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Analysis of Secondary Metabolite Compounds and Sunscreen Potential of Red Seaweed Extract (*Kappaphycus alvarezii*) on the North Coast of Jepara

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ABSTRACT

Red seaweed (*Kappaphycus alvarezii*) or Eucheuma cottonii is one of seaweed that is widely cultivated by people in Jepara. Several studies stated that the seaweed has a lot of secondary metabolite content, one of them is Phenolic. It is known that these metabolites have potential to be used as sunscreen.The riserch is experimental and purpose of this study was to analyze secondary metabolite compounds and the sunscreen potential of red seaweed (*Kappaphycus alvarezii*) with solvent variations (70% ethanol, ethyl acetate and N-Hexane) using the *Ultrasound Assisted Extraction* (UAE) method. The average of total phenol content of 70% ethanol extract was 8.6%; ethyl acetate extract was 3.25% and N-Hexane extract was 5.23%. Sunscreen activity of red seaweed extract (*Kappaphycus alvarezii*) based on the highest SPF value in 70% ethanol extract with a concentration of 1000 ppm was 7.72 (extra protection). The best erythema transmission percentage (%Te) value in 70% ethanol extract with a concentration of 1000 ppm was 1.457% (extra protection). The best pigmentation transmission percentage (%Tp) value in 70% ethanol extract with a concentration of 1000 ppm is 3.21% (sunblock).

Keywords: Secondary Metabolites, *Kappaphycus alvarezii*, Sunscreen, Ultrasonic Assisted Extraction

Introduction

Indonesia is a tropical country located on the equator line. This location causes Indonesia receive high intensity exposure of sunlight (Mumtazah et al., 2020). Sunlight contains ultraviolet (UV) light (Sineke et al., 2016). Ultraviolet (UV) light are electromagnetic wave radiation emitted by the sun. Not all ultraviolet light can reach the earth's surface. Ultraviolet light have several types, including UV-A, UV-B and UV-C (Lisna et al., 2023). Ultraviolet (UV) light can be classified into UV A with the wavelength between 320 - 400 nm, UV B with the wavelength of 290 - 320 nm and UV C with the wavelength of 10 - 290 nm (Isfardiyana et al., 2014). Prolonged exposure to UV light is able to cause acute and chronic health problems to the skin. Direct protection from sunlight on the skin is the use of sunscreen. Sunscreen is a cosmetic preparation that is used to reflect or actively absorb

the sunlight, especially in areas with ultraviolet and infrared wave emissions, so that it can prevent skin disorders due to ultraviolet light (Auliani et al., 2020). The effectiveness of sunscreen can be expressed by the percentage of erythema transmission, pigmentation transmission, and the Sun Protection Factor (SPF) value. These three parameters can be determined using the spectrophotometric method (Ahmad, 2015).

One of the secondary metabolite compounds that can be used as sunscreen is phenolic. Phenolic compounds are secondary metabolite compounds found in plants characterized by an aromatic ring structure with one or more hydroxyl groups (OH). The characteristics of phenolic compounds tend to be easily soluble in polar solvents, become colorless if it's reacted to pure polar solvent, meanwhile if it's exposed to air, oxidation will occur that causing dark color, forming a complex with proteins, very sensitive to enzyme oxidation, easily oxidized by strong bases, and able to absorb UV-Vis light (Julianto, 2019). Phenolic compounds in plants are used to protect plant tissue from damage due to sunlight radiation.

Phenolic compounds have the potential as sunscreens due to the presence of chromophore groups that can absorb UV light that reduce the intensity on the skin (Whenny et al., 2016). Phenolic compounds have conjugated bonds in the benzene core, when it is exposed to UV light, resonance will occur by means of electron transfer that causing the compounds have potential as a photoprotective in sunscreens (Kusriani et al., 2017). One of the plants that has phenolic secondary metabolites is seaweed. Seaweed contains a number of bioactive components such as phenolic compounds, natural pigments, sulfated polysaccharides, fibers and other bioactive components that have been studied to be worthwhile for health (Erniati et al., 2016). An example of seaweed that has phenolic secondary metabolites is red seaweed (*Kappaphycus alvarezii*). Red seaweed is a type of seaweed from the *Solieaceae* family which contains flavonoids, hydroquinone phenols, triterpenoids and tannins.

Methodology

Population dan Sample

This Researh is experimental. The sample utilized in this research were a collection of the freshest, healthiest, finest and aged about 40-45 days red seaweed (*Kappaphycus alvarezii*) obtained from the Central Institute of Brackishwater Aquaculture (*BBPPBAP*) Bulu District, Jepara Regency , Central Java.

Tools and Materials

The tools used in this study were: analytical scales (*Ohaus*), simplicia blender, sieve mesh No. 40, filter paper, measuring cup (*Pyrex*), beaker glass (*Pyrex*), Erlenmeyer (*Pyrex*), water bath (DHH - 4), test tube (*Pyrex*), glass funnel (Pyrex), dropper pipette, test tube rack, drying cabinet, crucible porcelain, spatula, *rotary vacuum evaporator* (RE 100 - Pro), moisture balance, micropipette, ultrasonic bath (Brason 2002), label paper, measuring flask (*Pyrex*), spatula, cuvette, and UV - Vis spectrophotometry (*Shimadzu*). The research materials used for extraction were red seaweed (*Kappaphycus alvarezii*) obtained from the Central Institute of Brackishwater Aquaculture (*BBPPBAP*) Jepara, 70% ethanol, ethyl acetate and N-Hexane. The research materials used for phytochemical screening were red seaweed extract (*Kappaphycus alvarezii*) and 1% $FeCl_3$. The research materials used to determine the total phenolic levels were red seaweed extract (*Kappaphycus alvarezii*) with variations of 70% ethanol solvent; ethyl acetate; and N-Hexane, ethanol p.a, gallic acid standard, (*Sigma - Aldrich*), Folin Ciocalteu reagent (*Merck*), Na_2CO_3 (Sodium Carbonate) 7.5% (*Merck*).

Research Procedures

1. Sample Preparation
   The sample used in this study was red seaweed (*Kappaphycus alvarezii*) obtained from the Central Institute of Brackishwater Aquaculture (*BBPPBAP*) Bulu District, Jepara Regency, Central Java.

2. Preparation of Dry Simplisia
   3 kg of fresh red seaweed were taken as the sample. It had to be wet-sorted and washed in the running water. Next, red seaweed were cut into small pieces and put on a tray. By using drying cabinet at a temperature of 50 ° C for 6 hours, the red seaweed got draining process.

3. Making Simplisia Powder
   The dry red seaweed (*Kappaphycus alvarezii*) was weighed and powdered. It was blended and sifted with a sieve mesh no. 40.

4. Moisture Content Determination
   Water content determination of red seaweed powder (*Kappaphycus alvarezii*) was carried out using a moisture balance. 1 gram sample were placed into the moisture balance at a temperature of 105°C, carried out for 3 times replication, the water content was set to maintain its quality, it was <12%.

5. Making Extract
   Extraction of red seaweed (*Kappaphycus alvarezii*) extracted using the Ultrasonic Assisted Extraction (UAE) method. Red seaweed powder was

taken 200 grams as much as 3 times then each of them was extracted with various solvents (70% ethanol, ethyl acetate and N-Hexane) as much as 850 mL. The sonication process was carried out for 2 minutes and repeated 3 times, then filtered and separated the extract from the residue. The residue was re-soaked with 750 mL of solvent in sonication for 2 minutes repeated 3 times, filtered and the filtrate was separated. Subsequently, the remaining material was remacerated with 750 mL. of solvent using the sonication method for 2 minutes repeated 3 times more. Then the filtrate obtained was concentrated with a *evaporator* at a temperature of 40°C until forming a thick extract. Then it could be wieghed and calculated the yield (Susiloningrum et al., 2021).

6. Phenolic Identification
   The phenolic test was carried out by taking 1 mL of red seaweed extract (*Kappaphycus alvarezii*) added 3 drops of 1% $FeCl_3$ solution. Color would change into green, red, purple, blue, dark blue, pitch black, blackish blue or blackish green that indicate a positive result for the presence of phenolic compounds (Wardhani et al., 2018).

7. Determination of Total Phenolic Level
   a. Making a stock solution of gallic acid.
      100 mg of gallic acid standard was taken and dissolved in distilled water into 100 mL. Next it was diluted, 10 mL of a 1000 ppm gallic acid stock solution was used to dilute it to 100 ppm, and 100 mL of distilled water was used to dissolve it Andriani et al., 2018).
   b. The maximum wavelength of gallic acid determination
      Following the addition of 0.3 mL of a 30 ppm gallic acid solution to a 5 mL volumetric flask, 1.5 mL of Folin-Ciocalteu reagent was introduced. The resulting solution was allowed to react for three minutes, after which 1.2 mL of 7.5% Na_2CO_3 was added and mixed thoroughly. The mixture was then left to stand at room temperature before measuring its absorbance across a wavelength range of 400-800 nm (Andriani et al., 2018).
   c. Determination of Operating Time
      A 5 mL volumetric flask received 0.3 mL of a 30 ppm gallic acid solution. Subsequently, 1.2 mL of a 7.5% Na_2CO_3 solution and 1.5 mL of Folin-Ciocalteu reagent were added, and the resulting solution was mixed thoroughly. The absorbance of the mixture was then recorded at one-minute intervals for one hour, measured at the wavelength of peak absorption. (Andriani et al., 2018).
   d. Determination of the standard curve of gallic acid

0.3 mL gallic acid solution was taken at concentrations of 20; 30; 40; 50 and 60 ppm. A 5 mL measuring flask was filled with each concentration, and 1.5 mL of the reagent Folin-Ciocalteu was added.. It should be mixed. Next, 1.2 mL of 7.5% Na_2CO_3 is added and shaken until homogeneous. The solution was then allowed to stand at room temperature for the duration of the experiment. The absorbance of the solution was then determined at the wavelength of maximum absorption for the acid (Andriani et al., 2018).

e. Calculating the overall phenolic content

It was determined by weighing 10 mg of 70% ethanol, N-hexane and ethyl acetat extract of red seaweed extract (*Kappaphycus alvarezii*). Each extract was then diluted in the ethanol p.a. up to 10 mL and homogenized. Next, 0.3 mL solution was taken using pipette and 1.5 mL of Folin Ciocalteu reagent was added, followed by 1.2 mL of 7.5% sodium carbonate (Na_2CO_3) solution. The solution was standed at operating time and three replications of the absorbance measurement were made at the maximum wavelength (Andriani et al., 2018).

8. Calculating the Value of the SPF
20 mg 70% ethanol, N-hexane and ethyl acetate extract of red seaweed (*Kappaphyvus alvarezii*) were put and dissolved into a 20 mL measuring flask with ethanol p.a up to the limit mark to obtain a stock solution of 1000 ppm. The stock solutions were pipetted 2; 4; 6; 8; and 10 mL respectively. Then each solution was added with ethanol p.a until 10 mL so that various solvent were obtained from the stock solution diluted in concentrations of 200; 400; 600; 800 and 1000 ppm. The solutions were tested with a wavelength of 290-320 nm every 5 nm interval using UV-Vis spectrophotometry (Taupik et al., 2022).

9. Calculating the Erythema Transmission Percentage
20 mg 70% ethanol, N-hexane and ethyl acetate extract of red seaweed (*Kappaphyvus alvarezii*) were put and dissolved into a 20 mL measuring flask with ethanol p.a up to the limit mark to obtain a stock solution of 1000 ppm. The stock solutions were pipetted 2; 4; 6; 8; and 10 mL respectively. Then each solution was added with ethanol p.a until 10 mL so that various solvent were obtained from the stock solution diluted in concentrations of 200; 400; 600; 800 and 1000 ppm. The solutions were tested with a wavelength of 292.5-317.5 nm every 5 nm interval using UV-Vis spectrophotometry (Taupik et al., 2022).

10. Determination of Pigmentation Transmission Percentage

20 mg 70% ethanol, ethyl acetate and N-hexane extract of red seaweed (*Kappaphyvus alvarezii*) were put and dissolved into a 20 mL measuring flask with ethanol p.a up to the limit mark to obtain a stock solution of 1000 ppm. The stock solutions were pipetted 2; 4; 6; 8; and 10 mL respectively. Then each solution was added with ethanol p.a until 10 mL so that various solvent were obtained from the stock solution diluted in concentrations of 200; 400; 600; 800 and 1000 ppm. The solutions were tested with a wavelength of 322,5 – 372,5 nm every 5 nm interval using UV-Vis spectrophotometry (Taupik et al., 2022).

Result and Discussion

1. Yield of Red Seaweed Extract (*Kappaphycus alvarezii*) can be seen in table 3.

Table 3. Extract Yield Result

Simplisia Name	Solvent	Powder (grams)	Extract (grams)	Yield
Red Seaweed (*Kappaphycus alvarezii*)	70% Ethanol	200	25	12,68
	Ethyl Acetate	200	25	12,60
	N – Hexane	200	27	13,54

The yield obtained from 70% ethanol extract was 12.68%, ethyl acetate extract was 12.60% and N-Hexane extract was 13.54%. The yield is stated to be good if > 10%. Based on the yield obtained from the three solvents, the yield was > 10%.

2. The organoleptic test results of red seaweed extract (*Kappaphycus alvarezii*) can be seen in table 4.

Table 4. Organoleptic Test Result

Sample	Solvent	Observation		
		Color	Smell	Form
Red Seaweed Extract (*Kappaphycus alvarezii*)	70% Ethanol	Dark Brown	Distinctive smell	Thick
	Ethyl Acetate	Dark Brown	Distinctive smell	Thick
	N-Hexane	Dark Brown	Distinctive smell	Thick

The results of organoleptic tests of red seaweed extract (*Kappaphycus alvarezii*) in each solvent were the sample having a dark brown color, a distinctive odor of red seaweed (*Kappaphycus alvarezii*) and forming a thick extract.

3. The results of Phytochemical Screening of Red Seaweed (*Kappaphycus alvarezii*) can be seen in table 5.

Table 5. Phytochemical Screening Results

Compound	Solvent	Reagent	Description	Result
Phenolic	70% Ethanol	$FeCl_3$.	+	Dark Black
	Ethyl Acetate	$FeCl_3$.	+	Dark Black
	N-Hexane	$FeCl_3$.	+	Dark Black

The phytochemical screening result of red seaweed (*Kappaphycus alvarezii*) with 70% ethanol, ethyl acetate and N-Hexane obtained positive results which were indicated by a color change to be dark black.

4. Determining the total phenolic content in Red Seaweed Extract. (*Kappaphycus alvarezii*) result can be seen in table 9.

Table 9. Determination of Total Phenolic Content Result

Sample	Absorbance	Gallic acid equivalent (ppm)	Total Phenolic Content (%)	Average (%)	SD
70% Ethanol	0,628	86,28	8,62 %		
	0,627	86,12	8,61 %	8,6 %	±0,02
	0,625	85,81	8,58 %		
Ethyl Acetate	0,285	32,68	3,26 %		
	0,282	32,21	3,22 %	3,25 %	±0,03
	0,286	32,84	3,28 %		
N-Hexane	0,410	52,21	5,22 %		
	0,412	52,53	5,25 %	5,23 %	±0,01
	0,411	52,37	5,23 %		

The determination of the maximum wavelength aimed to obtain the maximum absorbance when measuring the reaction between gallic acid and Follin-Ciocalteu reagent. Measurement of an analyte must have used the maximum wavelength because at the maximum wavelength its sensitivity was very high, o maximize the absorbance change per unit of concentration (Apriliyani et al., 2018).

In this study, the maximum wavelength measurement was carried out using a concentration of 30 ppm which was the concentration series to be used. The reagents added are Folin Ciocalteu and Na_2CO_3. The addition of Follin-Ciocalteu reagent aimed to maintain the wavelength in

the visible region and the addition of Na_2CO_3 aimed to form a complex compound so that there was a shift in the wavelength towards the visible (Listiana et al., 2022).

The study results showed that the maximum wavelength obtained was 774 nm. At a wavelength of 774 nm, the *Lambert-Beer* law was fulfilled (Rizkiana et al., 2018). The results of the study also indicated that the operating time showed stable absorption at 45 minutes. This aligned with the findings of the research by Aang et al., (2021) that the results of the operating time were obtained at 45 minutes. Determination of operating time aimed to determine the time needed by the sample to obtain the measurement time when the reaction had run optimally which was indicated by stable absorbance (Suharyanto & Prima, 2020).

Based on the absorbance results obtained, they were then turned into the linear regression equation y = 0.0064x + 0.0758. Then the average was calculated until the total phenolic content of 70% ethanol solvent was 8.6%; ethyl acetate solvent was 3.25%; and N-Hexane solvent was 5.23%. Based on the study, the highest phenolic content of red seaweed extract (*Kappaphycus alvarezii*) was obtained in 70% ethanol. It showed that there was a similarity in polarity between the phenolic compounds of red seaweed extract and 70% ethanol as indicated by the high dissolved phenolic compounds.

5. The results of the SPF Value of Red Seaweed Extract (*Kappaphycus alvarezii*) can be seen in tables 10, 11 and 12.

Table 10. SPF Value Results of Red Seaweed Ethanol 70% Extract

No	Solvent	Concentration (ppm)	SPF Value	Protection Categories
1.	70%Ethanol	200	3,08	Minimum Protection
2.		400	4,53	Medium Protection
3.		600	5,35	Medium Protection
4.		800	6,76	Extra Protection
5.		1000	7,72	Extra Protection

Table 11. SPF Value Results of Red Seaweed Ethyl Acetate Extract

No	Solvent	Concentration (ppm)	SPF Value	Protection Categories
1.	Ethyl Acetate	200	2,09	Minimum Protection
2.		400	3,30	Minimum Protection
3.		600	4,00	Minimum Protection
4.		800	4,87	Medium Protection
5.		1000	5,32	Medium Protection

Table 12. SPF Value Results of Red Seaweed N-Hexane Extract

No	Solvent	Concentration (ppm)	SPF Value	Protection Categories
1.	N-Hexane	200	2,19	Minimum Protection
2.		400	4,35	Medium Protection
3.		600	5,37	Medium Protection
4.		800	6,43	Extra Protection
5.		1000	7,00	Extra Protection

The SPF (Sun Protection Factor) values result obtained in the study showed that the 70% ethanol was higher than the N-Hexane and ethyl acetate solvents. This was because the SPF value was related to the total phenolic content value that had been obtained, where the total phenolic content of red seaweed extract (*Kappaphycus alvarezii*) with 70% ethanol solvent obtained higher results compared to N-Hexane and ethyl acetate solvents. It showed that there was a similarity in polarity between the phenolic compounds of red seaweed extract and 70% ethanol which was indicated by the high dissolved phenolic compounds against the SPF (Sun Protection Factor) value.

6. The Erythema Transmission Percentage (%Te) result of Red Seaweed Extract (*Kappaphycus alvarezii*) can be seen in tables 13, 14 and 15

Table 13. Result of Erythema Transmission Value of Red Seaweed 70% Ethanol Extract

No	Solvent	Concentration	%Te	Protection Categories
1.	70% Etanol	200 ppm	5,695%	Extra Protection
2.		400 ppm	5,212%	Extra Protection
3.		600 ppm	4,666%	Extra Protection
4.		800 ppm	2,299%	Extra Protection
5.		1000 ppm	1,457%	Extra Protection

Tabel 14. Result of Erythema Transmission Value of Red Seaweed Ethyl Acetate Extract

No	Solvent	Concentration	%Te	Protection Categories
1.	Ethyl Acetate	200 ppm	17,479%	*Fast Tanning*
2.		400 ppm	16,845%	*Fast Tanning*
3.		600 ppm	15,644%	*Fast Tanning*
4.		800 ppm	14,314%	*Fast Tanning*
5.		1000 ppm	13,328%	*Fast Tanning*

Tabel 15. Result of Erythema Transmission Value of Red Seaweed N-Hexane Extract

No	Solvent	Concentration	%Te	Protection Categories
1.		200 ppm	9,565%	Standard Suntan

2.		400 ppm	8,636%	Standard Suntan
3.	N-Hexane	600 ppm	6,706%	Standard Suntan
4.		800 ppm	5,417%	Extra Protection
5.		1000 ppm	4,798%	Extra Protection

The average erythema value of Red seaweed (*Kappaphycus alvarezii*) with various solvents was *fast tanning, standard suntan, extra protection* and *sunblock* categories. *Fast tanning* is in the range of 10 to 18 which means it has the ability to darken the skin quickly without causing redness as a protection for the skin. *Standard suntan* is in the range of 6 to 12 which means the ability to darken the skin from UV B radiation which will produce a little erythema without pain. *Extra protection* is in the range of 1 to 6 which means it has the ability to protect the skin from exposure to UV B light that can cause erythema by absorbing more UV light. *Sunblock* is in the range of <1 so that it can totally protect against sunlight, both UV A and UV B.

Based on the research results of the three solvents, 70% ethanol obtained the smallest erythema transmission percentage (%Te) compared to N-Hexane and ethyl acetate, which indicated that the smaller erythema transmission percentage the less UV-B light were transmitted, Therefore, the compound has great activity as a sunscreen.

7. The Percentage of Pigmentation Transmission (%Tp) result of Red Seaweed Extract (*Kappaphycus alvarezii*) can be seen in tables 16, 17 and 18.

Table 16. Results of Pigmentation Transmission Value of Red Seaweed 70% Ethanol Extract

No	Solvent	Concentration	%Tp	Protection Categories
1.		200 ppm	8,83 %	Sunblock
2.		400 ppm	7,45 %	Sunblock
3.	70% Etanol	600 ppm	6,49 %	Sunblock
4.		800 ppm	3,28 %	Sunblock
5.		1000 ppm	3,21 %	Sunblock

Table 17. Results of Pigmentation Transmission Value of Red Seaweed Ethyl Acetate Extract

No	Solvent	Concentration	%Tp	Protection Categories
1.		200 ppm	18,59 %	Sunblock
2.		400 ppm	17,81 %	Sunblock
3.	Ethyl Acetate	600 ppm	17,53 %	Sunblock
4.		800 ppm	13,43 %	Sunblock
5.		1000 ppm	12,89 %	Sunblock

Table 18. Results of Pigmentation Transmission Value of Red Seaweed 7N-Hexane Extract

No	Solvent	Concentration	%Tp	Protection Categories
1.		200 ppm	9,81 %	Sunblock
2.		400 ppm	9,18 %	Sunblock
3.	N-Hexane	600 ppm	7,74 %	Sunblock
4.		800 ppm	5,18 %	Sunblock
5.		1000 ppm	4,54 %	Sunblock

Based on the %Tp value, red seaweed extract (*Kapppaphycus alvarezii*) with 70% ethanol, ethyl acetate and N-Hexane had sunscreen effectiveness as a sunblock or total block because the %Tp value obtained from the five extract concentrations with a range of 3 - 40% which indicated that the red seaweed extract (*Kapppaphycus alvarezii*) could absorb UV-A light.

Based on the three solvents, 70% ethanol obtained a best percentage of pigmentation transmission (%Tp) value between ethyl acetate and N-Hexane because the results obtained were smaller than ethyl acetate and N-Hexane which indicated that UV-A light transmission decreased with decreasing pigmentation transmission percentage. thus that it is possible to say that the material is quite effective as a sunscreen.

Based on the study, there is a correlation between total phenolic content and sunscreen potential expressed in the SPF values, percentages of pigmentation transmission (%Tp) and erythema transmission (%Te). Phenolic compounds could act as sunscreens to prevent the adverse effects of UV radiation on the skin because antioxidants act as photoprotective. Because phenolic compounds include chromophore groups that can absorb UV light, they may be used as sunscreens, so that it could reduce the intensity on the skin (Whenny et al., 2016).

Phenolic compounds had conjugated bonds in the benzene core where when exposed to UV light, resonance occured by means of electron transfer, causing this compound having the potential to be photoprotective in sunscreens (Kusriani et al., 2017). Based on the results of this study, the highest total phenolic content and SPF value were obtained in 70% ethanol. It was higher than ethyl acetate and N-Hexane, it was 8.6% with an SPF value of 7.72 (extra protection). It happened because the SPF value was related to the total phenolic content value obtained, where the total phenolic content of red seaweed extract (*Kappaphycus alvarezii*) with 70% ethanol was higher than ethyl acetate and N-Hexane. This showed that there was a similarity in polarity between the phenolic compounds of red seaweed extract and 70% ethanol as indicated by the high dissolved phenolic compounds against the SPF (Sun

Protection Factor) value.

Based on the study results of Bahar et al (2021), it was explained that there was a positive correlation between the phenolic compound content and the SPF value of the extract. The extract which was detected containing stronger phenolic compounds, the SPF value would be increased. Phenolic compounds had the potential as effective sunscreens due to the existence of chromophore groups that have the ability to absorb ultraviolet light, so that it could reduce the intensity on the skin.

Sunscreen activity could also be determined based on the percentage values for pigmentation transmission (%Tp) and erythema transmission (%Te). In this study, the best (%Te) and (%Tp) were obtained in 70% ethanol at a concentration of 1000 ppm; it was 1.457% (extra protection) and 3.21% (sunblock). The results obtained were smaller than ethyl acetate and N-Hexane which indicated that the smaller percentage of erythema and the greater the pigmentation, the less UV-A radiation is transmitted, indicating strong sunscreen properties for the substance.

8. Data Analysis of Total Phenolic Content of Red Seaweed (*Kappaphycus alvarezii*)
   a. Normality Test
      The normality test result of the total phenolic content of Red Seaweed extract can be seen in table 19

Table 19. Normality Testof Total Phenolic Content

Statistical Test	Solvent	A	Significance	Description
Normality Test (*Shapiro-Wilk*)	70% Ethanol	>0,05	0,463	Normal
	Ethyl Acetate	>0,05	0,637	Normal
	N-Hexane	>0,05	0,637	Normal

The normality test on data in this study used the *Shapiro-Wilk* test because the number of data was less than 50. It is obtained that the significance value in the 70% ethanol was 0.463, ethyl acetate was 0.637, and N-Hexane was 0.637. From the data, the normality assumption was met significantly >0.05, so the data is normally distributed.

   b. Homogeneity Test
      The homogeneity test of the total phenolic content of the Red Seaweed (*Kappaphycus alvarezii*) extract can be seen in table 20.

Table 20. Homogeneity Test of Total Phenolic Content

Statistical Test	A	Significance	Description
Homogeneity Test(*Levene Statistic*)	>0,05	0,454	Homogeneous

The homogeneity test of data was conducted to determine the similarity or homogeneity of two or more data variants. The homogeneity test showed a result of 0.454 > 0.05, so it can be concluded that the data was homogeneous because it has the same variant.

c. Uji *One Way Anova*

The *One Way Anova* test result of the total phenolic content of Red Seaweed (*Kappaphycus alvarezii*) extract can be seen in table 21

Table 21. *One Way Anova* Test of Total Phenolic Content

Statistical Test	A	Significance	Description
One Way Anova Test	<0,05	0,001	Significant different

One Way Anova test was conducted to determine the difference in the average (*mean*) between two or more variables. The *One Way Anova* test showed a result of 0.001 <0.05, based on these findings, it can be said that there is a substantial discrepancy between solvent variations and total phenolic content because the results obtained were <0.05.

d. *Post Hoc* Test

The Post Hoc test result of the total phenolic content of Red Seaweed (*Kappaphycus alvarezii*) extract can be seen in table 22.

Table 22. *Post Hoc* Test of Total Phenolic Content

Solvent	Significance		
	Ethanol	Ethyl Acetate	N-Hexane
70% Ethanol		0,001*	0,001*
Ethyl Acetate	0,001*		0,001*
N-Hexane	0,001*	0,001*	

Post Hoc test was conducted to determine the pairs of groups that had significant differences further. In the *Post Hoc* test, it can be concluded that the data had significant differences between solvent variations and total phenolic content.

Conclusion

Based on the study it can be concluded as follows:

a. The analysis result of red seaweed extract (*Kappaphycus alvarezii*) was positive for containing phenolics.
b. The highest total phenolic content found in 70% ethanol, which was 8.6%. In N-Hexane, the total phenolic content was 5.23% and the lowest total phenolic content found in ethyl acetate, which was 3.25%.
c. There was sunscreen activity in red seaweed extract (*Kappaphycus alvarezii*) based on the highest SPF value in 70% ethanol extract with a concentration of 1000 ppm, which was 7.72 (extra protection). The best erythema transmission percentage (%Te) value was in 70% ethanol extract with a concentration of 1000 ppm, which was 1.457% (extra protection). The best pigmentation transmission percentage (%Tp) value for 70% ethanol extract with a concentration of 1000 ppm was 3.21% (sunblock).

Conflict of interest

No Conflict of interest

Reference

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