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Research Article

Free-Radical Scavenging Activity (FRSA) of Secondary Metabolite Extracted from Indonesian *Eucheuma Spinosum*

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ABSTRACT

Indonesia is the biggest country in the production of red seaweed of *Eucheuma spinosum*. The red seaweed has bioactive compounds that have a potential activity such as phenolic compounds as well as carrageenan and pigments. This paper reported phytochemical analysis of *E. spinosum* harvested by a local farmer in Sumenep Island, East Java and free-radical scavenging activity (FRSA) derived from 2,2'-diphenyl-1-picrylhdrazyl (DPPH) for several organic solvents. For extraction, dried powder is added with 5.0 mL of various solvents following by ultra-sonication assisted extraction for 30 minutes. The extract was separated by centrifugation for phytochemical analysis and radical scavenging evaluation. The prospecting of dichloromethane, ethyl acetate, and n-hexane extracts indicated the potency for radical scavengers. Alkaloids, terpenoids, and saponins were secondary metabolites that indicated the presence in the extracts. The best IC_{50} value was presented by ethyl acetate extracts (384,86 ppm) with 38.78% for 50 ppm, while IC_{50} values of n-hexane, methanolic, dicloromethane extracts were 410.12, 677.76 and 685.08 ppm, respectively.

Keywords: *Eucheuma spinosum*, phytochemical analysis, radical scavenging activity

1. Introduction

Seaweed farming has been broadly developed in Indonesia, specially *Eucheuma* species which has been cropped several decades ago [1]. *Eucheuma spinosum* or, other names, *Eucheuma denticulatum* (N. L. Burman) Collin [2] is the main source for carrageenan. The average carrageenan production is around 6500 tons per year [3]. Carrageenan is a natural polymer with many functions for additive, food products, and pharmaceuticals industry [4, 5]. The carrageenan structure consists of a sulfated polysaccharide [6, 7, 8]. Carrageenan is the primary metabolite product from *E. spinosum*, and still, there is another important product that has no specific function for plants, i.e. secondary metabolite product.

Recently, many studies have been reported other functionality of secondary metabolites from *E. spinosum* which could be a potential source. The secondary metabolites are used as an anti-oxidative compound [9, 10, 11, 12], anti-bacterial [13], anti-tumor [14], anti-hypercholesterolemic [15], anti-inflammation and amylase inhibition activity [16]. The secondary metabolite compounds contained in *E. spinosum* are alkaloids [17], flavonoids [13], isoprenoid [18], monoterpene [19], diterpene [20], and myrcene [21].

Researches of *E. spinosum* were reported from North Borneo (Sabah Malaysia) [9] and North Sulawesi [22, 23]. Methanolic extraction of the samples from different islands indicated the presence of phenolic and polyphenol (secondary metabolites) [9, 24]. Predicted compounds in both samples have a simple phenolic structure, such as flavonoid and condensed tannin. *E. spinosum* from Bali Island (Indonesia) was extracted using ethanol and the sample contained a phenolic group with the addition of carotenoid [25]. Carotenoid is categorized as a terpenoid group and it is composed of

poly-unsaturated hydrocarbon with 40 carbon chain. The two different structures of carotenoid are alpha- and beta-carotene [26].

The scavenging activity of free radicals is a measurement to test the ability of a compound to inhibit free radicals. The other free radical species can be generated from an electron or hydrogen radical. 2,2'-diphenyl-1-picrylhdrazyl (DPPH) has a molecular structure with the ability to transfer radical electron or hydrogen radical [27]. The radical electron can be accepted by secondary metabolite through sharing a radical proton and forms a hydrogenated-DPPH (**Figure 1**). This hydrogenated structure has different spectrum absorption to DPPH. Thus, it will reduce the absorption spectrum at DPPH's maximum wavelength (517 nm). Research on *E. spinosum* from Makassar reported had a strong antioxidant activity with an IC_{50} value of 75.98 ppm [28]. Underline to those facts and some potent bioactivities from *E. spinosum* or *E. denticulatum* (N. L. Burman) Collin, exploring secondary metabolite and evaluating the bioactivities of scavenging of free radical in the seaweed become an important step for further prospecting and development.

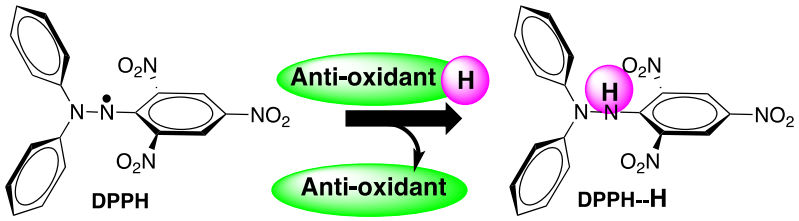

Figure 1. Schematic for anti-oxidative activity with DPPH

2. Materials and Methods

2.1. Materials

Eucheuma spinosum is purchased from a local farmer from Sumenep, East Java, Indonesia. The sample is dried under the sunlight and ground to be a dry powder. Some chemicals used are from Merck and Smartlab such as ethyl acetate, n-hexane (Smartlab), ethanol, methanol, chloroform (Smartlab), dichloromethane (Smartlab), DPPH, iron(III) chloride, acetic anhydride, and other phytochemical reagent fresh-prepared before used.

2.2. Sample Extraction

A 50 mg of dried powder of *E. spinosum* in the reaction tube is added 5.0 mL of ethyl acetate. This mixture is sonicated for 15 min and centrifuged for separation of the supernatant. The supernatant is further evaporated under reduced pressure to get ethyl acetate extract and the extract is measured its phytochemical and radical scavenging evaluation. This procedure is applied using different solvents such as methanol, dichloromethane, and hexane.

2.3. Phytochemical Evaluation

Procedure for phytochemicals screening is undertaken following Masruri et al. [29] with modification.

2.3.1. Alkaloid Test

A 100 mg of a crude extract is added 0.5 mL solution of hydrochloric acid 2%. This is further added with Dragendorf reagent. Positive alkaloid is indicated by the orange precipitate. Another reagent is also applied using Mayer reagent. The yellowish precipitate is a positive indication of the presence of alkaloids.

2.3.2. Flavonoid Test

A 100 mg of a crude extract is added 1-2 mL of aqueous methanol and heated. This mixture is further added with 0.5 mL of concentrated hydrochloric acid and magnesium powder. Positive flavonoid is detected by changing the solution color to red or orange.

2.3.3. Triterpenoid and Steroid Test

A 100 mg of a crude extract in the reaction tube is added with 0.5 mL of acetic anhydride and 1.0 mL of sulfuric acid. The presence of a brown or violet's ring in the solution is a positive indication of triterpenoid. Meanwhile, the existence of steroids is indicated by the formation of a green-blue solution.

2.3.4. Saponin Test

The presence of saponin is tested by the formation of permanent foam. A crude extract is added 0.5 mL of hydrochloric acid 2%. This mixture is shaken for 5 min. Relatively permanent foam formed is a positive indication of saponin.

2.3.5. Tannin Test

A 100 mg of a crude extract in the test tube is added with 1.0 mL of hot methanol and 1.0 mL of iron(III) chloride 1.0%. The presence of tannin in the extract is indicated by the formation of a dark-blue or dark-green solution.

2.4. Evaluation Radical Scavenging Activity

The radical scavenging activity of the *E. spinosum* extract is undertaken following Taroreh et al. [30] with a minor modification. A 1.0 mL of the extract (concentration variation 50; 100; 200; and 400 ppm) is added 2.0 mL of solution 0.08 mM of DPPH. This mixture is homogenized and incubated for 30 min at room temperature. The mixture is measured absorbance at 517 nm. A blank sample is prepared by addition 1.0 mL methanol into 2.0 mL of DPPH solution. The percent DPPH scavenging effect was calculated using **Equation 1**.

$$\text{Scavenging Activity (\%)} = \frac{\text{A blank-A sampel}}{\text{A blank}} \text{ x } 100\% \quad (1)$$

Where A_{blank} is absorbance value for solution DPPH in methanol and A_{sample} is absorbance value for sample.

3. Result and Discussion

3.1. Extraction and Phytochemicals Screening

Extraction with the different polarities of solvents is devoted to obtaining secondary metabolite into different solvent affinity. Most polar solvent (methanol) can dissolve secondary metabolites with high polarities, such as phenolic, flavonoid, tannin, and alkaloid compounds [12, 29]. Conversely, non polar solvent such as n-hexane can extract secondary metabolite with non polar properties, for example; triterpenoid, saponin, steroid, and less volatile alkaloid compounds [29, 31].

The phytochemical result is shown in **Table 1.** Methanol and ethyl acetate can be used to extract two groups of secondary metabolite i.e. triterpenoids and alkaloids for methanolic extract and also triterpenoids and saponins for ethyl acetate extract. Dichloromethane and n-hexane extracts share a similar result, they contained triterpenoids, alkaloids, and saponins. Based on previous research, *E. spinosum* extract contained alkaloids [31, 32], terpenoids [33], and saponins compounds [31].

Table 1. Secondary Metabolite of Some Crude Extracts of *Eucheuma spinosum*

Secondary Metabolite	Crude Extract			
	Methanol	Dichloromethane	Ethyl Acetate	n-Hexane
Flavonoid	-	-	-	-
Triterpenoid	+	++	+	++
Steroid	-	-	-	-
Alkaloid (Dragendroff)	+	+	-	+
Alkaloid (Mayer)	+	-	-	-
Tanin	-	-	-	-
Saponin	-	++	+	++

"++" means extract giving a deep in color or giving more precipitate, "+" means extract containing a fair compound or giving light color or giving less precipitate, and "-" means no indication of a certain compound in the extract.

3.2. Free Radical Scavenging Activity

The results of the antioxidant activity of methanolic and dichloromethane extract using DPPH scavenging are summarized in **Table 2** and **Table 3**. Overall, methanolic and dichloromethane extracts have a similar range of free radical scavenging activity, 27.68 and 27.22% for 50 ppm, respectively. Meanwhile, the highest activity obtained was 54.62 and 56.44% for the highest concentration of extract. The IC_{50} value of both extracts was in a similar range (677.76 ppm for methanolic extract and 685.08 ppm for dichloromethane extract. However, both extracts have different content of secondary metabolite. Saponin is detected in dichloromethane extract, but it did not detect in methanolic extract.

Based on the results, the percentage of antioxidant activity increased after increasing the concentration, it can be seen at the concentration of 800 ppm has the highest value of antioxidant activity. The ability of DPPH radicals is influenced by the amount of extract concentration. Generally, DPPH activity is increased with the addition of the extract to a certain concentration [23]. Previous research stated that *E. spinosum* using methanol and ethanol solvents (concentration of 50% and 95%) has radical scavenging activity (IC_{50}), IC_{50} value of *E. spinosum* using methanol 50 and 95% were 223.305 and

238.128 ppm, while IC_{50} value of *E. spinosum* using ethanol 50 and 95% were 113.882 and 97.522 ppm, respectively. The smaller the IC_{50} value, the higher the radical scavenging activity [34].

Table 2. Radical Scavenging Activity of Methanolic Extract

Concentration (ppm)	UV Absorbance measurement (a.u.)				Radical Scavenging activity (%)	IC_{50} (ppm)
	Blank	1	2	3		
50	0.7154	0.523	0.517	0.512	27.68 ± 0.76	
100		0.507	0.504	0.495	29.83 ± 0.87	
200		0.483	0.478	0.458	33.88 ± 1.85	677.76
400		0.442	0.433	0.427	39.34 ± 1.06	
800		0.332	0.331	0.311	54.62 ± 1.66	

Table 3. Radical Scavenging Activity of Dichloromethane Extract

Concentration (ppm)	UV Absorbance measurement (a.u.)				Radical Scavenging Activity (%)	IC_{50} (ppm)
	Blank	1	2	3		
50	0.7154	0.521	0.525	0.516	27.22 ± 0.63	
100		0.519	0.487	0.501	29.78 ± 2.24	
200		0.493	0.489	0.478	31.97 ± 1.09	685.08
400		0.484	0.465	0.459	35.84 ± 1.82	
800		0.311	0.311	0.313	56.44 ± 0.16	

Evaluation of the scavenging activity for hexane extract showed a better result than that of methanolic and dichloromethane extract. N-Hexane extract gave radical scavenging activity of 43.43% for 50 ppm (**Table 4**). The IC_{50} value of the n-hexane extract obtained was 410.12 ppm. However, the scavenging activity of the ethyl acetate extract (IC_{50} = 384.86 ppm) has slightly higher than n-hexane extract (**Table 5**). *E. spinosum* extracted with methanol and partitioned with ethyl acetate have a strong free radical scavenging activity value, IC_{50} of 41.94 ppm [35]. Ethyl acetate extract on *E. spinosum* contained the only terpenoid groups of secondary metabolite (triterpenoid and saponin). Terpenoids are phenolic compounds that have the potential to act as antioxidants or free radical scavengers [36, 37]. Other research stated that terpenoids are active as free radical scavengers with an IC_{50} value of 61 ppm [38] and an IC_{50} value of 6.751 ppm [39].

Table 4. Radical Scavenging Activity of n-Hexane Extract

Concentration (ppm)	UV Absorbance measurement (a.u.)				Radical Scavenging Activity (%)	IC_{50} (ppm)
	Blank	1	2	3		
50	0.7154	0.417	0.371	0.426	43.43 ± 4.12	
100		0.402	0.347	0.414	45.81 ± 4.99	
200		0.385	0.385	0.396	45.67 ± 0.89	410.12
400		0.355	0.353	0.36	50.24 ± 0.50	
800		0.311	0.311	0.312	56.48 ± 0.08	

Table 5. Radical Scavenging Activity of Ethyl Acetate Extract

Concentration (ppm)	UV Absorbance measurement (a.u.)				Radical Scavenging Activity (%)	IC_{50} (ppm)
	Blank	1	2	3		
50	0.7154	0.437	0.438	0.439	38.78 ± 0.14	
100		0.431	0.432	0.434	39.56 ± 0.21	
200		0.422	0.418	0.405	41.99 ± 1.24	384.86
400		0.389	0.389	0.389	45.62 ± 0.00	
800		0.221	0.223	0.221	69.01 ± 0.16	

4. Conclusion

Secondary metabolite of *E. spinosum* extract was successfully detected by the phytochemical test. Terpenoid group (triterpenoid and saponin) and alkaloid can be detected on methanolic, dichloromethane, and n-hexane extract. The radical scavenging activity of *E. spinosum* using four various solvents showed that ethyl acetate extract had the highest IC_{50} value of 384.86 ppm, and then followed by n-hexane extract (410.12 ppm), methanolic extract (677.76 ppm), and diclorometha extract (685.08 ppm).

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