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FULL PAPER

Bioactivity profile of three types of seaweed as an antioxidant, UV-protection as sunscreen and their correlation activity

[1,2,*]Sami F.J., [1]Soekamto N.H., [1]Firdaus and [3]Latip J.

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin, Makassar, Indonesia*
[2]*Department of Pharmaceutical Chemistry, Sekolah Tinggi Ilmu Farmasi Makassar, Indonesia*
[3]*School of Chemical Sciences and Food Technology, University Kebangsaan Malaysia*

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Abstract

Seaweed is one of the marine algae that have antioxidant sources because it contains bioactive compounds such as carotenoids, phenol compounds and their derivatives, polysaccharide sulfate, and vitamins. Algae consist of three major groups namely brown algae (phaeophyta), red algae (rhodophyta), and green algae (chlorophyta). The purpose of this study was to determine the bioactivity profile of seaweed extract *Eucheuma cottoni*, *Sargassum polycystum* and *Caulerpa racemosa* so that it could be developed as a cosmetic raw material. The three types of seaweed were extracted by maceration and bioactivity testing was carried out as an antioxidant using the ABTS radical reduction method and bioactivity testing as UV-protection with the parameters of the percentage transmission of erythema, pigmentation and Sun Protective Factor (SPF). The results showed that *C. racemosa* extract provided a very strong antioxidant activity and was able to protect the skin from UV exposure with an SPF value that was categorized as ultra-protection. Antioxidant activity of the three types of seaweed is positively correlated to the protective effect based on the SPF value.

1. Introduction

Seaweed is an aquatic organism that is classified into macro-algae class and its existence is very abundant as well as one of the valuable marine biological natural resources (Yanuarti *et al*., 2017; Tanna and Mishra, 2019). Seaweed consists of three major groups based on the color pigments namely brown algae (phaeophyta), red algae (Rhodophyta), and green algae (Chlorophyta) (Yende *et al*., 2014; Yap *et al*., 2019). Seaweed can be counted as a source of bioactive compounds because of their ability to produce secondary metabolites that vary greatly with very broad biological activity (Subrathraa and Poonguzhali, 2013). In general, seaweed contains fiber, protein, fat, vitamins and minerals, besides that there are polysaccharides such as carrageenan and alginate which are components that are often exploited (Alam Bhuiyan and Qureshi, 2016; Nofiani *et al*., 2018; Sinurat and Fadjriah, 2019; Tanna and Mishra, 2019). Several countries in Asia and Europe using seaweed as fertilizer for agriculture, as well as thickening and gel-forming agents for industrial applications such as food, meat products, milk product in food industries and some additional ingredients in cosmetics industries (Kraan, 2012; Kılınç *et al*., 2013; Tanna and Mishra, 2019). In addition, there are many types of secondary metabolites found in seaweed that are currently very widely used in the development of new drugs and cosmetics. Seaweed is one source of antioxidants because it contains bioactive compounds such as carotenoids, steroids, terpenoids, phenol compounds and their derivatives, flavonoids, coumarin as well as vitamins (MacArtain *et al*., 2008).

Several studies have reported that *E. cottoni* red seaweed has pharmacological activity as an anti-cancer (Kania *et al*., 2013; Arsianti *et al*., 2018), antioxidant (Matanjun *et al*., 2008; Wresdiyati *et al*., 2008; Muawanah *et al*., 2016) anti-inflammatory (Sudirman *et al*., 2018) and sunscreen (Pakki *et al*., 2018). Green seaweed such as *C. racemosa* has biological activities such as antioxidants (Kumar *et al*., 2011; Nofiani *et al*., 2018; Yap *et al*., 2019), antibacterial (Chan *et al*., 2018), and anti-inflammatory (Bitencourt *et al*., 2015). In additions brown seaweed such as *S. polycystum* has pharmacological effect as antioxidant (Chandini *et al*, 2008; Yende *et al.,* 2014; Sami *et al.*, 2019), anti-tumor (Yende *et al*., 2014), analgesic and anti-inflammation (Hong *et al*., 2011; Yende *et al*., 2014), neuroprotective

*Corresponding author.
Email: *fitriyantijumaetri_sami@yahoo.com*

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(Hong *et al*., 2011; Yende *et al*., 2014), sunscreen (Pakki *et al*., 2018) and anti-viral activity (Yende *et al*., 2014).

Nowadays, seaweed is still being developed in various industries, especially in the cosmetics industry. The phenolic content and its derivatives as well as the flavonoid content found in *E. cottoni, C. racemosa*, and *S. polycystum* seaweed species so that they can be developed as cosmeceutical antioxidants and skin protection from UV exposure. In our study, a study will be conducted on the effect of antioxidants on skin protection from UV rays so that natural ingredients derived from marine algae have contributed as raw materials for the development of safe cosmetics.

2. Materials and methods

2.1 Materials

The material used such as reagents were analytical grade Ethanol, Aquabidestillata, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Potassium Persulfate ($K_2S_2O_8$), and Samples of red seaweed *E. cottoni*, green seaweed *C. racemosa* and brown seaweed *S. polycystum* were obtained in Punaga Village, Mangarabombang District, Takalar Regency, South Sulawesi.

2.2 Sample preparation

Samples of red seaweed (*E. cottoni*), brown seaweed (*S. polycystum*) and green seaweed (*C. racemosa*) were sorted and fresh seaweed was washed using running water to remove dirt, moss, mud and sand. Furthermore, chopping was done to facilitate the drying process carried out for 3 days not exposed to direct sunlight. The samples were then washed again using fresh water to remove the salt content of seaweed, then dried again 5-6 days not exposed to direct sunlight (Suryaningrum *et al*., 2006).

2.3 Extraction process

Extraction was done by maceration process where each dried seaweed powder was put into a maceration vessel and added with 96% ethanol solvent, then allowed to stand for 3x24 hrs occasionally stirring, and then filtered. Each sample was re-macerated for up to 48 hrs using the same solvent. Each filtrate was taken then evaporated with a rotary evaporator to obtained crude extract of *E. cottoni, C. racemosa* and *S. polycystum.*

2.4 Phytochemical screening

Profiles of the secondary metabolites of flavonoids, tannins, steroids, terpenoids, saponins and alkaloids in all three types of seaweed were determined based on the colorimetric method using chemical reagents (Harborne, 1998).

2.5 Antioxidant activity

Antioxidant activity of three types of seaweed was carried out by radical scavenging colorimetric method. ABTS radical solution was prepared by mixing ABTS solution 2.76 mM (7.1015 mg ABTS in 5 mL aqubidestillata) with potassium persulfate 2.58 mM (3.5 mg $K_2S_2O_8$ in 5 mL aquabidestillata). Each solution was mixed and incubated for 12 hrs in a dark room at room temperature. After the incubation period the volume was sufficient to 25 mL with ethanol analytical grade. Each sample was made a concentration series by dropping a certain amount of volume and the mixture was added with ABTS solution of 1 mL. The volume was adjusted to 5 mL and incubated in a dark room at room temperature for 30 mins. The mixture was measured for absorption on a spectrophotometer at a wavelength of 750 nm. In this study, ethanol was used as a blank and quercetin as a positive control. The percentage of ABTS radical inhibition by the presence of a sample can be calculated using the following equation:

$$Inhibition\ (\%) = \frac{Abs\ blank - Abs\ sample}{Abs\ blank} \times 100\%$$

2.6 Sunscreen assessment

Testing the sunscreen activity of three types of seaweed is done by looking at the profile values of percentage of erythema transmission, pigmentation transmission and SPF (Eff *et al*., 2018; Pakki *et al*., 2018).

2.6.1 Transmission of erythema (%Te) and pigmentation (%Tp)

Each extract solution was made into a series of concentrations (1-1000 µg/mL) and then its transmission (%T) was measured using a UV spectrophotometer at wavelengths that could cause erythema and pigmentation (292.5 nm - 372.5 nm) with 5 nm interval. Based on the transmission value (%T) obtained the percentage of transmission erythema and pigmentation (%Te and %Tp) were calculated using the following formula:

$$\%Te = \frac{\Sigma erythema\ transmission\ (T \times Fe)}{\Sigma flux\ erythema\ (Fe)}$$

And

$$\%Tp = \frac{\Sigma pigmentation\ transmission\ (T \times Fp)}{\Sigma flux\ pigmentation\ (Fp)}$$

Where T = Transmission in certain wavelength and Fe/Fp = Flux erythema/Flux pigmentation value based on Table 1.

Table 1. Flux erythema/Flux pigmentation value on wavelength number

Wavelength (nm)	Flux erythema (Fe)	Flux pigmentation (Fp)
292,5	11,390	-
297,5	65,100	-
302.5	100,000	-
307,5	35,770	-
312,5	0,9734	-
317,5	0,5670	-
322,5	337,50	10,790
327,5	0,2890	10,200
332,5	0,1290	0,9360
337,5	0,0456	0,7980
342,5	-	0,6690
347,5	-	0,5700
352,5	-	0,4880
357,5	-	0,4560
362,5	-	0,3560
367,5	-	0,3100
372,5	-	0,2600
∑	236,850	69,420

2.6.2 Sun protective factor (SPF) assessment

Each sample solution was made in series of concentrations (1-1000 µg/mL) and subsequent absorption measurements were made using a UV spectrophotometer at wavelengths of 292.5-372.5 with a scale change distance every 5 nm once observed. The SPF value is calculated using the formula:

$$\text{Area under Curve (AUC)} = \frac{Aa + Ab}{2} \times dP_{a-b}$$

$$Log\ SPF = \frac{AUC}{\lambda n - \lambda 1} \times DF$$

here A_a= Absorbance in wavelength a nm, A_b = Absorbance in wavelength b nm, dP_{a-b} = Difference in wavelengths a and b, λn = The largest wavelength (with A ≥ 0.05), λ_1 = Smallest wavelength (290 nm), DF = Dilution factor (FP = 1 for extract)

2.7 Data analysis

The data of antioxidant and sunscreen assessment were analyzed by Microsoft Excel program with expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1 Extraction

Seaweed samples in the form of *E. cottoni, C. racemosa, S. polycystum* were extracted by maceration which aims to attract the chemical components contained in each sample. Based on the results of maceration using 96% ethanol was obtained percent of yield contained in Table 2. One of the successful process to pull out of chemical components into the sample is the selection of solvents. Table 3 shows that the *S. polycystum* sample gave a large percent yield than the others followed by *C. racemosa* and *E. cottoni*.

3.2 Chemical compounds

Each extract that has been obtained is then identified as the components of the compound by using specific chemical reagents. Table 2 shows that the chemical compounds into the three types of seaweeds. From the

Table 2. Percentage of yield samples extract

Samples Extract	Yield (%)
E. cottoni	11.6
S. polycystum	29.7
C. racemosa	24.8

Table 3. Profile of chemical compounds in three types of seaweeds

Sample	Steroids	Tannin	Saponin	Flavonoids	Alkaloids	Terpenoids
E. cottoni	+	-	+	+	+	-
S. polycystum	-	-	-	+	+	+
C. racemosa	+	+	-	+	+	-

+ = positive contain chemical compounds, - = negative contain chemical compounds

results of phytochemical screening, it can be seen that three types of seaweed extract from Indonesian marine contains several secondary metabolite components such as alkaloids, flavonoids, saponins, steroids, tannin and terpenoids. However, in this study, different results were obtained from previous studies of *E. cottoni* extract containing flavonoids and terpenoids group (Suryaningrum *et al*., 2006), *S. polycystum* extract contained flavonoid, saponins, steroids and triterpenoids compounds (Cahyaningrum *et al*., 2016), and *C. racemosa* extract containing phenol compounds, and tannins (Adhita Putera, 2015). This may be influenced by several factors such as the influence of the location of growth, improper harvest time and changing weather climate (Adhita Putera, 2015).

3.3 Antioxidant activity by ABTS radical scavenger

Antioxidant activity assessment by radical ABTS scavenging method is used to determining the antioxidant activity obtained by the oxidation of potassium persulfate with ABTS diazonium salt. Radical ABTS provides maximum absorption at a wavelength of 750 nm (Re *et al.*, 1999). The presence of antioxidant compounds from the sample is marked by the loss of blue color in the ABTS radical reagent (Mistriyani *et al*., 2018). Each serial concentration of three types of seaweed was then measured on a UV-Vis spectrophotometer with a quercetin as positive control. The amount of antioxidant activity is indicated by the IC_{50} value, which is the concentration of the sample

solution needed to inhibit 50% of ABTS free radicals. Figure 1 shows the IC_{50} value of three types of seaweed indicating that the lower of the IC_{50} value, the stronger its antioxidant activity.

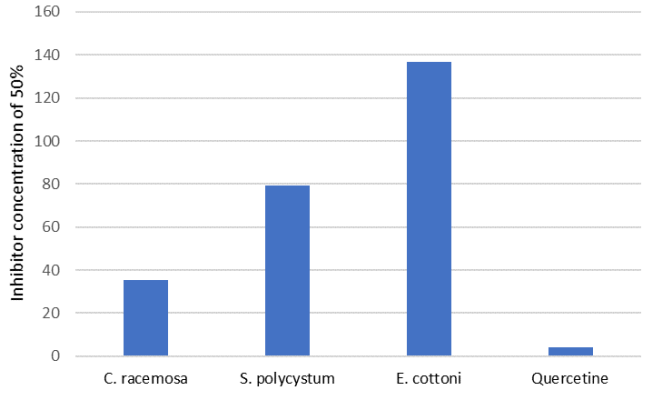

Figure 1. Inhibitor Concentration of 50% value of three types of seaweed. The data was express as mean±SD with triplicate (n=3)

The results of the antioxidant activity assessment of the three types of seaweed showed that *C. racemosa* extract provided a very strong antioxidant activity with an IC_{50} value of 35.55 µg/mL followed by *S. polycystum* and *E. cottoni* with IC_{50} values respectively of 79.29 µg/mL (strong activity) and 136.7 µg/mL (moderate activity). The very strong antioxidant activity of *C. racemosa* is supported by the presence of phenolic derivate compounds such as tannin and flavonoid which contribute to reducing free radicals by donating their protons (-H) (Apak *et al*., 2007; Nur *et al*., 2019).

3.4 Sunscreen assessment

Sunscreen activity assessment of the three types of seaweed was carried out based on three main parameters in vitro, namely determining the percentage of erythema transmission, percentage of pigmentation transmission and determining the SPF value (Kılınç *et al*., 2013; Donglikar and Deore, 2016; Eff *et al*., 2018; Pakki *et al*., 2018). In this study, the percentage of erythema and pigmentation transmission were evaluated by spectrophotometer analysis with serial concentration of seaweed samples (0.02-0.1%), flux erythema and pigmentation value on wavelength number can be seen in Table 3. Figure 2 shows the graph percentage of erythema transmission in difference concentration. The graph indicated that the higher the concentration of the sample indicates the lower percentage of erythema transmission. This shows that the samples have the ability to absorb UV rays so as to reduce the amount of exposure that can cause erythema on the skin. Similar results were also found in the percentage profile of pigmentation transmission (Figure 3). This also shows that seaweed samples have the ability to absorb UV radiation so that the reduced amount of excessive light exposure can cause pigmentation on the skin (Eff *et al*., 2018). The results of these assessments indicate that *C. racemosa* ethanol extract provides a decrease in the amount of UV radiation exposure so as to allow the adverse effects of erythema and pigmentation to be reduced.

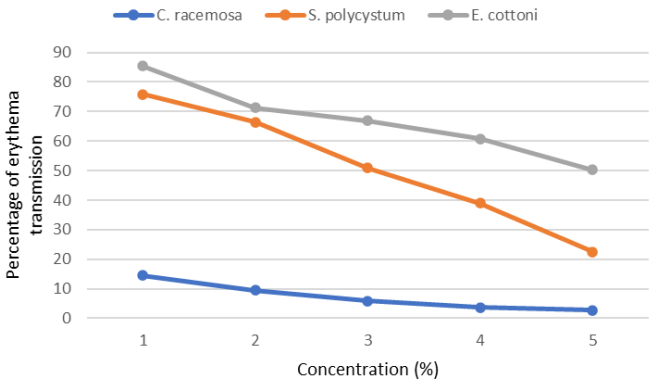

Figure 2. Profile the percentage of erythema transmission from three types of seaweed

The next sunscreens assessment parameter from three types of seaweed were evaluated is sun protective factor (SPF). SPF value is a measure of the level of protection against UV-B rays (290-230 nm) which is the most dangerous group of rays that can cause damage more quickly and easily than UV-A rays (230-400 nm). SPF assessment was performed on each extract of seaweed with the highest concentration of 0.1%. The choice of concentration is based on the results of optimization that have been done and based on the concentration that gives an effect on the percentage parameters of erythema transmission and pigmentation. The results of the evaluation of the SPF value can be seen in Table 4.

Table 4. SPF value of three types of seaweed with the concentration of extract 0.1%

Samples Extract	SPF Value	Categories*
E. cottoni	1.02±0.71	Low protection
S. polycystum	2.41±0.22	Minimal protection
C. racemosa	22.59±0.98	Ultra protection

*Source: Balsam and Sagarin (1972)

Based on Table 4 indicated that the seaweed *C. racemosa* type gave activity as UV protection with category ultra-protection followed by *S. polycystum* with category minimal protection and *E. cottoni* with category was low protection. Seaweed has activity as UV protection because it is thought that there are chemical compounds capable of absorbing was strong UV rays between UVA and UVB. In this study, the results obtained that *C. racemosa* seaweed provides the best activity compared to other types of seaweeds.

3.5 Correlation activity

Based on the results of phytochemical screening showed that the *C. racemosa* extract was positive contained tannins, flavonoids and steroids. Several studies have reported that tannin and flavonoid compounds have an aromatic ring in the form of chromophore which can absorb UV rays. When UV light interacts with aromatic compounds or compounds containing conjugated chromophore there will be resonance by electron transfer (Choquenet *et al*., 2008; Cefali *et al*., 2016; Nunes *et al*., 2018). In this study, the results obtained that the amount of antioxidant activity has a positive correlation with the protective effect against UV rays (sunscreen) which can be seen in Figure 4. Figure 4 indicates that the antioxidant activity of the three types of seaweed gives a correlation of 73.2% to the activity as UV Protection. This very supportive of skin repair when there is excessive exposure to UV light so that the content of compounds contained in seaweed can be developed as a medical cosmetics' raw material.

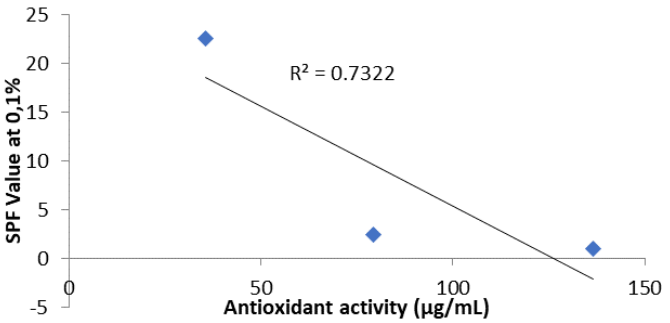

Figure 4. Correlation between SPF value against antioxidant activity of three types of seaweeds

4. Conclusion

The three types of seaweed i.e. *E. cottoni, S. polycystum* and *C. racemosa*, provide bioactivity against antioxidant activity and protective effects against UV exposure so that it has a perspective to be developed.

Conflict of interest

The authors declare no conflict of interest.

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