyl)methylideneamino]tetrazol-5-amine *Gracilaria vermiculophylla*		**Carroll *et al.*, 2021; ChemSpider ID: 17595192**
6	9.999	355.1555	C12H14N14	Unknown		
7	10.133	355.1549	C21H22O5	Xanthohumol *Gracilaria verrucosa*		**Mata *et al.*, 2023; ChemSpider ID: 555077**
8	10.421	355.1555	C21H22O5	Xanthohumol *Gracilaria verrucosa*		**Mata *et al.*, 2023; ChemSpider ID: 555077**

9	10.702	355.1559	C21H22O5	Xanthohumol *Gracilaria verrucosa*	**Mata *et al.*, 2023; ChemSpider ID: 555077**
10	10.225	355.1555	C21H22O5	Xanthohumol *Gracilaria verrucosa*	**Mata *et al.*, 2023; ChemSpider ID: 555077**
11	11.827	581.2536	C36H36O7	2,2′-{[4-**(**Allyloxy**)**phenyl]met hylene}bis[5-**(**4-methoxyphenyl**)**-1,3- cyclohexanedione]	**ChemSpider ID:4102854**
12	13.254	355.1541	C13H14N12 O	Unknown	
13	14.548	342.1975	C23H23N3	Unknown	
14	15.715	355.1541	C13H14N12 O	Unknown	

6. Antioxidant test

Based on Table (8), the inhibition value in the positive control (ascorbic acid) and *Gracilaria* sp. is increasing. In ascorbic acid, the highest value was 81.858 at a concentration of 10ppm, and the lowest value was 44.027 at a concentration of 2ppm, while in *Gracilaria* sp. the highest value was 80.4 at a concentration of 1500ppm, and the lowest value was 63.80 at a concentration of 500ppm.

Table 8. Antioxidant test of *Gracilaria* sp. extract

	Concentration (ppm)	**ABS**	**Inhibition (%)**
Ascorbic Acid	2	0.25	45.03
	4	0.21	55.10
	6	0.17	64.05
	8	0.14	69.90
	10	0.08	81.90
	Concentration (ppm)	**Abs**	**Inhibition (%)**
Gracilaria sp.	500	0.174	63.80
	750	0.150	67.50
	1000	0.130	72.5
	1250	0.110	76.5
	1500	0.109	80.4

7. SPF (Sun protection factor) value of extract and SPF (Sun protection factor) of sunscreen spray lotion extract of *Gracilaria* sp.

The results of the one way anova test analysis on the SPF value of the extract showed that there was a significant difference between treatments ($P< 0.05$). Based on Tables (9, 10), the analysis results show that the best extract concentration is the 6000ppm extract treatment with an SPF value of 27.75, because the SPF value is not significantly different from the positive control (Oxybenzone) with an SPF value of 28.03.

Table 9. Sun protection factor (SPF) value and SPF protection type of *Gracilaria* sp. extract

Concentration	SPF of Extract ± SD	Protection Type
2000 ppm	13.59 ± 0.03^{a}	Maximum
4000 ppm	22.59 ± 0.42^{b}	Ultra
6000 ppm	27.75 ± 0.32^{c}	Ultra
8000 ppm	37.15 ± 0.22^{d}	Ultra
Positive Control (Oxybenzone)	28.03 ± 0.10^{c}	Ultra

Notes: Different notations indicate significantly different treatments ($P < 0.05$).

Table 10. SPF values and SPF protection type of sunscreen spray lotion product based on *Gracilaria* sp. extract

Concentration	SPF of Extract ± SD	Protection Type
Basic sunscreen	2.90 ± 0.18^{a}	Minimal
Formulation 4000ppm	15.20 ± 0.29^{b}	Ultra
6000 ppm formulation	22.0 ± 0.29^{c}	Ultra
Formulation 8000ppm	31.30 ± 0.28^{d}	Ultra
Positive Control (commercial sunscreen SPF 15)	15.50 ± 0.05^{b}	Ultra
Positive Control (commercial sunscreen SPF 50)	36.60 ± 0.51^{e}	Ultra

Notes: Different notations indicate significantly different treatments (*P*< 0.05).

8. Sunscreen spray lotion evaluation test

The results of the evaluation of four sunscreen spray lotion formulations, namely Basic, formulations of 4000, 6000, and 8000ppm taken from the best concentration of extracts with the highest SPF value. There are three evaluation tests of sunscreen spray lotion preparations, namely organoleptics, pH, and homogeneity can be seen in Table (11). Organoleptic testing aims to observe the aroma, dosage form, and color changes in the preparation. Organoleptic is done with normal sensing to identify **(Salim *et al,* 2018)**. In Table (9), organoleptic results from sunscreen spray lotion *Gracilaria* sp. extract had a

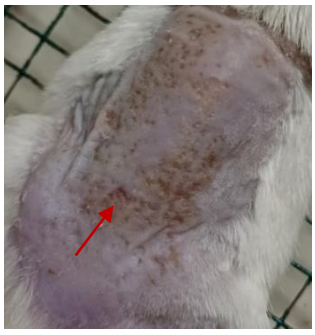
yellow to yellow-brown color, a distinctive aroma of red algae, and is semi-solid. Based on the results of organoleptic tests on 30 students of Faculty of Biotechnology, Universitas Kristen Duta Wacana Yogyakarta, the most preferred formulation was 6000ppm. Homogeneity testing is intended to determine whether the particles in the product are evenly distributed or not **(Yulianti *et al.*, 2015)**. In Table (11), homogeneity results of each formulation are homogeneous which is known from the absence of large particles or lumps in each formulation, and characterized by very evenly distributed particles and visually uniform **(Ariyanti *et al.*, 2022)**.

Table 11. Physical evaluation test of sunscreen spray lotion *Gracilaria* sp. extract

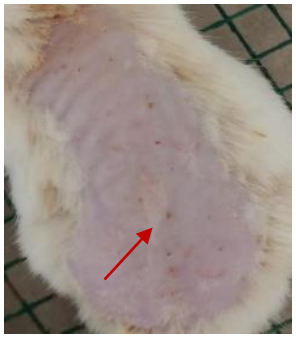
Test Type	Base	Extract 4000 ppm	Extract 6000 ppm	Extract 8000 ppm	SNI
Organoleptic	White, unflavored, semi-solid	Yellowish, algae-smelling, semi-solid	Brownish, algae-smelling, and semi-solid	Brownish color, algae-smelling, and semi-solid	-
pH	6.1	6.2	6.1	6.1	4.5-7.5
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

9. *In vivo* test of sunscreen spray lotion

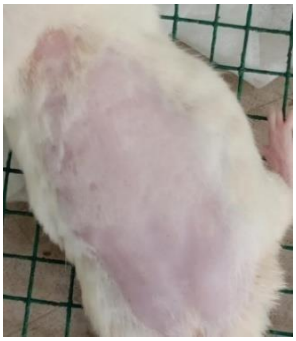
In vivo tests in this study, each group of mice was irradiated using UV-B lamps for 24 hours. The results of the one-way ANOVA test analysis on the erythema score showed that there was a significant difference between treatments ($P < 0.05$). Based on Table (12), the analysis results show that the erythema score in the treatment group of 6000 ppm and 8000ppm formulations is not significantly different from the positive control (SPF 50), and significantly different from the negative control. Fig. (2) shows that the formulations of 6000 and 8000ppm are highly capable of protecting the skin with an erythema score of 0 including no erythema. Sunscreen formulation of *Gracilaria* sp. extract at a concentration of 6000ppm is the best formulation, because it has the same protection as the positive control (SPF 50).



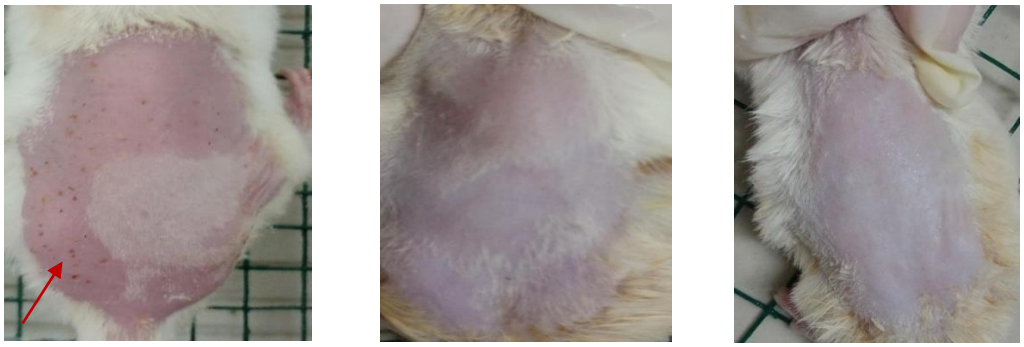
Negative control **(**no extract**)**



Positive control **(**SPF 15**)**



Positive control **(**SPF 50**)**



Extract treatment 4000 ppm Extract treatment 6000 ppm Extract treatment 8000 ppm

Fig. 2. Morphological appearance of BALB/c mice skin after treatment. The red arrow indicates the presence of erythema

Table 12. Erythema scoring of sunscreen spray lotion containing *Gracilaria* sp. in mice

Number	**Treatment**	**Average SD**
1	Negative control **(**base**)**	4 ± 0.00^{a}
2	Positive control **(**SPF 15**)**	1 ± 1.00^{b}
3	Positive control **(**SPF 50**)**	0 ± 0.00^{c}
4	Extract treatment 4000ppm	1 ± 0.00^{b}
5	Extract treatment 6000ppm	0 ± 0.00^{c}
6	Extract treatment 8000ppm	0 ± 0.00^{c}

DISCUSSION

The extraction process using 70% ethanol produced a relatively high yield from *Gracilaria* sp, as detailed in Table (2). This high extraction yield is primarily due to the polarity of the solvent used. Ethanol at 70% concentration, which contains a significant amount of water, is more polar and therefore better suited for extracting both polar and semi-polar compounds **(Nahor *et al.*, 2022)**. When compared to previous studies, such as that by **Kaimudin *et al.* (2020)** who obtained a 5.47% yield using ethyl acetate, and **Susanto *et al.* (2021)**, who reported a 1.57% yield using 96% ethanol on *Gracilaria* sp. from Wediombo Beach, the present results indicate the enhanced effectiveness of 70% ethanol in extracting a wider range of bioactive compounds. Moreover, the yield variation among studies can also be influenced by the type of algae species, the location where samples were collected, and the polarity of the extraction solvent **(Hidayati & Darmanto, 2017; Tarigan *et al.*, 2023; Ullah *et al.*, 2023; Meiyasa *et al.*, 2024)**. These findings suggest that not only the solvent but also the environmental and biological context play a crucial role in determining the yield of extractable materials.

Phytochemical screening, an essential initial step in the study, confirmed the presence of various bioactive compounds including tannin, phenols, flavonoids, alkaloids, and saponins, as shown in Table (3). These results contrast with previous findings by **Insani *et al.* (2022)**, who identified phenol, saponin, and steroid compounds using methanol as the solvent, and **Susanto *et al.* (2021)**, who detected saponins and alkaloids using 96% ethanol. Such differences highlight the influence of solvent type and polarity, geographic location, and sampling time on the phytochemical composition. These observations are in line with the findings of **Fithriani *et al.* (2015)** and **Susanto *et al.* (2021)**, which confirm that environmental and methodological variables affect the profile of secondary metabolites in seaweeds.

In terms of flavonoid content, the extract yielded a total of 3.95 ± 0.04 mg QE/g, a value lower than that reported by **Purwaningsih and Deskawati (2020)**, who obtained 25.23 mg QE/g from ethyl acetate extracts of *Gracilaria* sp. This difference is likely due to solvent properties. Flavonoids differ in polarity; aglycone forms are less polar and dissolve more readily in semi-polar solvents like ethyl acetate, whereas flavonoid glycosides (which are sugar-bound and more polar) are better extracted using 70% ethanol **(Purwaningsih *et al.*, 2015; Widiyana & Illian, 2022)**. Therefore, the solvent type plays a crucial role in determining the class of flavonoids extracted. Moreover, additional factors such as the algae species, environmental conditions at the collection site, and the specific extraction method used also contribute to variations in flavonoid content.

The total phenolic content of the extract was recorded at 11.23 ± 0.41 mg GAE/g. This value is lower than that found by **Lailiyah (2014)**, who observed 14.91 mg GAE/g in *Sargassum piluliferum* with methanol, and by **Purwaningsih and Deskawati (2020)**, who reported 28 mg GAE/g using ethyl acetate. These discrepancies are again attributable to differences in solvent polarity, algae species, and geographic factors. Findings from **Septiyanti *et al.* (2019)** and **Mahendran *et al.* (2021)** further support the notion that phenolic content can vary widely based on species type, location, and extraction methods. Thus, while 70% ethanol is effective in extracting a range of polar compounds, it may not maximize phenolic yield as well as some other solvents.

The GC-MS analysis of the extract identified 11 chemical compounds, among which Cholesteryl alcohol, Ethyl oleate, and Tetradecanoic acid **(**myristic acid**)** were recognized for their antioxidant properties. Cholesteryl alcohol was also identified as having UV protective capabilities **(George *et al.*, 2012)**. These compounds provide strong evidence supporting the use of *Gracilaria* extract in formulations aimed at dermal antioxidant protection and UV defense. Complementary to this, LC-MS analysis revealed 14 chromatographic peaks, from which 5 compounds were identified. Notably, xanthohumol was found a compound well-documented for its dual antioxidant and UV-protective properties **(Adamiak & Sionkowska, 2022; Piekara & Piasecka-**

Kwiatkowska, 2024). These compound identifications reinforce the extract's potential as a multifunctional bioactive agent suitable for topical application.

The antioxidant activity of the extract, though lower than that of pure ascorbic acid, was substantial and exhibited a dose-dependent relationship. As extract concentration increased, DPPH radical inhibition also increased, indicating the presence of active compounds capable of neutralizing free radicals **(Hasanuddin, 2023)**. Despite relatively low total flavonoid and phenolic content, the antioxidant performance remained high, likely due to the presence of additional bioactive compounds such as alkaloids and saponins. Alkaloids function as primary antioxidants through hydrogen atom donation, while saponins serve as secondary antioxidants by inhibiting lipid peroxidation **(Agbo *et al.*, 2015; Widiastini *et al.*, 2021)**. This synergistic interplay among various bioactive constituents contributes to the extract's overall antioxidant capacity.

The photoprotective ability of the extract was assessed through SPF measurements at different concentrations. The SPF value was found to increase proportionally with extract concentration. The 8000ppm formulation demonstrated the highest SPF of 31.30, which is close to the SPF 50 standard used as the positive control, recorded at 36.60. This UV protection is attributed to the bioactive compounds identified in the phytochemical analysis. Phenolic compounds, with their aromatic rings and conjugated double bonds, are effective UV absorbers **(Loho *et al.*, 2021)**. Flavonoids, as the largest subgroup of phenolics, possess chromophore structures capable of absorbing UV radiation and preventing oxidative damage **(Suryanto *et al.*, 2013; Dharmawan *et al.*, 2023)**. Additionally, alkaloids contribute to UV protection due to their basic structure and nitrogen-containing rings, while antioxidants in general neutralize UV-induced free radicals **(Pontoan, 2016)**. Statistical analysis using one-way ANOVA confirmed significant differences in SPF values among formulations ($P< 0.05$), validating the efficacy of the extract in a dose-dependent manner.

The physical stability of the sunscreen spray lotion is another critical factor in its suitability for use. The pH of all tested formulations was measured at 6, which falls within the acceptable range of 4.5 to 7.5 according to SNI 16-4399-1996 standards **(Salim *et al.*, 2018; Lumantow *et al.*, 2023)**. This pH level is considered safe for human skin, avoiding risks such as irritation or dryness that can occur with products that are too acidic or alkaline **(Salim, 2018)**. Moreover, the formulations met additional SNI criteria, including ease of application and homogeneity, confirming the product's usability and safety for topical application.

The *in vivo* efficacy of the sunscreen formulations was demonstrated through tests conducted on BALB/c mice. The results clearly indicated that the formulations were effective in protecting the skin from UV-B radiation. This protective effect is attributed to the presence of phenolic compounds and flavonoids in the extract. Phenols, with their conjugated aromatic structures and hydroxyl groups, absorb UV-B light in the 290–320 nm range and stabilize free radicals formed by UV exposure **(Dunaway *et al.*, 2018)**.

Similarly, flavonoids act as chromophores, absorbing UV radiation and converting it into harmless heat, thereby minimizing the potential for oxidative skin damage **(Amini *et al.*, 2020)**. Mice treated with 6000 ppm extract showed no visible signs of erythema after UV-B exposure, highlighting the effectiveness of the formulation in real-world conditions. The results demonstrate the formulation's capability to both absorb harmful radiation and mitigate its damaging effects.

In summary, the findings of this study support the potential of *Gracilaria* sp. extract, particularly that obtained from Krakal Beach, Yogyakarta, Indonesia as a multifunctional natural ingredient in sunscreen products. The extract contains a range of bioactive compounds, including phenolics, flavonoids, alkaloids, and saponins, that contribute to both antioxidant and UV-protective activities. The use of 70% ethanol proved effective in extracting these compounds, and the resulting formulations met national standards for sunscreen quality. *In vivo* assessments confirmed the extract's ability to offer significant photoprotection and antioxidant benefits. These results underscore the viability of developing natural, safe, and effective sunscreen formulations based on local marine resources such as *Gracilaria* sp.

CONCLUSION

This study demonstrated that the extract of *Gracilaria* sp., collected from Krakal Beach, Yogyakarta, has significant potential as a natural active ingredient in sunscreen formulations. Extraction using 70% ethanol effectively yielded bioactive compounds such as phenolics, flavonoids, alkaloids, and saponins, which contribute to both antioxidant and UV-B protective properties. Although the total phenolic and flavonoid contents were moderate, the extract exhibited strong antioxidant activity, likely due to the synergistic effects of multiple bioactive compounds. The extraction was performed by maceration with 70% ethanol. Sunscreen sprays were formulated with extract concentrations of 4000, 6000, and 8000ppm. Evaluations included phytochemical screening, total phenolic and flavonoid content, antioxidant activity **(**with an inhibition percentage of 80.4%**)**, SPF measurement, physical stability, and *in vivo* testing. Results confirmed the presence of phenols, flavonoids, alkaloids, and saponins, with total phenolic and flavonoid contents of 11.23 mg GAE/g and 3.95 mg QE/g, respectively. The 8000ppm formulation exhibited the highest SPF value of 37.15, meeting the Indonesian National Standard (SNI) criteria, while the 6000 ppm formulation achieved an SPF of 26.75 and showed zero (0) erythema *in vivo*, indicating effective UV-B protection in BALB/c mice. These findings highlight the potential of *Gracilaria* sp. ethanol extract as a safe and natural UV filter for sunscreen formulations.

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