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 antioxidat [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 antioxidat 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 alginine [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.
Chemicals and Reagents
Solvents used are ethanol, ethyl acetate, and hexane. HCl, H₂SO₄, FeCl₃; Folin-Ciocalteau; 1.1-diphenyl-2-picrylhydrazyl (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:

\[ \text{Percentage of the DPPH scavenging} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100 \]

Where; \( A_0 \) =absorbance of control and \( A_t \) = absorbance of sample solution. The DPPH solution without sample solution was used as control. IC₅₀ 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 their polyphenols, have antioxidant activity [17-19]. The phenolic content in the CE was shown in Table 2.

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. 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-butanol).

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

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Algae</th>
<th>Alkaloid Wagner Meyer</th>
<th>Flavonoid H₂SO₄</th>
<th>Fenolat FeCl₃</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. filipendula</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1.69±0.159a</td>
</tr>
<tr>
<td>S. dublicatum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1.08±0.406b</td>
</tr>
<tr>
<td>Et-OH</td>
<td>S.crassifolium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>S. filipendula</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>S. dublicatum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>Et-act</td>
<td>S.crassifolium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>S. filipendula</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>S. dublicatum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>n-Hex</td>
<td>S.crassifolium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>S. filipendula</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
<tr>
<td>Padina sp</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.92±0.099b</td>
</tr>
</tbody>
</table>

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

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

LSD: 1.43; 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)

**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 (IC₅₀) value. IC₅₀ value of CE of five seaweeds is presented in Table 3.

Table 3. Inhibitory concentration (IC₅₀) value (ppm) of CE with various solvent

<table>
<thead>
<tr>
<th>Algae</th>
<th>Et-OH</th>
<th>Et-acetate</th>
<th>n-Hexan</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.filipendula</td>
<td>39.11±2.01a</td>
<td>93.87±1.38ad</td>
<td>135.46±2.13bd</td>
</tr>
<tr>
<td>S.dublicatum</td>
<td>63.56±1.08bc</td>
<td>75.67±0.88d</td>
<td>138.37±1.02b</td>
</tr>
<tr>
<td>S.crassifolium</td>
<td>73.48±0.57cd</td>
<td>95.76±2.06e</td>
<td>184.96±2.03c</td>
</tr>
<tr>
<td>S.binderi</td>
<td>49.21±1.22ac</td>
<td>82.99±1.01cd</td>
<td>176.59±1.05c</td>
</tr>
<tr>
<td>Padina sp</td>
<td>54.98±1.59b</td>
<td>85.40±1.04de</td>
<td>113.72±0.58e</td>
</tr>
</tbody>
</table>

LSD: 12.10; 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)

The ethanolic extract of S. filipendula showed significantly higher levels of DPPH free radical scavenging activity or lower IC₅₀ value (p<0.05). Furthermore, all ethanolic extract showed higher activity (lower IC₅₀ value) than the others. The ethanolic extract of S. filipendula showed the highest scavenging activity of DPPH radicals, with an IC₅₀ 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 IC₅₀; when the TPC was high, the IC₅₀ 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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**REFERENCES**


