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Research Article

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Phenol Content and Antioxidative Activity in the Extract of Multiple Sargassum Species

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ABSTRACT

Free radicals are harmful to the human body since it causes damage to cells, and antioxidant compounds can reduce free radicals in the body so that the public demand for antioxidant products increases. However, the available antioxidants are synthetic which are potentially toxic when excessively accumulated in the body. Therefore, it is necessary to trace natural compounds that are natural antioxidants Phenol is one source of antioxidant compounds in seaweed. Jepara coastal waters have abundant seaweed resources, one of which is Sargassum seaweed. This study aims to conduct an evaluation study of the total content of phenol compounds and the antioxidant capacity of various extracts of Sargassum from Jepara beach. This research is expected to be the basis for research on antioxidant supplements for preventive actions. The study was conducted in June 2018, which consisted of sampling, extraction, determination of total phenol and antioxidant activity. Sampling in the study used a purposive method. Antioxidant capacity and total phenol were determined using laboratory experimental methods. The results of the analysis showed that the treatment of different types of extracts resulted in significantly different inhibition percentages ($p<0.050$). The three extracts with the best percentage of inhibition, sorted from the smallest to the largest, were extracts with ethyl acetate solvents on S. polycistum, S. crassifolium, and S. duplicatum. The extract concentration was directly proportional to the percentage inhibition value ($p<0.050$). In other words, the higher the concentration of the extract, the higher the phenol content produced ($p<0.050$). Correlation of total phenol and the percentage of inhibition is linear, with the regression equation and the resulting value of the relationships differing significantly ($p<0.05$).

Keywords: *Sargassum, Extract, Phenol, Antioxidant, DPPH.*

INTRODUCTION

Free radicals are compounds that are reactive because their outer shells have free electrons [1]. Free radicals that cause oxidative stress can be prevented by antioxidants. Antioxidants are compounds able to bind it to free radicals and are highly reactive molecules that inhibit oxidation and cell damage reactions [1,2]. Its natural antioxidant compounds are present in the body but this can also be taken from the external intake. Plants or natural marine products are one of the external sources of antioxidants.

Phenolic compounds and flavonoids contained in plants or natural marine products are known to counteract the effects of free radicals. Its content plays an important role in testing antioxidant activity. The higher the phenolic compound content in a sample, the higher the antioxidant activity. This is indicated by a smaller IC_{50} value.

One of the marine products are widely consumed is seaweed. Sargassum is a type of brown seaweed that has not been widely consumed and used directly and its distribution is abundant in Indonesia. Sargassum stock contains antioxidant bioactive compounds and its distribution includes the Indonesian coastline [3]. Sargassum has been proven to be rich in bioactive compounds sources of antioxidants, fucoxanthin [4] and polyphenol [5]. Fu et al. [6] showed that *S. polycystum* seaweed had phenolic components (37.41 ± 0.01 mg GAE/g) and high antioxidant activity (2.00 ± 0.01 µmol TEAC/g). Xu and Chang [7] stated that solvents are commonly employed in the extraction of polyphenol from plants are aquades, ethanol, methanol, acetone, and ethyl acetate. Diachanty et al. [8] found that extraction using 100% ethanol on *S. polycystum* and *Padina minor* seaweed produced low extract yield, but high antioxidant activity.

The difference in polarity of extraction solvents can affect the solubility of chemical elements in the sample and its extraction results, making the selection of the appropriate solvent as an important factor in optimizing yield, total phenol content and antioxidant activity

[9]. This study aimed to determine the levels of phenolic compound and antioxidant activity of hexane solvent extract, ethyl acetate, and methanol on *S. polycistum*, *S. crassifolium,* and *S. duplicatum*.

MATERIALS AND METHODS

The sample was collected according to the purposive method and it was washed with tap water to remove the epiphytes and the remaining debris. The samples are namely: *S. polycistum*, *S. crassifolium* and *S. duplicatum*. A whole thallus of seaweed was taken for taxonomic identification based on morphology study. Before natural drying under the shade, seaweeds were cut to pieces (± 5 cm). Further, the dried seaweeds were ground with a multi-use blender to obtain powder material.

Extraction of S. polycistum, S. crassifolium, and S. duplicatum

Three seaweed (dry) of 100 g was extracted gradually by using 300 mL n-hexane, ethyl acetate, and methanol, respectively. The extraction was performed for 24 hr at room temperature in obscurity. The extracted seaweed was filtered by Whatman filter paper to separate the solute from the residues. The remaining residues of dry seaweed material were re-extracted using the same process and solvents. Filtrates obtained from the extraction process were pooled. N-hexane, ethyl acetate, and methanol filtrates were evaporated to dryness with vacuum rotary evaporator at 40°C in 500 mmHg. N-hexane, ethyl acetate and methanol extracts of Sargassum were dried using N2 before storage at -20°C [10].

Total phenol content

Seaweed extracts were added with 1 ml of 96% ethanol and 5 ml of distilled water. The extract was added with 0.5 ml of 50% Folin-Ciocalteu reagent, homogenized and left for 5 minutes, added with 1 ml Na_2CO_3 5% and left in a dark room for ± 60 minutes. The standard used in the total phenol content in this study is gallic acid. Absorbance was measured using a spectrophotometer of 725 nm wavelength. The absorbance rate was then converted into total phenol content, stated in mg gae/g of the sample weight. The analysis of the total phenolic compound was carried out using the methods [11].

DPPH methods

DPPH methods are performed to assay antioxidant activity. This assay begins with determining the maximum wavelength by preparing a 3 mm DPPH solution of 3 ml, ± 10 minutes. The λ_{max} of the solution is set and the measurement results of λ_{max} are recorded for use at a later stage [12]. Stability time of antioxidant measurements was determined by making 200 ppm extract solution as much as 25 ml, and 4.5 ml of the solution was taken and 0.2 mm DPPH solution of 1.5 ml was added, then determined the stability time after incubation and before incubation at a time span of 5-60 minutes, at intervals of 5 minutes. The sample is measured at λ_{max} and at the stability time that has been obtained. Measurement of antioxidant activity was carried out by dissolving each extracted sample in its solvent with a concentration of 100 ppm. The extract was taken as much as 4.5 ml and added with 1.5 ml DPPH solution (with a comparison of extracts dissolved with certain concentrations to DPPH solution at 3: 1), with a concentration of 0.2 mm in 99% v/v ethanol. After it was incubated at 37°C during the stability time obtained in the previous stage, the solution was put into cuvets to measure the rate of absorbance at λ_{max}, which is at 515 nm. and All tests were performed in triplicate [13]:

$$Antioxidant\ activity\ (\%) = \frac{(ao - ac)}{ac} \times 100\%$$

Note:

ao: absorbance of the control

ac: absorbance of the sample

The treatment of different antioxidant test concentrations [10] modified to be used in determining the IC_{50} value. Sargassum extract obtained from the extraction process with hexane, ethyl acetate, and methanol was dissolved in methanol with concentrations of 100, 250, 500, 1000, and 2500 ppm [14]. The dilution process is carried out to obtain an extract solution that is in accordance with the concentration in the test treatment of antioxidant activity. Ascorbic acid and BHT were used as positive controls. Ascorbic acid as a positive control was used with concentrations of 1, 2, 5, 10, and 25 ppm. BHT synthetic antioxidants as a positive control were used with concentrations of 5, 10, 25, 50, and 100 ppm [15].

RESULTS AND DISCUSSIONS

Total phenol content of fresh samples

The sample was analyzed to determine the total phenol content. The results of one-way ANOVA analysis of the total phenolic compound of the three seaweeds showed that the treatment of different types of seaweed had a significantly different total phenolic compound value ($p<0.050$).

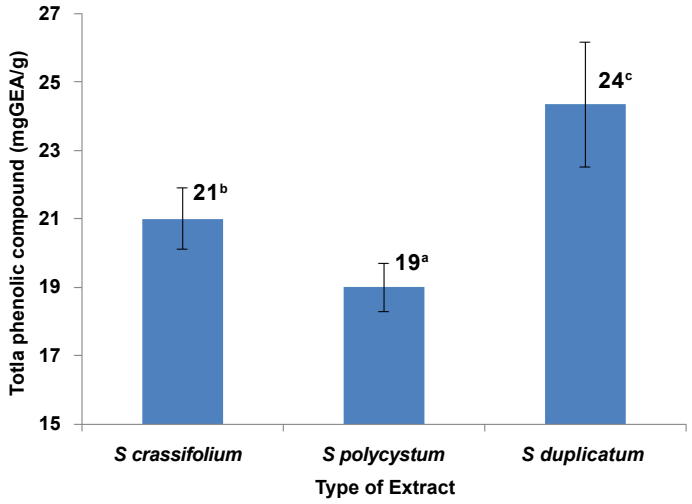

Figure 1: Levels of total phenolic compounds in fresh Sargassum seaweed samples.

The total phenolic compound in the sample, sorted from the largest to the smallest, is *S. duplicatum*=24 mg GEA/g BB, *S. crassifolium*=21 mg GEA/g BB) and *S. polycistum*=19 mg GEA/g BB (Figure 1) Polyphenol component contained in seaweed is catechol. Catechol is categorized into phenol antioxidants [16]. Based on observations on the parameters of its habitat, the high content of the total phenolic compound is 3 factors namely: sunlight, the presence of herbivores, and plant health. Accumulation of phenolic compounds in plants can be caused due to various abiotic stresses such as light [17]. The association of sunlight with antioxidant activity has been explained in many studies [18].

Analysis of percent inhibition

Analysis two-way ANOVA test showed that the treatment of different types of seaweed had a significantly different total phenolic compound ($p<0.050$). *S. duplicatum* was the highest percent inhibition. The ANOVA test showed that the use of different types of seaweed had a significantly different total phenolic compound ($p<0.050$). The lowest percent inhibition was found in hexane solvent and the highest was ethyl acetate solvent. Ethyl acetate fraction of seaweed contains protochlorophyllide as one of the active antioxidant substances. Protochlorophyllide structurally it is one of chlorophyll which consists of a porphyrin ring which is bound with a flat, magnesium atom in the middle, bound by a nitrogen ring on each side [19].

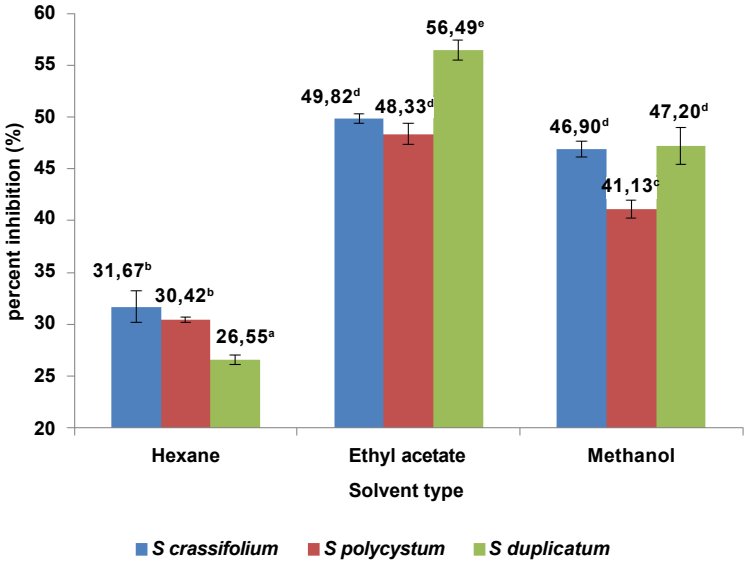

Figure 2: Antioxidant activity of extracts against DPPH Radicals.

The results of one-way ANOVA analysis of the percent inhibition showed that the treatment of different types of extracts had a significantly different percent inhibition ($p<0.050$). Percent inhibition from the smallest to the largest was extracted with ethyl acetate solvents on seaweed *S. polycistum, S. crassifolium* and *S. duplicatum* are 48.33%, 49.82%, and 56.49% respectively (Figure 2).

The existence of antioxidant compounds in a material can be determined by an assay of antioxidant activity. An assay of antioxidant activity in Sargassum extract was carried out using the DPPH method. It's can be used to assay the ability of antioxidant compounds as

antidote protons for radical compounds or hydrogen atom donors. After an assay using this method, Sargassum extract was concluded to have antioxidant activity because the extract was able to donate hydrogen atoms which were marked by changes in coloration from purple to pale yellow. The level of discoloration of purple DPPH is an indication of the presence of free radical inhibition activities by antioxidant samples. The purple color turns yellow when the DPPH odd radical electrons become paired with hydrogen atoms from the antioxidant antidote to free radicals to form DPPH-H [20].

Analysis of IC_{50}

Based on the analysis percent inhabitation of extracts, three of the best extracts were obtained, extracts with ethyl acetate as solvent. Then testing the antioxidant activity of each extract with the concentration difference treatment was carried out to determine the IC_{50} value. A comparison with positive controls of BHT and Ascorbic Acid is also carried out in this test. The regression form of BHT antioxidant activity, Ascorbic Acid and each extract against DPPH radicals sequentially are presented in the graphs.

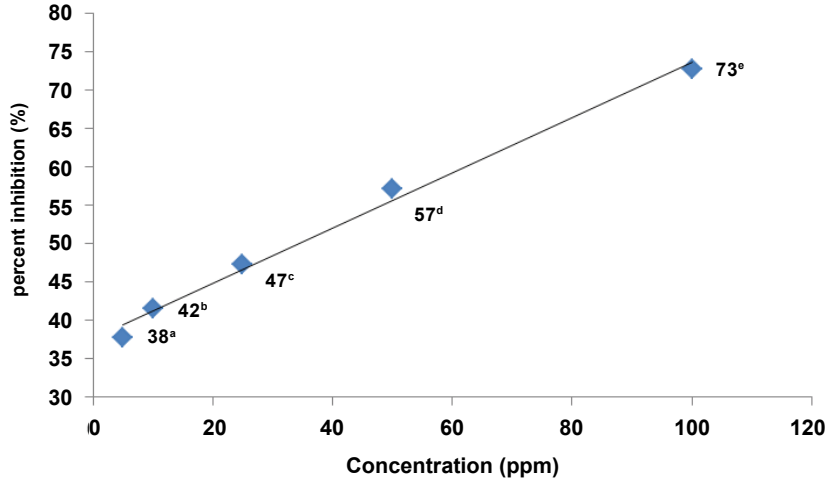

Figure 3: Regression of BHT antioxidant activity against DPPH radicals.

The results of the analysis of the inhibition percentage of BHT positive control (Figure 3) showed that the higher the BHT concentration, the higher the percentage of inhibition resulted. These results showed that there is a positive correlation between BHT concentration and percentage inhibition. One-way ANOVA analysis showed that BHT concentrations had significantly different inhibition percentage are ($p<0.050$), which were 38%, 42%, 47%, 57%, and 73% respectively.

BHT is a chemical compound with antioxidative properties. This synthetic antioxidant is mostly added to food because it effectively inhibits free radical activity and is synergistic with other antioxidants. But the use of synthetic antioxidants can cause poisoning at certain doses. The maximum safe threshold of BHT in food is 200 ppm [21].

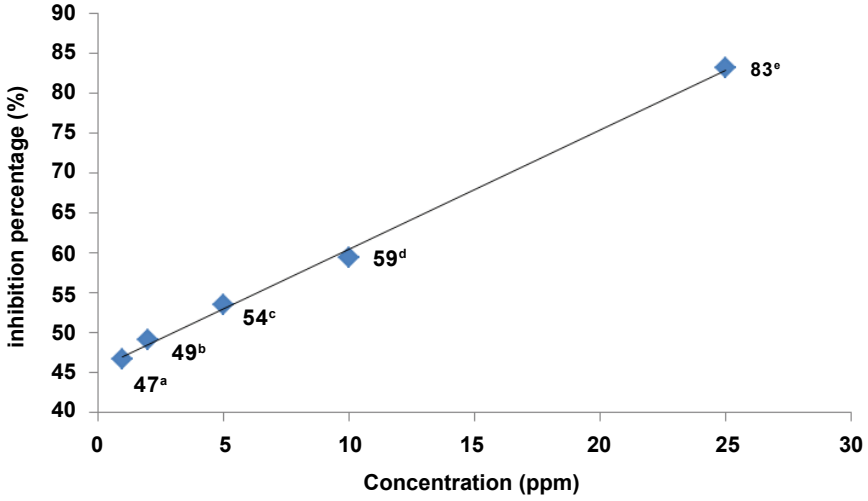

Figure 4: Regression of ascorbic acid antioxidant activity against DPPH radicals.

The results of the analysis percent inhibition are ascorbic acid positive control (Figure 4) that the higher the ascorbic acid concentration. There is a positive correlation between the concentration of ascorbic acid and the percent inhibition in the form of linear regression. Analysis of one-way ANOVA showed that the difference in concentrations of ascorbic acid had significantly different inhibition percentage values ($p<0.050$), which were equal to 47%, 49%, 54%, 59%, and 83% respectively.

The inhibition percentage analysis of the three best extracts shows that higher the concentration of the extract, the higher is the percentage inhibition value (Figure 5). In other words, there is a linear regression correlation positive correlation between the concentration of extract and percent inhibition.

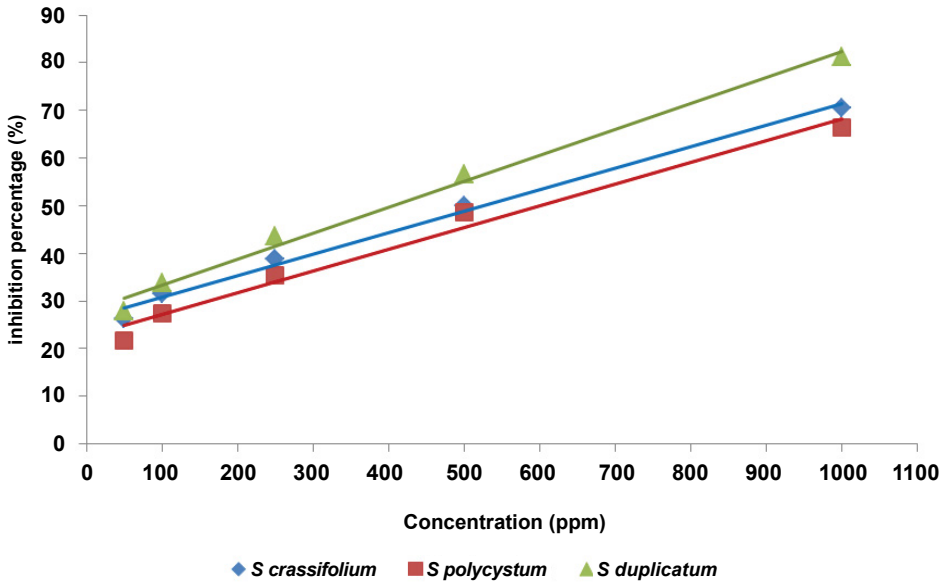

Figure 5: Antioxidant activity of sargassum extract with ethyl acetate solvents on DPPH radicals.

The results of One-way ANOVA analysis showed that the differences in the concentration of ethyl acetate solvent extract on *S. crassifolium* had a significantly different inhibition value ($p<0.050$), which were 26%, 32%, 39%, 50%, and 70% respectively. One-way ANOVA analysis showed that ethyl acetate solvent extract on *S. polycistum* concentrations have significantly different percent inhibition ($p<0.050$), which were 22%, 27%, 35%, 49%, and 66% respectively. The results of one-way ANOVA analysis showed ethyl acetate solvent extract on *S. duplicatum* concentrations had significantly different percent inhibition ($p<0.050$), which were 28%, 34%, 44%, 56%, and 81% respectively.

The antioxidant mechanism of the extract was caused by the presence of natural phenolic compounds. It can inhibit the oxidation process by donating hydrogen atoms which will bind peroxide groups to produce more stable compounds [22].

Based on the correlation analysis between the concentration and the percent inhibition, the regression equation and the correlation value are calculated (Table 1). The analysis showed that positive controls and extracts have different regression equations and correlation values. Based on the regression equation, the IC_{50} values are then determined. Percent inhibition is the ability of a substance to counteract free radical activity, which is related to the concentration of a substance. IC_{50} is the concentration of substrate solution or sample which will cause a reduction of DPPH activity by 50%.

Table 1: Antioxidant activity of IC_{50} *Sargassum* extracts with ethyl acetate solvents.

Treatment	y=a+b*x	R^2	R	IC_{50}
BHT	y=0,3599 x+37,596	0,9924	0,9962	34[a]
Ascorbic acid	y=1,4540 x+46,257	0,9960	0,9980	601[d]
S. crassifolium	y=0,0451 x+26,273	0,9921	0,9960	526[c]
S. polycystum	y=0,0458 x+22,485	0,9807	0,9903	601[d]
S duplicatum	y=0,0544 x+27,886	0,9910	0,9955	407[b]

One-way ANOVA analysis, IC_{50} positive control and extract values showed a significant difference ($p<0.050$). If the sample was compared between positive controls and extracts, BHT has a better antioxidant activity with an IC_{50} value of 34 ppm. The comparison between the three extracts showed a significant difference in IC_{50} values ($p<0.050$), with the smallest antioxidant activity sequence to the largest in *S. polycistum, S. crassifolium,* and *S. duplicatum* with IC_{50}=607 ppm, 526 ppm, and 406 ppm respectively. The percent inhibition of free radical activity increases with an increasing concentration of extract [23]. The lower IC_{50} value it's higher the antioxidant activity. The highest percent inhibition and lowest IC_{50} values confirm that BHT is a strong antioxidant. Antioxidant compounds that make up the BHT ingredients can contribute greatly to potency of the compound as an antioxidant.

Analysis of total phenolic compounds

The results showed a linear regression line, which means that there is a correlation between the concentration of the extract and the total content of the phenol compound (Figure 6).

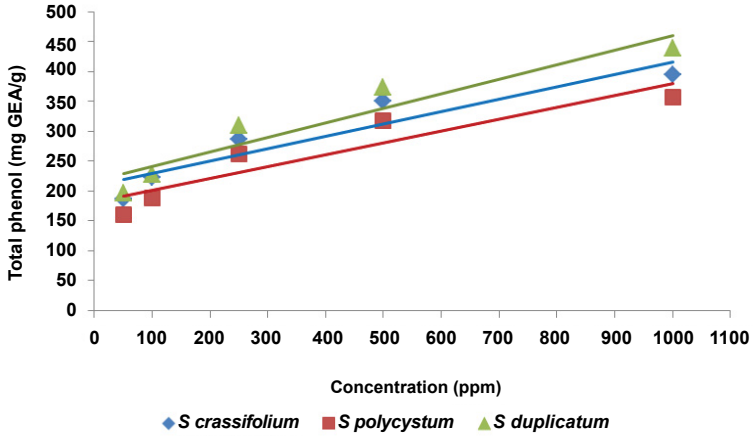

Figure 6: Antioxidant activity of sargassum extract with ethyl acetate solvents on DPPH radicals.

The analysis of the relationship between the concentration of ethyl acetate solvent extract and total phenolic compound, the regression equation, and the correlation value were obtained (Table 2).

Table 2: Regression equations of the total phenol content of sargassum extract with ethyl acetate solvents.

Treatment	y=a+b*x	R^2	R
S. crassifolium	y=0,2072 x+209,26	0,8772	0,9366
S. polycystum	y=0,1985 x+181,79	0,8567	0,9256
S. duplicatum	y=0,2443 x+216,17	0,9031	0,9503

One-way ANOVA analysis showed that the differences in the concentration of ethyl acetate solvent extract on *S. crassifolium* had significantly different phenol content values ($p<0.050$) were 187 mg GEA/g, 223 mg GEA/g, 286 mg GEA/g, 350 mg GEA/g, and 394 mg GEA/g respectively. The results showed that of ethyl acetate solvent extract on *S. polycistum* concentrations had significantly different phenol content values ($p<0.050$), were 161 mg GEA/g, 188 mg GEA/g, 262 mg GEA/g, 318 mg GEA/g, and 357 mg GEA/g respectively. The results of one-way ANOVA analysis showed that of ethyl acetate solvent extract on *S. duplicatum* concentrations had significantly different phenol content values ($p<0.050$), which were 197 mg GEA/g, 228 mg GEA/g, 309 mg GEA/g, 373 mg GEA/g dan 438 mg GEA/g respectively.

Phenylpropanoid compounds are formed in response to tissue damage or predation of herbivorous animals and it's the formation of chlorogenic acids, alkyl ferulate esters, and bonds of phenolic esters which may act in defensive measures or provide precursors for the synthesis of suberin and polyphenolic barrier [24]. The relationships between stress on plants and the level of antioxidants is still a complex unit, variable and dynamic puzzle [25]. But plants form secondary metabolites and antioxidant polyphenols for physiological, metabolic reasons, as well as in response to stress and predatory animal attacks.

Analysis correlation of total phenolic compound and percent inhibition

The results of the analysis correlation and its regression equation and the correlation values (Figures 7-9) and its regression equation and the correlation values (Table 3).

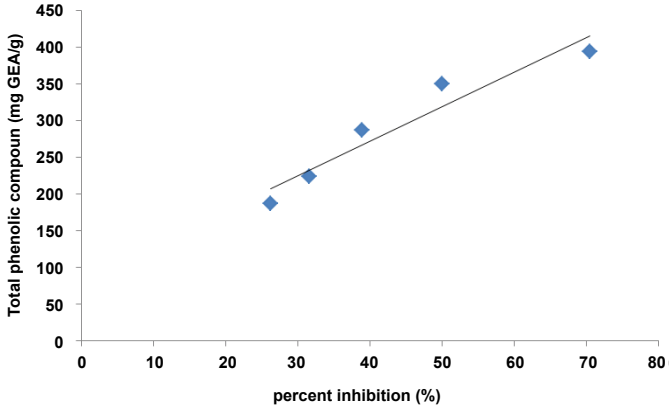

Figure 7: Correlation between total phenolic compound of *S. crassifolium* extract with ethyl acetate solvent and inhibition percentage of DPPH radicals.

The results of the correlation analysis between the total phenol of *S. crassifolium* extract on percent inhibition showed that the higher the total phenol extract, the higher the percent inhibition value obtained. There is a positive correlation between the concentration of the total phenolic of extract and the percent inhibition (Figure 7). Analysis of one-way ANOVA showed that the difference in total phenol of extract of 187 mg GEA/g, 223 mg GEA/g, 286 mg GEA/g, 350 mg GEA/g, and 394 mg GEA/g had significantly different inhibition percentage values ($p<0.05$) were 26%, 32%, 39%, 50%, and 70% respectively.

Table 3: Regression equations and correlation of the total phenolic compound of sargassum extract with ethyl acetate solvents towards inhibition percentage.

Treatment	y=a+b*x	R^2	R
S. crassifolium	y=4,6962 x+84,218	0,922	0,9602
S. polycystum	y=4,4762 x+78,632	0,9326	0,9657
S. duplicatum	y=4,4762 x+78,632	0,9460	0,9726

The analysis of the relationship between the concentration of ethyl acetate solvent on Sargassum extract and inhibition percentage, the regression equation, and the correlation value were obtained (Table 3).

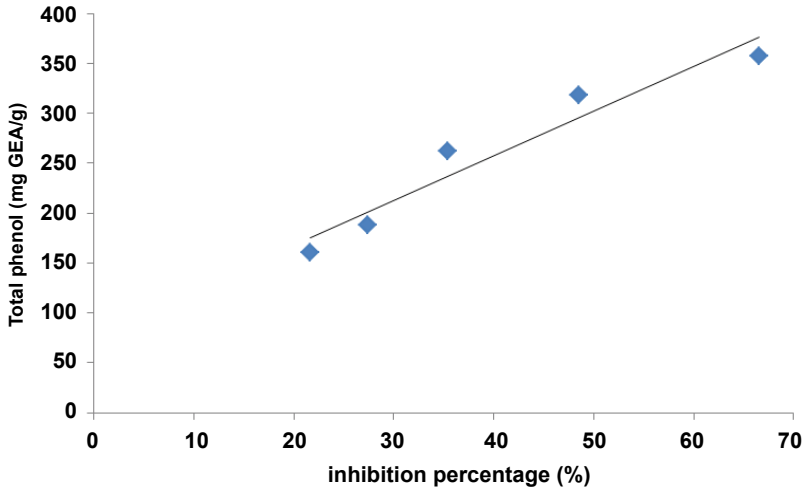

Figure 8: Correlation between total phenolic compound of *Sargassum polycystum* extract with ethyl acetate solvent and percent inhibition of DPPH radicals.

The results of the correlation analysis between the total phenol extract of *Sargassum polycystum* to the percent inhibition (Figure 8), it can be concluded that the higher the total phenol extract, the higher the percent inhibition value obtained. Figure 8 showed that there is a positive correlation between the concentration of total phenol of extract and the percentage of inhibition in the form of linear regression. Analysis of one-way ANOVA showed that the difference in total phenol of extract of 161 mg GEA/g, 188 mg GEA/g, 282 mg GEA/g, 318 mg GEA/g, and 357 mg GEA/g had significantly different inhibition percentage values ($p<0.050$), which were 22%, 27%, 35%, 49%, and 66% respectively.

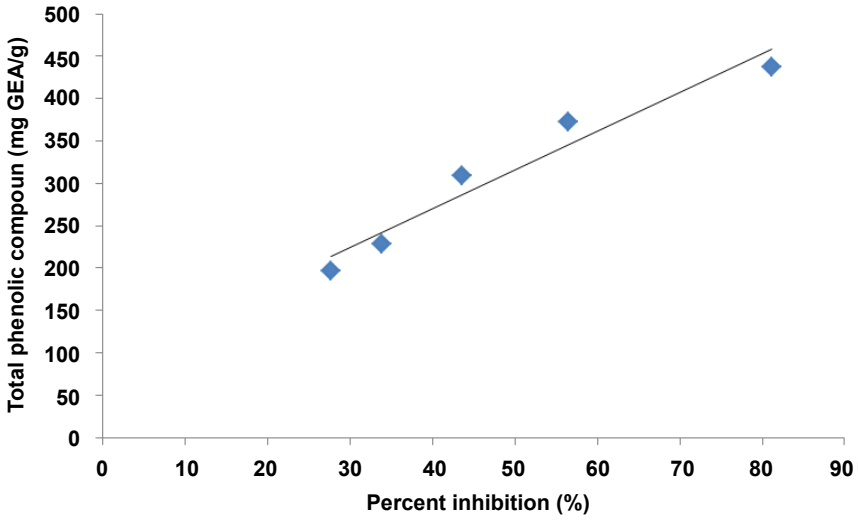

Figure 9: Correlation between total phenolic compound of *Sargassum duplicatum* extract with ethyl acetate solvent and inhibition percentage of DPPH radicals.

The results of the correlation analysis between the total phenol of *Sargassum duplicatum* extract on percent inhibition presented in Figure 9 showed that the higher the total phenol extract, the higher the percent inhibition value obtained. The results show that there is a positive correlation between the concentration of total phenol of extract and the percentage of inhibition. Analysis of one-way ANOVA showed that the difference in total phenol of extract of 197 mg GEA/g, 228 mg GEA/g, 309 mg GEA/g, 373 mg GEA/g, and 438 mg GEA/g had significantly different inhibition percentage values ($p<0.05$), which were 28%, 34%, 44%, 56%, and 81% respectively.

The results showed that Sargassum crude extract had antioxidant activity. This finding is supported by Diachanty et al. [8] on crude extract of *S. polycystum* and *P. minor* with ethyl acetate solvent which showed better IC_{50} values of 42.0 µg/mL and 65.9 µg/mL. The higher concentration of ethyl acetate seaweed extraction resulted in high levels of total phenol and antioxidant activity because the polyphenol component which has antioxidant activity is easier to extract from organic solvents with moderate polarity than water [6]. This is because water cannot separate other non-phenolic components in the extraction process. According to Lee et al. [26] phenol is a chemical compound that has the potential as an antioxidant, although the compound is not the only trigger for antioxidant activity. Triterpene compounds, pentacyclics, vitamin C, pigments such as chlorophyll, sulfuric compounds, or nitrogen can act as antioxidants. Correlation analysis between total phenol and antioxidant activity using the DPPH method aims to determine the significance of the correlation between total phenol and antioxidant activityThe results showed that Sargassum crude extract had antioxidant activity. This finding is supported by Diachanty et al. [8] on crude extract of *S. polycystum* and *P. minor* with ethyl acetate solvent which showed better IC_{50} values of 42.0 µg/mL and 65.9 µg/mL. The higher concentration of ethyl acetate seaweed extraction resulted in high levels of total phenol and antioxidant activity because the polyphenol component which has antioxidant activity is easier to extract from organic solvents with moderate polarity than water [6]. This is because water cannot separate other non-phenolic components in the extraction process. According to Lee et al. [26] phenol is a chemical compound that has the potential as an antioxidant, although the compound is not the only trigger for antioxidant activity. Triterpene compounds, pentacyclics, vitamin C, pigments such as chlorophyll, sulfuric compounds, or nitrogen can act as antioxidants. Correlation analysis between total phenol and antioxidant activity using the DPPH method aims to determine the significance of the correlation between total phenol and antioxidant activity.

CONCLUSION

The results of the analysis showed that the treatment of different types of hexane extract, ethyl acetate and methanol had significantly different inhibition percentage values ($p<0.050$). The best percentage of inhibition if sorted from the largest to the smallest is extracted with ethyl acetate solvent on *S. duplicatum, S. crassifolium,* and *S. polycistum* seaweed. The higher the concentration of the extract, the higher the inhibition percentage ($p<0.050$) and the total phenol content of the compound produced. There is a positive correlation between the total phenolic compound and percent inhibition ($p<0.050$).

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