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Original Article

Polyphenol Content and Antioxidant Activities of Crude Extract from Brown Algae by Various Solvents

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ABSTRACT

Five species of brown algae, Sargassum filipendula, S. dublicatum, S. crassifolium, S. binderi and Padina sp were collected from the coastal of Sumenep. Seaweeds were extracted with ethanol, ethylacetic and n-hexan. The extracts of each sample were examined for phytochemical, total phenolic content (TPC) by using Follin Ciocalteau method and antioxidant activities with measuring the scavenging activity of DPPH free radicals. The ethanolic, ethylacetic and hexane extracts of all type of algae showed the presence of alkaloids, flavonoids and phenols. The ethanolic extracts of S. filipendula showed higher phenolic contents and antioxidant activities than the others. Total phenolic content and IC50 value of ethanolic extract from S. filipendula were 12.87 ± 1.04 mg GAE/g and 39.11 ± 2.04 ppm, respectively.
Key words: Antioxidant Activity, Brown Algae, Phenolic Content, Solvent.

INTRODUCTION

The natural antioxidants are increasingly favored by the public because in addition to its ability to scavenge free radicals and also safer to use. Some studies have shown that algae is one source of bioactive components that have activity as antidiabetik [1], antitumor [2], antibacteri [3] and antioxydant [3-8]. Brown Algae are one of the sources of natural antioxidants that its availability is very abundant. Polyphenols extracted from Brown algae has reported antioxydant activity that is able to inhibit the activity of free radicals. The content of polyphenols in Brown algae can reach 15 percent dry weight bahan [9-11]. The content of these compounds varies greatly and depends on many factors, including the type or species.

Brown algae known as an alginat major producers, but some kind of brown algae *Sargassum* sp as less favorable as a raw material in the industrial alginate [12]. *Sargassum filipendula, S. dublicatum, S. crassifolium, S. binderi* and *Padina* sp are a plentiful Brown algae grows in the coastal waters of Madura. As one of the largest seaweed production center in East Java, the commodities business activities on the island are still focused on the red algae *Eucheuma cottoni, E. spinosum* and *Gracilaria* sp. The local of brown algae is still not widely utilized and only a small part was used as animal feeds or plant fertilizers. Drying is a method of post-harvest handling and produced raw material which the added value is very low. Some research has been done but still partial and sufficient information is not yet available as well as a type of red algae dominated. This research is aimed to obtain of brown algae potential information from the coastal of Madura as a source of the natural antioxidants. In addition, to determine a suitable solvent for the extraction of antioxidants from brown algae.

MATERIALS AND METHODS

Brown Algae

Samples of brown algae (*Sargassum filipendula, S. dublicatum, S. crassifolium, S. binderi* and *Padina* sp) were collected from Sumenep Madura in East Java, and the coolbox is cooled during transport to the laboratory. Sample was washed and drained in the shade below the room temperature. The samples are mixed with the blender and stored in an airtight container until time of use.

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Chemicals and Reagents

Solvents used are ethanol, ethyl acetate, and hexane. HCl, H_2SO_4, $FeCl_3$; Folin-Ciocalteau; 1.1-diphenyl-2-picryhydrazyl (DPPH), gallic acid reagents were obtained from Sigma Chemical Co, USA. All chemicals were of analytical grade.

Preparation of Sample Extracts

Extraction was carried out by the methods of maceration using shakers for 24 hours. Solvents used are ethanol (Et-OH), ethylacetic (Et-acetic) and n-hexane (Hex). Approximately 50 g of the sample is extracted with 300 ml of solvent. The extracts obtained were separated by rotary evaporator under controlled temperatures between 60° C. Crude extract (CE) measured the percentage yield, total polyphenol, antioxidant activity and phytochemical.

Phytochemical Screening

Crude extracts of sample were screened for the presence of alkaloids, flavonoids and phenols [13]. It was done to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and discoloration reaction. General reactions in these analyses revealed the presence or absence of these compounds in the crude extracts.

Determination of Total Phenolic

The total phenolic compounds were determined spectrophotometrically using Folin-Ciocalteu reagent [14]. The crude extracts (250 µL) each of sample was mixed with 1250 µL deionized water, 250 µL ethanol and 125 µL of the Folin-Ciocalteu reagent. The mixture was incubated at room temperature for 5 min and then 250 µL of 5% sodium carbonate solution was added. The mixture was allowed to stand for 1 hour in the dark at room temperature. The absorbance of the discolor produced was measured with a spectrophotometer at 725 nm. The Total Phenolic Content (TPC) of the extracts were determined from regression equation of standard curve $y = 0.0085\ x - 0.018$ and expressed as mg of Galic Acid Equivalents (GAE) per g of sample.

Measurement of DPPH Radical Scavenging Activity (RSA)

DPPH RSA was assessed according to the method described by Shen et al. [15]. An aliquot (0.1 ml) of each sample with different concentration (25, 50, 100 and 200 ppm) was added to 3.0 ml of ethanolic DPPH solution. The mixture was shaken and left to stand for 30 min at room temperature in a dark room. The scavenging effect on the DPPH radical was read using spectrophotometer (Spectroquant Pharo 300) at 517 nm. The capability to scavenging the DPPH radical was calculated by the following equation:

Percentage of the DPPH scavenging = $\left[\frac{Ao-As}{Ao}\right] x\ 100$

Where; A_C =absorbance of control and A_S = absorbance of sample solution. The DPPH solution without sample solution was used as control. IC_{50} value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated by using the plotted graph of RSA against the concentration of samples.

Statistical Analyses

Data analysis was used analysis of variance (Anova, using Design Expert 7.0 software) and significant differences between the means of parameters were determined by using Least Significant Different (LSD) test.

RESULTS

Extracts yield and Phytochemical

Crude extracts (CE) of the five seaweeds and its phytochemical assay can be seen in Table 1. Yield of the crude extracts were influenced by the type of solvent ($P<0.01$). The ethanolic extracts of the all types of brown algae have the highest compared with the others. This indicated that most extract dissolved in high polarity solvent more than in low polarity solvent. Amount of the ethanolic extract was about 1.697 ± 0.159 percent of fresh sample. This result is lower than reported by Ye et al. [16], but higher than the results that had been reported by Boonchum et al [17]. This occurs because of differences in material preparation and extraction methods. The results of crude extracts revealed that the presence of alkaloids, flavonoids and phenolic compound in all extracts studied.

Total Phenolic Content (TPC)

Phenolic compounds are widely distributed in the plant kingdom and been reported to have several biological activities including antioxidant properties. Some reports revealed that marine algae extracts, especially their polyphenols, have antioxidant activity [17-19]. The phenolic content in the CE was shown in Table 2.

Total polyphenol content in the crude extracts were influenced by species and kind of the solvent ($P<0.05$). Table 1 showed that the ethanolic extracts of *S. filipendula* have the highest total phenolic content, i.e. 12.87 mgGAE/g extract. Ethanolic extract of *Padina* sp and *S. binderi* exhibited higher TPC of 10.16 ± 1.13 and 9.09 ± 0.14 mgGAE/g extract, respectively, as compared with other species. The crude extract of hexane from all kinds

of samples showed the lowest total polyphenol content (Table 2). These results indicated that phenolic compounds in *S. filipendula, Padina* sp *and S. binderi* more soluble in polar solvent (ethanol), while its in *S. dublicatum* and S. crassifolium are both soluble in Et-acetic (semi polar solvent). According by reports of Ye et al [16] that the phenol compound in brown algae (*Sargassum pallidum*) found to be higher in the polar solvent (n-buthanol).

Table 1. Yield of the crude ethanolic, etylacetic and hexanic extracts of brown algae; and phytochemical assay.

Solvent	Algae	Alkaloid		Flavonoid	Fenolat	Yield
		Wagner	Meyer	H_2SO_4	$FeCl_3$	[%]
	S. filipendula	+	+	+	+	
	S. dublicatum	+	+	+	+	
Et-OH	*S.crassifolium*	+	+	+	+	1.69±0.159[a]
	S.binderi	+	+	+	+	
	Padina sp	+	+	+	+	
	S. filipendula	+	+	+	+	
	S. dublicatum	+	+	+	+	
Et-act	*S.crassifolium*	+	+	+	+	1.08±0.406[b]
	S.binderi	+	+	+	+	
	Padina sp	+	+	+	+	
	S. filipendula	+	+	+	+	
	S. dublicatum	+	+	+	+	
n-Hex	*S.crassifolium*	+	+	+	+	0.92±0.099[b]
	S.binderi	+	+	+	+	
	Padina sp	+	+	+	+	

LSD: 0.279; Data are expressed as mean ± SD, standard deviation; [A–b] Column wise values with same superscripts of this type indicate no significant difference (P > 0.05)

Table 2. Total phenolic content (mg GAE/g extract) of CE with various solvent

Algae	Et-OH	Et-acetic	n-Hexane
S.filipendula	12.87±1.04[a]	5.35±0.08[f]	1.85±0.27[g]
S.dublicatum	7.87±0.12[cd]	6.61±1.05[de]	1.82±0.05[g]
S.crassifolium	6.81 ±1.07[de]	5.24±0.52[f]	1.08±0.17[g]
S.binderi	9.09±0.14[bc]	5.86±0.37[e]	1.14±0.11[g]
***Padina* sp**	10.16±1.13[b]	6.02±0.18[e]	1.81±0.12[g]

LSD: 1.43; Data are expressed as mean ± SD, standard deviation; [A–g] Column wise values with same superscripts of this type indicate no significant difference (P > 0.05)

DPPH radical scavenging activity

The DPPH free-radical is a stable free-radical, which has been widely accepted as a tool for estimating the free radical scavenging activity (RSA) of antioxidant [18]. Antioxidant activity of crude extract as assessed by DPPH method and expressed as inhibitory concentration (IC_{50}) value. IC_{50} value of CE of five seaweeds is presented in Table 3.

Table 3. Inhibitory concentration (IC_{50}) value (ppm) of CE with various solvent

Alga	Et-OH	Et-acetate	n-Hexan
S.filipendula	39.11±2.01[a]	93.87±1.38[ef]	135.46±2.13[h]
S.dublicatum	63.56±1.08[bc]	75.67±0.88[d]	138.37±1.02[h]
S.crassifolium	73.48±0.57[cd]	95.76±2.06[f]	184.96±2.03[i]
S.binderi	49.21±1.22[ac]	82.99±1.01[de]	176.59±1.05[i]
***Padina* sp**	54.98±1.59[b]	85.40±1.04[def]	113.72±0.58[g]

LSD: 12.10; Data are expressed as mean ± SD, standard deviation; [a–g] Column wise values with same superscripts of this type indicate no significant difference (P > 0.05).

The ethanolic extract of *S. filipendula* showed significantly higher levels of DPPH free radical scavenging activity or lower IC_{50} value ($p<0.05$). Furthermore, all ethanolic extract showed higher activity (lower IC_{50} value) than the others. The ethanolic extract of *S. filipendula* showed the highest scavenging activity of DPPH radicals, with an IC_{50} value, i.e. 39.11±2.01 ppm, followed by ethanolic extracts of *S. binderi, Padina* sp, *S. dublicatum, S. crassifolium* and the other extracts. This is supported by the data total polyphenol content (Table 2); TPC *S. filipendula* had the highest well, i.e. 12.87 mgGAE/g extract. In this study, a correlation was found between the TPC and IC_{50}; when the TPC was high, the IC_{50} was low. In accordance with the results of the study [17, 18 and 20],

who reported that the higher total polyphenol content also tends to be higher antioxidant activity. Correlation of both factors very closely, $R^2 = 0.8359$ and shown to follow the equation:

$y = 161.6324 - 11.4697x$, where $y = IC_{50}$ $[ppm]$ and$x = TPC$ $[mgGAE/g]$.

This was due to the high level amount of polyphenol constituents present in *S. filipendula* which were capable of functioning as free radical scavengers [2].

DISCUSSION

Ethanolic extract generally exhibited higher yield. The yield of *S. filipendula* was highest (1.69±0.159 %), total polyphenol content, i.e. 12.87±1.04 mgGAE/g extract, and IC_{50} value was i.e. 39.11±2.01 ppm. *S. filipendula*, *S. binderi* and *Padina* sp can be used as an alternative source of natural antioxidants.

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