Polysaccharide isolation of Brown seaweed: *Sargassum* sp and its photoprotection activity

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Abstract: Sun radiation contains ultraviolet rays consisting of UV-A, UV-B, and UV-C. This ultraviolet light can cause adverse effects on the skin such as the appearance of sunburn (erythema), pigmentation, skin aging, and can also cause skin cancer (melanoma). To reduce the adverse effects on the skin, it is necessary to use sunscreen that protects the skin from exposure to ultraviolet rays. Brown seaweed is one of the natural ingredients that can be used as a natural ingredient for sunscreen. This is because genetically brown seaweed is more often exposed to sunlight so it can synthesize components that can absorb ultraviolet light. *Sargassum* sp. It is known to have polysaccharide compounds such as alginate and fucoidan which have potential as antioxidants and anti-inflammatory. The purpose of this study was to determine the photoprotective activity of the polysaccharide compounds found in *Sargassum* sp.

1. Introduction

Solar radiation is a combination of ultraviolet radiation and visible light that can reach the earth’s surface with a wavelength of ≥295 nm [1]. Ultraviolet light itself is divided into 3 categories, namely UV-C, UV-B, and UV-A. Ultraviolet rays are classified as carcinogenic substances and can cause several other effects such as mutagens, lowering the immunity of the skin, and accelerating the rate of aging of the skin [2]. The effect of UV exposure from sunlight can increase Reactive Oxygen Species (ROS) and cause oxidative stress. Oxidative stress is a condition of an imbalance between ROS and antioxidants present in the skin. Excessive ROS will be toxic and cause cell damage [3]. To inhibit the ROS process, it is necessary to add antioxidant compounds in cosmetic ingredients used on the skin.

The application of sunscreen to the skin can change the body’s reaction to sun exposure. Sunscreen is a chemical that can absorb or block UV rays and exhibit various immunosuppressive effects from sunlight [4]. The raw materials used as UV filters consist of synthetic raw materials and natural raw materials. However, UV filters made from synthetic raw materials are known to have toxic and carcinogenic potential. It is necessary for further research on natural raw materials from the manufacture of UV filters. Brown Algae is one of the natural ingredients that can be used as an ingredient for making sunscreen. Genetically, Brown Algae are more often exposed to sunlight, therefore brown algae can synthesize components that can absorb UV rays. One of the brown algae that have the potential as a natural ingredient for sunscreen is *Sargassum* sp. [5].

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In this research, the photoprotection activity test focused on alginate and fucoidan polysaccharides. Polysaccharides in brown algae that act as antioxidants such as alginate and fucoidan are known to have the ability to ward off UV radiation and have the potential to reduce inflammation [6].

2. Methodology

The research process includes the sample extraction process and the photoprotective test process.

2.1 Extraction

The materials used in this study were seaweed genera *Sargassum* sp. obtained from the coast of Poteran Island, Madura, Indonesia. The seaweed sample was washed, dried and grinded into powder. The powder sample of *Sargassum* sp. was immersed in HCl (0.01 N) and CaCl₂ (2%) for 4 hours at room temperature and at 70°C. Samples then were centrifuged. Alginate extraction was carried out by dissolving the pellet in Na₂CO₃ (3%) then heated at 50°C. Sample then dried at 60°C. After drying, the membrane dialysis process was carried out for 4x24 hours at a temperature of 4°C. The fucoidan extraction was carried out by evaporation for 1-2 hours at a temperature of 40-50°C using a vacuum rotary evaporator to produce a paste-shaped extract, then a membrane dialysis process was carried out for 4x24 hours at a temperature of 4°C [7,8].

The results of this extraction process are then weighed to determine the weight and yield calculations are carried out [9]. Yield calculation is done by using the formula:

\[
\text{Yield} = \frac{\text{weight of alginate/fucoidan}}{\text{weight of powder sample}} \times 100\% \quad (1)
\]

2.2 Photoprotection Activity Test

The photoprotection activity test was carried out by calculating the SPF (Sun Protection Factor) value and calculating the percentage values of %Te and %Tp obtained from the absorbance value of the sample on the UV-Vis spectrophotometer. This SPF value measurement was carried out at a wavelength of 290-320 nm with 5 nm intervals [10]. Meanwhile, the measurement of %Te and %Tp was carried out at a wavelength of 292.5 – 337.5 nm with 5 nm intervals. The concentration of the test solution used was 4000 ppm with an arithmetic series of 1 ppm, 100 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm, and 4000 ppm.

The SPF value can be calculated using the equation according to Mansur calculation as follows [11]:

\[
\text{SPF} = CF \times \sum_{\lambda=290}^{320} EE(\lambda) \times I(\lambda) \times \text{Abs}(\lambda) \quad (2)
\]

Note: CF = Correction Factor = 10; EE = Erythmogenic Effect of radiation (Table 1); I = Spectrum of Solar Intensity (Table 1); Abs (\lambda) = absorbance value.

| \(\lambda\) (nm) | EE \times I |
|-----------------|------------|
| 290             | 0.0150     |
| 295             | 0.0817     |
| 300             | 0.2874     |
| 305             | 0.3278     |
| 310             | 0.1864     |
| 315             | 0.0839     |
| 320             | 0.0180     |
| \(\sum_{\lambda} EE \times I\) | 1          |
Table 2. Category SPF Protection on sunscreen [13].

| SPF   | Category of Protection |
|-------|------------------------|
| 2 – 4 | Minimum Protection     |
| 4 – 6 | Medium Protection      |
| 6 – 8 | Extra Protection       |
| 8 – 15| Maximum Protection     |
| ≥ 15  | Ultra Protection       |

The percentage of erythema transmission is the ratio of the amount of UV-B light energy transmitted by sunscreen preparations on the erythema spectrum in the wavelength range of 292.5 – 317.5 nm [14].

The value of %Te itself is obtained by calculation using the formula [15]:

\[
\%Te = \frac{\sum \text{Transmisi erytema}}{\sum \text{Fluks erytema}} = \frac{\sum T \times Fe}{\sum Fe} \tag{3}
\]

Note: T = Sample Transmission; Fe = Erythema Effectiveness Factor (Tabel 3).

Table 3. Erythema Effectiveness Factor [15]

| λ (nm) | Fe (µW/cm²) |
|--------|-------------|
| 292.5  | 1.139       |
| 297.5  | 6.51        |
| 302.5  | 10          |
| 307.5  | 3.577       |
| 312.5  | 0.973       |
| 317.5  | 0.567       |
| 322.5  | 0.455       |
| 327.5  | 0.289       |
| 332.5  | 0.129       |
| 337.5  | 0.0456      |

The percentage of pigmentation transmission is the ratio of the amount of UV-A light energy transmitted by sunscreen preparations on the pigmentation spectrum in the wavelength range of 322.5 – 372.5 nm [14].

The %Tp value itself is obtained by calculation using the formula [15]:

\[
\%Tp = \frac{\sum \text{Transmisi pigmentasi}}{\sum \text{Fluks pigmentasi}} = \frac{\sum T \times Fp}{\sum Fp} \tag{4}
\]

Note: T = Sample Transmission; Fp = Pigmentation Effectiveness Factor (Tabel 4).

Table 4. Pigmentation Effectiveness Factor [15]

| λ (nm) | Fp (µW/cm²) |
|--------|-------------|
| 322.5  | 1.079       |
| 327.5  | 1.02        |
| 332.5  | 0.936       |
| 337.5  | 0.798       |
| 342.5  | 0.669       |
| 347.5  | 0.57        |
| 352.5  | 0.488       |
| 357.5  | 0.456       |
| 362.5  | 0.356       |
| 367.5  | 0.31        |
| 372.5  | 0.26        |
Table 5. Protection category on indicators %Te and %Tp

| %Te a) | %Tp b) | Category     |
|--------|--------|--------------|
| <1     | 3-40   | Sunblock     |
| 1-6    | 42-86  | Proteksi ultra|
| 6-12   | 45-86  | Suntan       |
| 10-18  | 45-86  | Tanning      |

3. Results and Discussion

Extracted polysaccharides of brown algae Sargassum sp. showed that alginate extract was more abundant than fucoidan extract. This is because alginate is the main constituent of the cell wall of brown algae, while fucoidan is found in parts of the cell wall, namely fibrillar tissue and intercellular spaces of brown algae. Alginates alone account for about 40% of the dry weight of brown algae, while fucoidan is only about 5-10% of the dry weight of brown algae [16].

The following is the extraction result from alginate and fucoidan brown algae Sargassum sp.

Table 6. Sargassum sp. polysaccharide extraction results.

| Polysaccharide | Mass (gr) | Yield (%) |
|----------------|-----------|-----------|
| Alginate       | 40        | 1.2       | 3.00     |
| Fucoidan       | 40        | 0.11      | 0.28     |

The protocol of extraction used several different solvents for alginate and fucoidan extracts. The solvents used during the initial immersion process were HCl (0.01 N) and CaCl₂ (2%). The immersion process was also carried out at room temperature and hot temperature (70°C). The use of acid, calcium chloride salt and heating treatment during the extraction process can affect the amount of yield produced [17].

SPF test on alginate and fucoid samples and Sargassum sp. begins by diluting each sample. The absorbance value for each sample using a spectrophotometer with a wavelength of 290 nm - 320 nm and then calculate the SPF value. The following is the result of calculating the SPF value on alginate and fucoid samples and Sargassum sp.

Table 7. SPF value of alginate and fucoidan Sargassum sp.

| Concentration | Alginate SPF | Protection | Fucoidan SPF | Protection |
|---------------|--------------|------------|--------------|------------|
| 4000          | 32.218       | ultra      | 65.709       | ultra      |
| 3500          | 26.805       | ultra      | 22.056       | ultra      |
| 3000          | 26.711       | ultra      | 25.961       | ultra      |
| 2500          | 24.038       | ultra      | 18.189       | ultra      |
| 2000          | 11.823       | maximum    | 14.921       | maximum    |
| 1500          | 7.239        | extra      | 28.459       | ultra      |
| 1000          | 11.716       | maximum    | 13.328       | maximum    |
| 500           | 10.688       | maximum    | 15.905       | ultra      |
| 100           | 1.509        | false      | 2.888        | minimum    |
| 100           | 8.225        | extra      | 8.887        | maximum    |

The results from the table above show that the alginate sample of Sargassum sp. the highest SPF value and protection category are shown by samples with a concentration of 4000 ppm with an SPF value of 32.218 with ultra protection category, while the lowest SPF value and protection category are shown by samples with a concentration of 100 ppm with an SPF value of 1.509 and the protection category shows False because the SPF value is too low. so it does not fall into the category of protection.
The extract of fucoidan *Sargassum* sp. the highest SPF value and protection category were shown by the sample with a concentration of 4000 ppm with an SPF value of 65.709 and the ultra protection category, while the lowest SPF value and protection category were shown by the sample with a concentration of 100 ppm with an SPF value of 2.888 and the protection category showed *minimum*.

SPF is a universal indicator that describes the effectiveness of a product or substance that is UV protection. The length of time the skin is protected by a sunscreen is largely determined by the SPF value listed on the product. Without sunscreen, the skin turns red and burns within 10 minutes in the sun [18].

Erythema and pigmentation transmission tests were carried out at a concentration of 2500 ppm for alginate and fucoid samples and *Sargassum* sp. The absorbance value was calculated using a spectrophotometer at a wavelength of 292.5 nm – 337.5 nm for the %Te indicator and a wavelength of 322.5 nm – 372.5 nm for the %Tp indicator. The following is the result of calculating the values of %Te and %Tp in alginate and fucoid samples and *Sargassum* sp.

| Sample               | %Te Value | Category | %Tp Value | Category |
|----------------------|-----------|----------|-----------|----------|
| Alginate *Sargassum* sp. | 0.436     | Sunblock | 3.919     | Sunblock |
| Fucoidan *Sargassum* sp. | 1.573     | Sunblock | 5.874     | Sunblock |

The results from the table above show that at a concentration of 2500 ppm alginate and fucoid of *Sargassum* sp. show the value of %Te and %Tp respectively in the category of Sunblock. Erythema is an inflammatory reaction that occurs due to excessive exposure to ultraviolet radiation on the skin. Inflammatory reactions that arise due to ultraviolet radiation include the appearance of redness of the skin, warm skin, and tightened skin conditions. Erythema or sunburn can be experienced by light-skinned people as well as dark-skinned people. Pigmentation of the skin is indicated by the presence of a *tanning* reaction. The *tanning* process on human skin depends on wavelength radiation. UVB rays cause skin pigmentation. When the skin is exposed to UVB radiation, no pigment production occurs in the skin other than an erythema reaction that occurs. The process of pigmentation occurs after the skin is exposed to sunlight [19].

4. Conclusion
The polysaccharides of *Sargassum* sp. Collected from Poteran island were isolated into alginate and fucoid with different yield. Those polysaccharides promise as a source of photoprotection compound. It is shown from the protection activity as sunblock.

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