



PHOTOPROTECTIVE POTENTIAL OF *Eucheuma cottonii* EXTRACT FROM LAMPUNG BAY AS NATURAL SUNSCREEN AGENTS

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Abstract

Eucheuma cottonii is a seaweed species that contains bioactive compounds such as flavonoids, hydroquinone phenols, and triterpenoids. However, the presence of mycosporine-like amino acids (MAAs) in *E. cottonii* from Lampung Bay, Indonesia, remains unexplored, despite their high potential for use in sunscreen creams to enhance the economic value of seaweed. This study aimed to identify MAAs and determine the photoprotective activity of *E. cottonii* collected from Lampung Bay. MAAs were extracted using hot maceration (40°C) in either 25% methanol or 25% ethanol. Phytochemical analysis, MAAs quantification, and SPF testing were performed to evaluate photoprotective activity. Extraction using hydroethanol yielded a higher rendement than that using hydromethanol. Phytochemical testing confirmed the presence of phenolic and flavonoid compounds in the extracts. MAAs were detected using TLC, but further separation and testing were required to confirm the results. The highest SPF values of 20.18±0.99 (25% ethanol) and 16.48±0.53 (25% methanol extract) fell into the ultra-protection category. These results indicate that *E. cottonii* from Lampung Bay has a strong potential as a raw material for sunscreen formulation.

Keywords: flavonoid, phytochemical, MAAs, seaweed, SPF

Potensi Fotoprotektif Ekstrak *Eucheuma cottonii* dari Teluk Lampung sebagai Agen Tabir Surya Alami

Abstrak

Eucheuma cottonii adalah spesies rumput laut yang diketahui mengandung senyawa bioaktif misalnya flavonoid, fenol hidrokuinon, dan triterpenoid. Namun, keberadaan amino acid-like mycosporine (MAAs) pada *E. cottonii* dari Teluk Lampung, Indonesia, belum dieksplorasi. Senyawa ini memiliki potensi tinggi sebagai bahan baku krim tabir surya sehingga dapat meningkatkan nilai ekonomi rumput laut tersebut. Penelitian ini bertujuan mengidentifikasi MAAs dan menentukan aktivitas fotoprotektif *E. cottonii* dari Teluk Lampung. Ekstraksi MAAs menggunakan metode maserasi panas (40°C) dengan pelarut 25% metanol dan 25% etanol. Uji fitokimia, MAAs, dan SPF dilakukan untuk mengevaluasi aktivitas fotoprotektif. Hasil ekstraksi menunjukkan bahwa pelarut hidroetanol menghasilkan rendemen yang lebih besar dibandingkan hidrometanol. Uji fitokimia mendeteksi adanya senyawa fenolik dan flavonoid. MAAs terdeteksi menggunakan TLC, namun memerlukan pemisahan dan pengujian lebih lanjut untuk konfirmasi. Nilai SPF tertinggi adalah 20,18±0,99 (ekstrak 25% etanol) dan 16,48±0,53 (ekstrak 25% metanol), keduanya

termasuk kategori perlindungan ultra. Hasil yang diperoleh menunjukkan bahwa *E. cottonii* dari Teluk Lampung sangat potensial sebagai bahan baku formulasi tabir surya.

Kata kunci: fitokimia, flavonoid, MAAs, rumput laut, SPF

INTRODUCTION

Ultraviolet (UV) radiation is a form of electromagnetic radiation characterized by wavelengths shorter than those of visible light but longer than those of X-rays. It is classified into three categories based on wavelength: UVA, UVB, and UVC. Although the sun is the primary source of UV radiation, it can also be generated artificially using devices such as tanning beds and germicidal lamps (D’Orazio *et al.*, 2013; Zaffina *et al.*, 2012; Ngoc *et al.*, 2019). The benefits of UV radiation include its essential role in vitamin D synthesis in the skin, which is vital for maintaining bone health and supporting the immune function. Additionally, UV light is employed in sterilization and disinfection processes because of its ability to eradicate bacteria, viruses, and other pathogens, thereby proving valuable in medical and water treatment contexts. However, UV exposure has several disadvantages.

Excessive exposure, particularly to UVB and UVA, can result in skin damage, sunburn, premature aging, ocular damage (such as cataracts), and an elevated risk of skin cancer, including melanoma. Prolonged exposure may also suppress the immune system, potentially diminishing the body’s capacity to combat certain diseases. Consequently, protective measures, such as the application of sunscreen and limiting exposure to intense sunlight, are necessary (Ombra *et al.*, 2017; Wright & Norval, 2021; Tsai & Chien, 2022). Sunscreens work by blocking or absorbing UV rays, thereby reducing skin damage and protecting the skin from the harmful effects of UV radiation. Regular sunscreen use is an essential preventive measure for maintaining skin integrity and minimizing the long-term risks associated with sun exposure (Olsen *et al.*, 2017; Nitulescu *et al.*, 2023; Raymond-Lezman & Riskin, 2024). Some UV-sunscreen substances have raised concerns regarding their potential adverse effects on human and environmental health (Ghazipura *et al.*, 2017; Yuan *et al.*, 2022).

The increasing global interest in sustainability and eco-conscious consumer products has driven ongoing research on bioactive natural compounds as potential alternatives to synthetic agents in sunscreen formulations. These efforts aim to identify effective, biologically active substances derived from natural compounds that offer UV protection while minimizing environmental impact (Kageyama & Waditee-Sirisattha, 2019; Rosic *et al.*, 2023). Mycosporine-like Amino Acids (MAAs) are a group of flavonoid-derived compounds that are generally present in most red algae. All MAAs share various central structures, namely the cyclohexenone/cyclohexenimine ring, which is responsible for UV absorption (Reymon *et al.*, 2018; Sipahutar *et al.*, 2019; Vega *et al.*, 2021; Alrosyidi & H, 2021; Kasanah *et al.*, 2022). These compounds have gained attention because of their beneficial bioactivities, such as anticancer, wound healing, DNA damage protection, and anti-UV activities (Peng *et al.*, 2023). Research has consistently demonstrated that a diverse array of macroalgal species possess the remarkable capability to synthesize one or multiple types of mycosporine-like amino acids (MAAs). Intriguingly, the specific composition of these metabolites exhibits considerable variability contingent on the geographical distribution and environmental conditions in which these macroalgae are found.

Eucheuma cottonii and *Kappaphycus alvarezii* are the primary sources of carrageenan and contribute substantially to the global seaweed aquaculture industry. Indonesia is currently one of the largest seaweed producers in the world (Sambodo & Arlesia, 2019; Safiati *et al.*, 2020; Yanuarti *et al.*, 2021). This red alga contains flavonoids, phenols, tannins, α -carotene, β -carotene, phycobilins, neoxanthin, and zeaxanthin, which exhibit potential anti-cancer, antioxidant, and anti-inflammatory activities (Sambodo & Arlesia, 2019; Sari *et al.*, 2020; Loho *et al.*, 2021; Sami *et al.*, 2021; Nusaibah *et al.*, 2023). The



chromophore groups that constitute flavonoids can absorb UV light waves (Quintero-Rincón *et al.*, 2023; Vega *et al.*, 2021; Yanuarti *et al.*, 2021). Its phenol content and derivatives can absorb UVA and UVB radiation, making it a potential alternative natural UV filter (Reymon *et al.*, 2018; Pratama *et al.*, 2019; Sami *et al.*, 2021; Loho *et al.*, 2021). This red macroalga is increasingly being examined as a potential source of safer alternatives to synthetic UV filters because of its antioxidant and photoprotective properties (Maharany *et al.*, 2017; Shafie *et al.*, 2022).

However, the extraction process of natural products is integral to the yield and composition of extracted metabolites. Its efficacy is significantly dependent on variables such as the solvent employed, extraction duration, and temperature (Al Qoh *et al.*, 2025; Quitério *et al.*, 2022). Furthermore, the geographical location, age, season, and genetics of organisms affect the content and amount of secondary metabolites, including MAAs. Metabolites serve specific functions in the bioactivity of organisms (Loho *et al.*, 2021; Sami *et al.*, 2021). Although *E. cottonii* is a known source of various bioactives, the MAAs profile of specimens cultivated in Lampung Bay, Indonesia, has never been systematically explored. This study addresses this critical research gap by evaluating the photoprotective activity and identifying the presence of MAAs in hydroethanol and hydromethanol extracts from this region, providing insights into its potential use in the cosmeceutical and other relevant industries.

MATERIAL AND METHODS

Seaweed Extraction

Fresh *E. cottonii* seaweed were shipped from Lampung Bay, Bandar Lampung City. The samples were then dried at room temperature for three days. The samples were ground using a Miller FCT-Z200 grinder. Hot solvent extraction was performed using a water bath, following the procedure described by Sun *et al.* (2021) with minor modifications. Briefly, 12.5 g of *E. cottonii* powder was extracted separately in 250 mL of 25% ethanol or 25% methanol for 2 h at

40 °C. The extraction process was repeated twice under identical conditions to maximize the metabolite recovery. The combined extracts were filtered through Whatman No. 1 filter paper, concentrated using a rotary evaporator, and further reduced in volume using a water bath maintained at 40 °C until viscous residues were obtained. The percentage yield of the crude extracts was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Volume extract (mL)}}{\text{Sample before extraction (g)}} \times 100$$

Qualitative Phytochemical Screening

Qualitative phytochemical screening of the crude extracts was conducted following the protocols described by Kabebe *et al.* (2021) with minor modifications. The presence of phenolics was determined by adding 0.05 mL of the extract to two drops of 5% FeCl₃ solution. A color change to green, blue-green, purple, red, or black was considered positive. Flavonoid detection was performed by mixing 0.05 mL of the extract with 50 mg of Mg powder and 0.2 mL of amyl alcohol. A color change to red, yellow, or orange indicated a positive result. For alkaloid screening, 0.1 mL of the extract was combined with 10 mL of chloroform and five drops of ammonia, followed by the addition of five drops of concentrated sulfuric acid and thorough mixing.

The solution was then tested separately using three alkaloid reagents: Dragendorff, Mayer, and Wagner. Positive reactions were confirmed by the appearance of orange-red, white, and brown precipitates. Alkaloid presence was confirmed when at least two of the three tests yielded positive results (Forestryana & Arnida, 2020). Steroid and triterpenoid detection was performed using 0.1 mL of the extract mixed with two mL of chloroform, followed by the addition of five drops of acetic anhydride and three drops of 2 N sulfuric acid. A color change to blue indicates the presence of steroids, whereas a red color indicates the presence of triterpenoids.

Detection of Mycosporine-like Amino Acids (MAAs)

The presence of MAAs in *E. cottonii* extracts was assessed using thin-layer chromatography (TLC) and UV-Vis spectrophotometry, following the method described by Sun *et al.* (2021), with slight modifications. For TLC analysis, aliquots of each extract were spotted on silica gel G plates (Merck) and developed in a solvent system consisting of methanol, ethanol, and distilled water in an 8:10:0.5 (v/v) ratio. After development, the plates were air-dried, sprayed with Dragendorff's reagent, and incubated at room temperature for 15–20 min. The appearance of yellow or violet spots was interpreted as a positive indication of MAAs presence.

UV-Vis absorbance was measured using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies) equipped with Scan software. Samples were prepared at a final concentration of 500 ppm in distilled water, and the absorbance was recorded over the wavelength range of 200–400 nm. Characteristic absorption maxima between 310 and 360 nm were considered indicative of MAAs.

Sun Protection Factor (SPF) Determination

Crude *E. cottonii* extracts were diluted in the same solvent used for their extraction (25% ethanol or 25% methanol) to final concentrations of 2,000; 3,000; 4,000; and 5,000 ppm. Distilled water was used as the blank. The absorbance of each solution was measured between 290 and 320 nm using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies). The measured range corresponds to the UV-B spectrum, which

is known to induce erythema and photo-induced carcinogenesis. The SPF values were calculated from the absorbance using the Mansur equation (Sayre *et al.*, 1979).

$$\text{SPF} = \text{FK} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{A}(\lambda)$$

Where $\text{EE}(\lambda)$ is the erythemal effect spectrum, $\text{I}(\lambda)$ is the solar intensity spectrum, $\text{A}(\lambda)$ is the sample absorbance at each wavelength, and CF is the correction factor (10). The SPF values were classified according to the standards listed in Table 1.

Statistical Analysis

All experiments were conducted in triplicate ($n = 3$), including yield calculations, SPF determination, and spectrophotometric detection of MAA. Data are presented as mean \pm standard deviation (SD). Differences in extraction yields between solvents were analyzed using an independent samples t-test. The effects of solvent type and extract concentration on SPF values were evaluated using two-way ANOVA, followed by Tukey's HSD post hoc test ($\alpha = 0.05$). The treatments included solvent (two levels: ethanol and methanol), concentration (four levels: 2,000; 3,000; 4,000; and 5,000 ppm), and their interactions. Statistical analyses were performed using SPSS v.25, and significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Yield Extraction

Eucheuma cottonii thalli collected after 35 days of long-line cultivation at approximately 1 m depth exhibited dark reddish-brown coloration, irregular branching patterns with pointed or blunt

Table 1 SPF protection category indicator (Kasitowati *et al.*, 2021; Yanuarti *et al.*, 2017)

SPF value	Sunscreen protection
2-4	Minimum
4-6	Moderate
6-8	Extras
8-15	Maximum
≥ 15	Ultra



nodules, and smooth cylindrical cartilaginous structures. The spines covering the thallus were irregularly distributed and bore simple to complex branches measuring 8–25 cm long (Figure 1). The resulting extracts were viscous and gel-like, suggesting carrageenan coextraction. This phenomenon is likely attributable to the high water content of the solvents and the extraction temperatures that promote carrageenan solubilization. Following solvent evaporation, the extracts were yellowish-brown, and hydroethanolic extraction produced significantly higher yields than hydromethanolic extraction (Figure 2).

This result is consistent with other studies that found that ethanol and hydroethanolic extracts generally show promising results, often outperforming methanol or methanolic extractions. The extract yields recorded in this study were substantially lower than those reported in previous studies (Sun *et al.*, 2021; Sami *et al.*, 2021; Beladini *et al.*, 2021). Solvent polarity has been shown to influence both MAA recovery and overall yield, with

ethanol–water mixtures typically producing higher yields in *Gracilaria* spp., whereas methanol–water systems perform better in *Bangia fusco-purpurea*, *Gelidium amansii*, and *Gracilaria confervoides* (Sun *et al.*, 2021; Roullier *et al.*, 2011). In studies focusing on red macroalgae and cyanobacteria, the use of ethanol and hydroethanolic solvents has been shown to be significantly effective in extracting photoprotective compounds, such as mycosporine-like amino acids (MAAs), phenolic compounds, and scytonemin. These compounds are known for their UV-absorbing properties, which contribute to photoprotection against UV radiation. Specifically, water was found to be the best solvent for extracting MAAs and phenols; however, a hydroethanolic solvent (ethanol:dH₂O in a 4:1 ratio) achieved higher yields of scytonemin, a compound not typically found in methanol extracts (Vega *et al.*, 2020).

Thus, while the choice between ethanol/hydroethanolic and methanol/methanolic

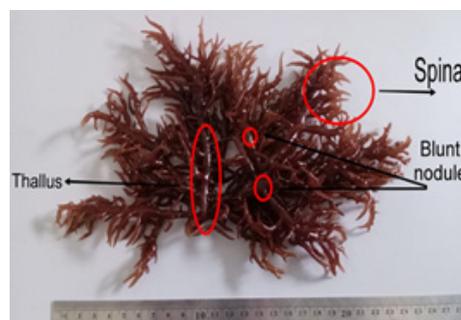


Figure 1 Morphology of *Eucheuma cottonii* from Lampung Bay, Sumatra, Indonesia. Thalli exhibit dark reddish-brown coloration and pointed nodules, with arrows

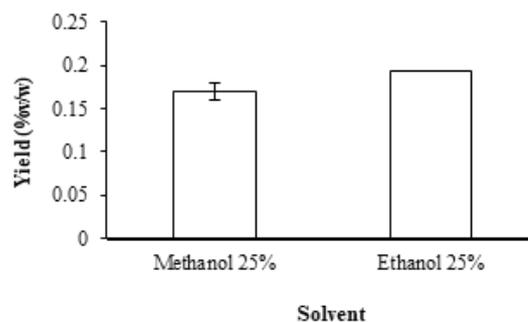


Figure 2 Comparison of crude extraction yields for 25% ethanol and 25% methanol extracts of *E. cottonii* after evaporation at 40°C with waterbath

extractions may depend on the desired compounds, ethanol and hydroethanolic solvents are often preferable for extracting a broader range of photoprotective compounds from seaweed, particularly when targeting compounds such as MAAs and scytonemin (Vega *et al.*, 2020; Vega *et al.*, 2021). This is attributable to its intermediate polarity, which facilitates the selective solubilization of UV-absorbing polyphenols and flavonoids, thereby augmenting the extract with photoprotective chromophores.

These findings suggest that species, environmental conditions during seaweed growth, and methodological parameters such as solvent ratio, extraction time, and temperature may have contributed to the lower yields observed here. Although prolonged heating during solvent extraction and evaporation can degrade heat-sensitive metabolites, most MAAs are thermostable (Sinha *et al.*, 2000), implying that environmental or physiological factors may play a more significant role. Therefore, future research should prioritize controlled experimental designs and standardized extraction protocols to isolate the effects of growth conditions and optimize the recovery of targeted metabolites (Al Qoh *et al.*, 2025; Quitério *et al.*, 2022).

Phytochemical Composition of *E. cottonii*

Qualitative phytochemical screening of 35-day-old *E. cottonii* samples from Ruguk Bay, Lampung revealed the presence of several major secondary metabolite groups (Table 3). The characteristic red and orange color reactions confirmed the presence of phenolic

and flavonoid compounds, respectively, in the extracts obtained using 25% methanol and 25% ethanol. These results confirm that both methanol-water and ethanol-water mixtures are effective solvents for polar metabolites, including phenolics and flavonoids, which are widely reported to contribute to the antioxidant and photoprotective activities of marine macroalgae.

In contrast, neither solvent system demonstrated strong efficiency in extracting less-polar metabolites, such as steroids and triterpenoids. This observation is consistent with previous reports indicating that hydroalcoholic mixtures have a limited capacity to solubilize lipophilic secondary metabolites (Moldovan *et al.*, 2019; Moriasi *et al.*, 2020). It should be emphasized that the qualitative nature of these assays may not detect compounds present at very low concentrations in the samples. Furthermore, variability in metabolite profiles may arise from environmental conditions such as seawater temperature, nutrient availability, and light intensity, which have been shown to influence the biosynthesis of phenolics, flavonoids, and terpenoids in red algae (Kebede *et al.*, 2021; Mokuia *et al.*, 2021). These findings highlight the importance of optimizing extraction protocols and considering ecological factors when evaluating the bioactive potential of *E. cottonii*.

Detection of Mycosporine-like Amino Acids

Mycosporine-like amino acids (MAAs) are nitrogen-containing secondary metabolites with characteristic UV absorption spectra

Table 3 Phytochemical results of *E. cottonii*

Secondary metabolite compounds	Test result		Description
	Ethanol 25%	Methanol 25%	
Phenolic	+	+	Red color formation
Flavonoids	+	+	Orange color formation
Alkaloids	-	-	Dragendorff Reagent : white precipitate, Mayer : white precipitate, Wagner : white precipitate
Triterpenoids	-	-	No color change, white ring formed
Steroids	-	-	No color change, white ring formed



between 310 and 365 nm, functioning as photoprotective compounds that mitigate UV-induced damage in photosynthetic organisms (Kräbs *et al.*, 2002; Peng *et al.*, 2023). In this study, MAAs were preliminarily detected using thin-layer chromatography (TLC) and UV-visible (UV-Vis) spectrophotometry. TLC analysis of 500 ppm hydromethanolic and hydroethanolic extracts of *E. cottonii* revealed the presence of purple and yellow spots (Figure 3), indicating the presence of nitrogenous compounds. These color reactions result from the interaction of Dragendorff's reagent with nitrogen-containing metabolites, such as MAAs (Sun *et al.*, 2021; Sari, 2021).

Although Dragendorff's reagent is conventionally used for alkaloid detection (Forestryana & Arnida, 2020), its use here is justified by the nitrogenous nature of MAAs, which consist of cyclohexenone or cyclohexenimine cores conjugated to amino acids or amino alcohols (Vega *et al.*, 2021). Therefore, it is important to note that Dragendorff's reagent may yield false positives, as it can also react with other nitrogen-containing constituents, such as purines, proteins, betaines, and ammonium salts, resulting in a range of colorimetric responses (Raal *et al.*, 2020). This underscores the necessity for confirmatory analyses, as the presence of other co-extracted compounds could have influenced these results.

Spectrophotometric analysis further corroborated the presence of MAAs, with the UV absorption patterns of *E. cottonii* extracts resembling those previously reported for *Gelidium amansii* (Sun *et al.*, 2021), albeit at lower intensities, suggesting a comparatively lower concentration of MAAs. Such variability may be attributed to differences in species-specific biosynthetic capacity, extraction efficiency, pH, and potential matrix interference (Reymon *et al.*, 2018; Vega *et al.*, 2021). TLC-based Rf value analysis revealed similar compounds in methanol and ethanol extracts, as indicated by a ≤ 0.2 difference between their purple-spot Rf values (0.82 and 0.89, respectively), whereas the yellow spots exhibited an Rf difference > 0.2 , suggesting the presence of distinct compounds between the solvents (Miranti *et al.*, 2023) (Table 4).

Mycosporine-like amino acids (MAAs) are highly polar, water-soluble compounds that typically remain near the origin (Rf close to 0) on standard silica plates when using common solvent systems, which makes differentiation challenging. Although TLC is often cited for purity checking, specific Rf values for individual MAAs are seldom reported in the literature, as the field has largely shifted to HPLC-MS methods for characterization. Furthermore, Rf values are highly dependent on factors such as the plate type, chamber saturation, temperature,

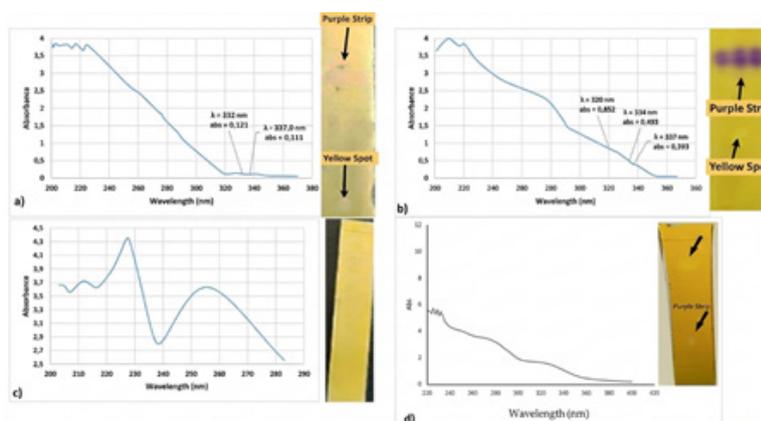


Figure 3 Preliminary Detection of MAAs using UV-Vis spectrophotometry and TLC chromatography. (a) UV-Vis spectrum of the 25% methanol extract and TLC plate ($\lambda_{\max} = 332$ nm and purple spot = 0.83). (b) UV-Vis spectrum of 25% ethanol extract and TLC plate ($\lambda_{\max} 334$ nm and purple spot Rf 0.89). (c) Blank water (d) UV absorbance pattern from reference *G. amansii* (Sun *et al.*, 2021).

Table 4 The Rf values of ethanol and methanol extracts of *E. cottonii*

Sample name	Spot type	Solvent travel distance (cm)	Compound travel distance (cm)	Rf	Interpretation
25% Ethanol	Purple strip	8	7.1	0.89	Presence of nitrogen-containing compound, likely MAAs; similar compound to methanol extract ($\Delta Rf \leq 0.2$).
	Yellow	8	6.5	0.82	Presence of nitrogen-containing compound, likely MAAs; similar compound to methanol extract ($\Delta Rf \leq 0.2$).
25% Methanol	Purple strip	8	6.6	0.82	Presence of nitrogen-containing compound, likely MAAs; comparable to ethanol extract.
	Yellow	8	4.3	0.54	Possible distinct MAA compound or co-extracted nitrogenous metabolite ($\Delta Rf > 0.2$ compared to methanol extract).

and precise composition of the mobile phase (Jofre *et al.*, 2020; Orfanoudaki *et al.*, 2019; Volkmann & Gorbushina, 2006).

Although the detection of MAAs remains preliminary and requires confirmation, these findings demonstrate the potential of combining TLC and UV-Vis spectrophotometry for rapid MAAs screening in *E. cottonii*. Nevertheless, further work involving high-resolution chromatographic and spectrometric techniques (e.g., HPLC, LC-MS/MS, and NMR) is necessary to precisely identify, quantify, and characterize the MAAs present. Such investigations would provide critical insights into their bioactivity, stability, and potential applications in photoprotection, cosmetics, and pharmaceuticals.

Sun Protection Factor (SPF) Evaluation

The Sun Protection Factor (SPF) quantifies the ability of a substance to shield the skin from UV-B radiation, which is primarily responsible for erythema, photoaging, and DNA damage in human skin cells (Quintero-Rincón *et al.*, 2023; Alrosyidi & Syaifiyatul,

2021; Pakki *et al.*, 2018). The highest mean SPF was found in 25% ethanol at 5000 ppm, and the lowest was found in the extract of 25% methanol at 2000 ppm. The mean SPF values of the hydroethanolic and hydromethanolic extracts of *Eucheuma cottonii* at different concentrations are summarized in Table 5.

The visual plot of the mean SPF and concentration clearly shows the strong effect of concentration on the SPF value. Both solvent systems exhibited a concentration-dependent increase in SPF values, indicating enhanced UV-B absorption at higher extract concentrations (Figure 4). However, the obtained SPF values were lower than those reported by Kasitowati *et al.* (2021), who observed an SPF of 19.65 ± 0.26 using macerated ethanol (96%) of *Eucheuma* extracts, and Ramdani *et al.* (2021), who reported an SPF of 31.01 from 50% ethanol maceration (1:10 solvent ratio, 24 h extraction) of *Eucheuma cottonii*. These differences highlight the influence of extraction method, solvent polarity, and extraction duration on the recovery and composition of bioactive photoprotective compounds.

Table 5 The average SPF value and protection category of *E. cottonii* extracts

Concentration (ppm)	SPF average value of hydroethanol extract	Protection category	SPF average value of hydromethanol extract	Protection category
5,000	20.18±0.99	Ultra	16.47±0.53	Ultra
4,000	16.88±0.74	Ultra	12.17±0.28	Maximum
3,000	11.05±1.44	Maximum	8.39±0.06	Extra
2,000	7.47±0.35	Extra	5.46±0.02	Medium

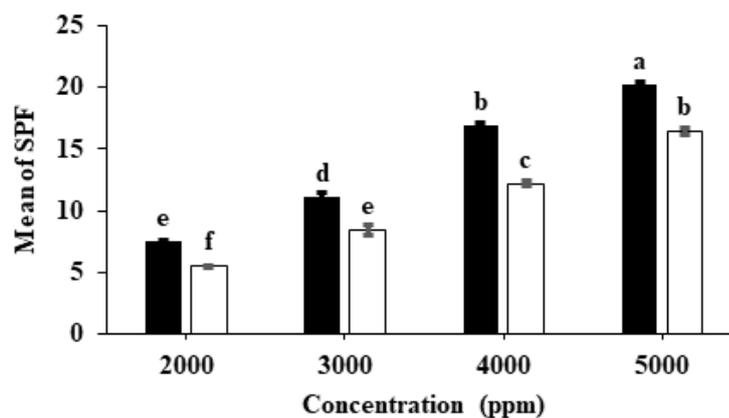


Figure 4 The average values of sun protection factor (SPF) for extracts prepared using 25% ethanol (■) and 25% methanol (□) at four different concentrations (2,000; 3,000; 4,000; and 5,000 ppm);

The error bars represent the standard deviation of the mean;

Letters (a, b, c, etc.) above the bars indicate the results of the Post-Hoc Tukey HSD test $\alpha=0.05$, where conditions not sharing a common letter are significantly different.

Hydroethanolic and hydromethanolic extracts at 2000–5000 ppm demonstrated measurable and concentration-dependent photoprotective activity, suggesting their potential as natural sunscreen agents. Consistent with previous findings, SPF values were positively correlated with extract concentration, supporting the principle that increasing the dose of bioactive-rich crude extracts improves UV absorption capacity and overall photoprotection (Messias *et al.*, 2023; Morocho-Jácome & Freire, 2021; Nunes *et al.*, 2018). These results reinforce the feasibility of using *E. cottonii* extracts as natural UV filters, although optimization of the extraction conditions is essential to maximize their SPF efficacy and align with the values reported in earlier studies. To place this result in a practical context, the SPF value is significantly

higher than the minimum requirement for a 'broad-spectrum' label in many regulatory regions (Pirotta, 2020). Furthermore, this performance was comparable to or exceeded the photoprotective activity reported for several commercial single-ingredient natural extracts currently used in the cosmetics industry. This indicates that crude *E. cottonii* extract from Lampung Bay, even without extensive purification, is a potent UV filter that warrants immediate consideration as a functional raw material for natural sunscreen production.

CONCLUSION

This study demonstrates that the hydroethanol solvent is more effective than hydromethanol for extracting photoprotective compounds, particularly mycosporine-

like amino acids (MAAs), from *Eucheuma cottonii* cultivated in Lampung Bay, Indonesia. The extracts exhibited measurable UV-protective capacity, supporting their potential application as active ingredients in natural-sunscreen formulations. Nevertheless, further work is warranted to isolate, identify, and structurally characterize the specific MAAs present, and to optimize extraction parameters for maximum yield and efficacy. Such investigations are essential for advancing the integration of *E. cottonii*-derived compounds into cosmeceutical and pharmaceutical applications.

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