Identification photoprotective activity of marine seaweed: *Eucheuma* sp.

R D Kasitowati\(^1,2,4\), A Wahyudi\(^1\), R Asmara,\(^3\) D Aliviyanti\(^1,2\), F Iranawati\(^1\), M A P Panjaitan\(^1\), D C Pratiwi\(^1,2\), S Arsad\(^1\)

\(^1\)Faculty of Fisheries and Marine Science, Universitas Brawijaya, Malang, Indonesia
\(^2\)Coastal Resilience and Climate Change Adaptation, Universitas Brawijaya, Malang, Indonesia
\(^3\)Faculty of Agriculture, Universitas Brawijaya, Malang, Indonesia

\(^4\)Corresponding author: raraskasitowati@ub.ac.id

**Abstract.** Skin cancer had become one of the most occurring type of cancer all around the world. The needs for alternative additional substance in sunscreen, especially from natural ingredients become important. Seaweed is one of marine natural resource known for its many benefits and widely used in cosmetic industries. The purpose of this research are to determine the photoprotective activity of *Eucheuma* sp. extract obtained from different type of solvents against ultraviolet radiation and its effect. *Eucheuma* sp. extracted by maceration using two different solvents (methanol and ethyl acetate). Filtrate obtained from maceration evaporated until the consistency are pasta-shaped and tested using spectrophotometer to determine the SPF, % of Te, and % Tp. The result of spectrophotometer tests evaluated and analyzed to determine which solvent generate the best value of SPF, % of Te, and % of Tp. The result of this this research showed that the highest SPF value are present in ethyl acetate extract (SPF 26.4). The best value of Te and Tp percentage are also present in the ethyl acetate extract with their respective values: 0.88% and 18.64%. The conclusion of this research are *Eucheuma* sp. extract had a good potential of photoprotective activity.

1. **Introduction**

Ultraviolet radiation is basically a part of the solar radiation spectrum, which affects all life on earth and direct radiation exposure cannot be avoided. Ultraviolet radiation has a dual role; a beneficial role because it plays a role in the formation of vitamin D and, at the same time, a negative role because it harms humans with its carcinogenic properties [1,2]. Adverse effects for humans due to excessive exposure to ultraviolet radiation include atrophy, pigmentation (tanning), inflammatory effects such as erythema (sunburn), wrinkles on the skin, and chronic effects such as skin cancer. Skin cancer itself is the most common type of cancer in humans and its incidence has continued to increase in this decade [3,4]. Non-melanoma skin cancer occurs up to 3 million cases and 132,000 cases of melanoma skin cancer occur worldwide [5].

Several studies have reported the association of ultraviolet radiation with skin cancer caused by damage to the DNA level due to oxidation stress and increased free radicals. Human skin basically has the ability to ward off free radicals with melanin and enzymatic antioxidants [6]. However, the damage often goes beyond the antioxidant capacity of the skin. Therefore, preventive measures are taken by increasing antioxidant defenses to fight free radicals. This method can be done by using products that contain antioxidant compounds or photoprotective compounds, one of which is sunscreen [7].
The demand for products with photoprotective compounds, especially those derived from natural sources, has recently received special attention because of concerns about the side effects of synthetic compounds found in products such as commercial sunscreens [8,9]. Therefore, various biological materials were tested to explore potential photoprotective activities. Several studies that have been conducted on terrestrial and aquatic biological resources prove that some organisms are capable of producing compounds with photoprotective activity and have been applied to pharmaceutical products [10,11].

From several biological resources that are capable of producing compounds with photoprotective activity, seaweed is one of the organisms that has received a lot of attention. Seaweed lives in very dynamic intertidal areas, producing various kinds of bioactive compounds in response to the environment [12]. Some of the bioactive compounds produced are known to have photoprotective activity that can reduce the detrimental effects of excess ultraviolet radiation [13,14]. Indonesia is one of the largest seaweed exporting countries in the world with the abundant abundance of various types of seaweed, one of the popular types of seaweed is Eucheuma sp. The genera of Eucheuma sp. has been reported to have a high content of antioxidant compounds and other bioactive compounds with potential photoprotective activity [15,16]. This study aims to obtain Eucheuma sp. through the maceration method with three different types of solvents (methanol and ethyl acetate) to determine the best SPF, %Te, and %Tp values of the extracts obtained.

2. Material and methods

2.1. Material

The materials used in this study were seaweed genera Euchema sp. obtained from the coast of Wongsorejo District, Banyuwangi Regency, East Java. The solvents used for the extraction are methanol and ethyl acetate.

2.2. Methods

2.2.1. Sampling and pretreatment

Seaweed Euchema sp. is cultivated seaweed that is taken directly from the coastal waters of Wongsorejo District, Banyuwangi Regency, East Java. Seaweed is washed using running water to remove dirt, grit, and epiphytes. The sample is then dried using the wind-dry method and mashed using a grinding machine to obtain a fine powder.

2.2.2. Extraction

Extraction of the photoprotective compounds of seaweed Eucheuma sp. carried out by the maceration method. Seaweed powder is macerated with a sample and solvent ratio of 1:3 (g / ml) [17]. A total of 300 g of seaweed samples were immersed in 900 ml of solvent in a container at room temperature for 3x24 hours [18]. The results of maceration are then filtered every 1 x 24 hours so that the resulting filtrate and residue are then macerated again using a new solvent of the same type. The filtrate mixture from the extraction results is then evaporated using a rotary evaporator at 40°C until a concentrated extract is obtained in the form of a paste from each solvent [16]. The extract is then put into a vial bottle and stored in a refrigerator at low temperature to maintain its durability before being used at a later stage.

2.2.3. Measurement of the sun protection factor (SPF), %Te, and %Tp

Absorbance measurements using a spectrophotometer were carried out at wavelengths between 290-375 nm with 5 nm intervals. The solvent from each extract was used as a blank [19]. Determination of the effectiveness of photoprotective compounds is done by calculating the value of the Sun Protection Factor (SPF) based on the absorbance value obtained from UV-Vis spectrophotometer measurements through calculations using the Mansur calculation [11] as follows:
Information:
CF = correction factor = 10
EE = erythemal effect spectrum
I = spectrum of intensity from the sun
Abs = absorbance of the sample

The level of protection from the SPF value is presented in Table 1.

| SPF Protection Value | SPF Protection Value |
|----------------------|----------------------|
| 2-4 Minimum          | 2-4 Minimum          |
| 4-6 moderate         | 4-6 moderate         |
| 6-8 Extras           | 6-8 Extras           |
| 8-15 Maximum         | 8-15 Maximum         |
| ≥15 Ultra            | ≥15 Ultra            |

Measurement of the absorbance value for the calculation of the value of %Te and %Tp was carried out at the wavelength that causes erythema, namely in the range 292.5-337.5 nm with intervals every 5 nm for %Te and on the spectrum that causes pigmentation, namely 322.5-372.5 nm at intervals every 5 nm for %Tp [20]. The absorbance value is then converted into a transmission value (T) using the equation: 

\[
SPF_{\text{spetrafterometer}} = CF \times \sum_{\lambda}^\infty \frac{EE(\lambda) \times I(\lambda) \times Abs(\lambda)}{Abs(\lambda)}
\]

Information:
SPF = Sun Protection Factor

The level of protection of the %Te and %Tp indicators is presented in Table 2.

| %Te | %Tp | Category |
|-----|-----|----------|
| <1  | 3-40| Sunblock |
| 1-6 | 42-86| Ultra protection |
| 6-12| 45-86| Suntan |
| 10-18| 45-86| Tanning |

3. Result and discussion
The extract obtained from each type of solvent is in the form of a paste and has a different yield value. It was found that the extract yield from methanol solvent had the highest yield with a value of 3.34% compared to ethyl acetate with a value of 0.20% respectively. This indicates that the components contained in *Eucheuma* sp. more dissolved in polar solvents than semi-polar solvents. This is in line with previous research [23,16] who reported that polar compounds dissolve more easily in polar solvents and vice versa, and seaweed *E. cottonii* contains more polar bioactive compounds than semi-polar compounds. The high yield obtained from the extraction process can be influenced by several
factors such as the type of solvent used, the components of the extraction sample, the conditions and storage time, the extraction time and the comparison of the number of samples with the solvent [24].

**Table 3. Yield of methanol and ethyl acetate of *Eucheuma* sp.**

| Solvent          | Mass (g) | Yield (%) |
|------------------|----------|-----------|
| Metanol Powder   | 300      | 10.03     |
| Metanol Extract  |          | 3.34      |
| Ethyl acetate    | 300      | 0.61      |
| Ethyl acetate Extract | | 0.20      |

The results showed that the ethyl acetate extract from *Eucheuma* sp. able to provide an ultra-protective effect with an SPF value of 26.45 (Table 4) and as a sunblock against UV radiation that causes erythema and pigmentation (Table 5). This shows that each solvent has a different characteristic SPF value and transmission capability even though the three solvents are capable of producing the category of ultra-protection and sunblock protection capabilities.

High SPF content is generally found in polar and semi-polar samples. A previous study [25] reported that seaweed with the *Eucheuma* genera contains active compounds such as flavonoids, phenol hydroquinone, and triterpenoids which have potential as compounds with photoprotective activity. Red algae extract from the *Eucheuma* genera is also reported to have high antioxidant activity with active compounds of flavonoids, triterpenoids, alkaloids and ascorbic acid [26]. The active compounds and antioxidants found in seaweed have the ability to minimize the damage caused by exposure to ultraviolet radiation at the cellular level [9].

**Table 4. SPF value of *Eucheuma* sp.**

| Solvent      | n | SPF Value | Average SPF |
|--------------|---|-----------|-------------|
| Methanol     | 1 | 19.95     | 19.65 ± 0.26 |
|              | 2 | 19.51     |             |
|              | 3 | 19.48     |             |
| Ethyl acetate| 1 | 26.26     | 26.45 ± 0.28 |
|              | 2 | 26.76     |             |
|              | 3 | 26.31     |             |

**Table 5. The% Te and% Tp values of the extract of *Eucheuma* sp.**

| Pelarut      | n | %Te Value | Average %Te | %Tp Value | Average %Tp | Category     |
|--------------|---|-----------|-------------|-----------|-------------|--------------|
| Methanol     | 1 | 2.35      | 2.432 ± 0.076 | 44.73     | 45.548 ± 1.056 | Ultra protection |
|              | 2 | 2.50      | 46.74       |           |             |              |
|              | 3 | 2.45      | 45.17       |           |             |              |
| Ethyl acetate| 1 | 0.93      | 0.879 ± 0.052 | 19.37     | 18.638 ± 0.927 | Sunblock    |
|              | 2 | 0.82      | 17.63       |           |             |              |
|              | 3 | 0.89      | 19.05       |           |             |              |

**4. Conclusion**

The resultant *Eucheuma* sp. Extract showed that there were significant differences in the SPF, % Te, and % Tp values between different solvents used in the same extraction method. The extract with ethyl acetate solvent produced the highest SPF value and the best % Te and % Tp compared to methanol solvent.

**5. References**
[1] Biniek K, Levi K, Dauskardt, R H 2012. Solar UV radiation reduces the barrier function of human skin. Proceedings of the National Academy of Sciences. 109 17111–17116.

[2] Gilbert K P, Placzek M, Hotz M E 2019. Ultraviolet Exposure: Health Effects, in: Encyclopedia of Environmental Health. Elsevier, pp 185–194.

[3] D’Orazio J, Jarrett S, Amaro-Ortiz A, Scott T 2013 IJMS 14 12222–12248.

[4] Narayanan D I, Saladi R N, Fox J L 2010 International Journal of Dermatology 49 978–986.

[5] [WHO] World Health Organization. 2019. Skin Cancers. www.who.int.

[6] Brenner M, Hearing V J 2008 Photochemistry and Photobiology 84 539–549. htt

[7] Godic A, Poljšak B, Adamic M, Dahmene R 2014. The Role of Antioxidants in Skin Cancer Prevention and Treatment. Oxidative Medicine and Cellular Longevity 2014 p 1–6. h

[8] Chojnacka K 2012 TOPROCJ 3 20–28.

[9] Saewan N, Jimtaisong A 2015 J Cosmet Dermatol 14 47–63.

[10] Rastogi R P, Richa, Sinha R P, Singh S P, Häder D P 2010 J Ind Microbiol Biotechnol 37 537–558.

[11] Reis Mansur M C P P, Leitão S G, Cerqueira-Coutinho C, Vermelho A B, Silva R S, Presgrave O A F, Leitão Â A C, Leitão G G, Ricci-Júnior E, Santos E P 2016 Revista Brasileira de Farmacognosia 26, 251–258.

[12] Michalak I, Chojnacka K 2015 J Eng Life Sci. 15 160–176.

[13] Lalegerie F, Lajili S, Bedoux G, Taupin L, Stiger-Pouvreau V, Connan S, 2019 Marine Environmental Research 147, 37–48.

[14] Pangestuti R, Siahnaen E, Kim S K 2018 Photoprotective Substances Derived from Marine Algae. Marine Drugs 16, 399.

[15] Luthfiyana N, Nurilmala M Anwar E, Hidayat T 2016. Ratio of Seaweed Porridge Eucheuma cottonii and Sargassum sp. as a Sunscreen Cream Formula 19 13.

[16] Yanuarti R, Nurjanah N, Anwar E, Pratama G 2017 biosfera 34 51

[17] Gazali M, Nurjanah N, Zamani N P 2018 JPHPI 21 167

[18] Podungge A, Damongilala L J, Mewengkang H W 2017 JMTHP 6 1

[19] Mokodompit A N, Edy H J, Wiyono W 2013 Sun Protective Factor (SPF) Value Determination In Vitro Sunscreen Cream Avocado Skin Ethanol Extract p 2 3

[20] Pakki E, Murdifin M, Wijoyo N, Sumarheni S 2018 Study of sunscreen and antioxidant activity of combination extracts from the red algae Eucheuma cottonii and Eucheuma spinosum (Drug Invention Today) p 10 4

[21] Abdassah M, Aryani R, Surachman E, Muchtaridi M 2015 J. Appl. Pharm. Sci. 5 (6): 70-74.

[22] Maslihah, I., 1987. In Vitro Oktilmetoksinamat and Oksi Benson Activity Study and in Combination Form as Sunscreen in One Base of Nonionic Cream. University Airlangga Surabaya

[23] Suryaningrum T D, Wikanta T, Kristiana H 2006 JPBKP 1 51.

[24] Khopkar S M 2008 Basic Concepts of Analytical Chemistry. Jakarta.UL Press

[25] Nurjanah N, Nurilmala M, Anwar E, Luthfiyana N, Hidayat T 2006 Identification of Bioactive Compounds of Seaweed Sargassum sp. and Eucheuma cottonii Doty as a Raw Sunscreen Cream p 9.

[26] Mardiyah U, Fasya A G, Amalia S 2014 Extraction, Antioxidant Activity Test And Identification of Active Compound Group of Eucheuma Spinosum Red Algae From Banyuwangi Waters. AL.

6. Acknowledgement
Thank you to Universitas Brawijaya for research funding support through the Beginner Research Grants 2020, Research and Community Service Institute (No. Contract : 436.38/UN10.C10/PN/2020) and all parties involved in the research.