# PHYTOCHEMICAL ACTIVITY

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**Submission date:** 03-Sep-2021 08:44AM (UTC+0700)
**Submission ID:** 1640440275
**File name:** 29-Article_Text-27-3-10-20210217.pdf (383.36K)
**Word count:** 8378
**Character count:** 47613

Journal of TROPICAL BIODIVERSITY E-ISSN : 2774-4116

# Phytochemical, Antibacterial and Antioxidant Activities Test of Three Macro-Algae *Phaeophyceae* Extracts From Pulau Tidung Coastal Kepulauan Seribu

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## Abstract

Macro-algae is a *Thallophyta* plant that has been known to have potential as a food and medicine ingredient, one of that is in the Kepulauan Seribu, particularly on Pulau Tidung coastal. Therefore this study aimed to explore the potential of three types of macro-algae: *Hormophysatriquerta, Padinaaustraslis, and Sargassumbinderi* (*Phaeophyceae*) on the Pulau Tidung coastal by revealing the phytochemical content, antibacterial, and antioxidant activity. The design used in this study was completely randomized factorial designs. The phytochemicals tested were flavonoid, alkaloid, tannin, saponin, quinon, steroid, and terpenoid. Antibacterial were tested by diffusion wel method on Muller Hinton agar in a petri dishes with a size of 100 mm and a thickness of 10 mm. The three tested macro-algae were extracted using methanol solvent and divided into four concentrations: 5%, 10%, 15%, and 20%. The test bacteria used were *Staphylococcus aureus* ATCC 6539 and *Escherichia coli* ATCC 8739. Observation of the antibacterial test was carried out 24 hours after incubation at 37$^0$C. Antioxidant activity at concentrations of 100, 200, 300, 500, 700 ppm was carried out by the DPPH (1,1-diphenil-2-*pikrylhidrazyl*) method. The results of phytochemical tests showed that flavonoids and steroids were found in the three macro-algae tested, while saponins were only found in *S. binderi*. The results of the inhibition zone showed that the three macroalgae extract tested did not inhibit *E. coli* bacteria. The largest inhibition zone was shown at a concentration of 20% against *S. aureus* bacteria, namely, *H. triquerta* = 10.09 ± 0.89, *P. austraslis* = 11, 46 ± 1.09, and *S. binderi* 8.00 ± 1.29. The regression results of the tested macro-algae antioxidant activity showed that the $IC_{50}$ values were 13,290 ppm (*H. triquerta*), 3,800 ppm (*P. australis*), and 4,053 ppm (*S. binderi*).

**Keywords:** Antibacterial, Antioxidant, Phytochemical, Macro-algae

## Introduction

Indonesia produces 20% or 3.9 million tons of macro-algae from eight macro-algae producing countries in the world (FAO, 2012). Macro-algae is plants that can not be distinguished between roots, stems, and leavessothatis included in *Thallophyta* (Lee, 2008). Algae classification based on pigmentation, flagella, chemical wall structure, and storage areas is *Cyanophyta* (blue-green algae), *Rhodophycophyta* (red algae), *Chlorophycophyta* (green algae), *Charophyta, Euglenophycophyta, Pyrrhophycophyta, Chryptophatoms* (gold and yellow algae including diatoms), and *Phaeophyta* (brown algae) (Knappe, 2004). Of the

eight macro-algae divisions, only three divisions whose members live as benthic macro-algae (seaweed), namely *Chlorophyta, Phaeophyta, and Rhodophyta* (Handayani *et al*., 2014).

One of the islands in Indonesia is the KepulauanSeribu. The Thousand Islands are located north of Jakarta bay and the Java Sea, between 06000'40"and 05054'40" south latitude and 106001'19"east longitude.The total area of the KepulauanSeribuAdministrative Regency is approximately 11 times the land area of Jakarta, the land area reaches 897.71 ha and the territorial waters reach 6997.50 $km^2$ (Jakarta.go.id, 2016). One of the islands in Kepulauan Seribu is Pulau Tidung. Pulau Tidung waters have a high diversity of macro-algae. Based on the research conducted by Handayani and Widowati (2016) on the island of Tidung, Kepulauan Seribu, there are 21 types of macro-algae from three divisions, namely *Chlorophyta, Phaeophyta,* and *Rhodophyta.*

According to Perez *et al*. (2016), micro-algae is a producer of biomass in the marine environment that has the potential to be a source of diversity and unique compounds. Macro-algae is a source of food for animals and fish that live in the sea. Humans have also used several types of seaweed. Heretofore, 22 types of macro-algae have been recorded as food ingredients, industrial and medicinal materials (Handayani*et al*., 2014).

Many of the ingredients contained in macro-algae such as alginates, carrageenan, and agar has been in use for several centuries in traditional medicine, pharmaceuticals, and food. The drug content in macro-algae is considered the effect of phytochemical compounds. Phytochemicals are natural bioactive compounds in plants such as flavonoid, saponin, and steroid (Singh, 2011). Several studies have stated that macro-algae have potential as an antibacterial ingredient as stated by Ismail et al (2016) that macro-algae also have antimicrobial or antibacterial uses. Antibacterial is usually considered as the effectiveness of bacterial cell death (Ivanova and Crawford, 2015). For antibacterial testing, bacteria representing the gram-positive group are usually used, namely *Staphylococcus aureus*, and representing the gram-negative group is *Escherichia coli* (Widowati, 2015).

Macroalga can be used as an anticancer (Brown *et al*., 2014). According to Shamsabadi *et al*. (2013), the ethanol extract of the red algae *Eucheumacottoni* has a strong effect on anti-cancer.Based on the research of Ermakova*et al*. (2011), the regulation enzyme's characteristics, fukoidan-isolatedcompounds from water extraction and chromatography with ion-exchange of brown algae*Eclonia cava, Sargassumhornery,* and *Costariacostata*have a role in inhibiting colony formation in melanoma and colon cancer. Both macro-algae and micro-algae contain pharmacologically active compounds such as fluorotanin, fatty acids, polysaccharides, peptides, and terpenes that fight bacterial invasion (Blunt*et al*., 2015; Shannon and Abu-Ghannam, 2016). According to Manivannan *et al*. (2011), macro methanol extract of brown algae or *Padinagymnospora* has a strong inhibitory power against *Bacillus subtilus* bacteria using antibiotic-discscontrol.

Macro-algae have been used as antioxidants (Francavilla *et al*., 2013). Antioxidant compounds are electron-giving or electron donor compounds. Biologically, antioxidants are counteracted compounds to reduce the negative impact of oxidants in the body. Antioxidants work by donating one electron to oxidant compounds that inhibit the activity of these oxidant compounds (Winarsi, 2007). Antioxidants are divided into two, namely endogenous and exogenous (Fraunberger *et al*., 2016). Endogenous antioxidants are antioxidants produced

within or caused by factors from within the organism, for example, superoxide dismutase, catalase, glutathione peroxidase, glutathione, and others while exogenous antioxidants are antioxidants produced outside of organisms, for example, vitamin C, vitamin E, carotenoids and polyphenols (Bouayed and Bohn, 2010; Schmidt *et al*., 2015).Measurement of antioxidants was carried out utilizing the DPPH (*1,1-diphenil-2-pikrylhidrazyl*, inhibition method by measuring the absorbance to obtain inhibition concentration ($IC_{50}$).$IC_{50}$ is the concentration of an antioxidant substance which can cause 50% of DPPH to lose its radical properties and vitamin C is used as a positive control (Salazar-Aranda *et al*., 2011).

Research on the macro-algae potential in Indonesia is still very limited, especially in terms of phytochemical, antibacterial, and antioxidant content. For this reason, a study was carried out that continued the research conducted by Handayani and Widowati (2016) regarding the inventory and identification of macroalgae species on the PulauTidung-Kepulauan Seribu to explore the potential of biological sources.This study aimed to explore the macro-alga potential of *Hormophysatriquerta, Padinaaustralis*, and *Sargassumbinderi* obtained from the Pulau Tidung coastal- Kepulauan Seribu by revealing the content of active compounds, antibacterial and antioxidant activity.

The hypothesis proposed in this study are:

1. There are differences in the active compounds between macro-algae types *H. triquerta, P. australis*, and *S. binderi*.
2. Extracts of *H. triquerta, P. australis,* and *S. binderi* can inhibit the growth of *Staphylococusaureus* and *Escherichia coli* bacteria cells.
3. The greater the concentration of *H. triquerta, P. australis*, and *S. binderi* extracts, the higher the inhibition of *S. aureus* and *E. coli* bacteria.
4. Extracts of *H. triquerta, P. australis*, and *S. binderi* have antioxidant activity.

## Materials and Method

### A. Research Setting

The research was conducted at the Chemistry Laboratory, the Biology Faculty Microbiology Laboratory, the Nasional Universitas Nasional Research Laboratory, and the Bogor Agricultural Institute's Center for Biopharmaca Study Laboratory from September 2016 to November 2016.

### B. Research instruments

#### 1. Material

The materials used in this study were 500 grams of dry macro-algae simplicia (*H. triquerta, S. binderi, P. australis*), *E. colistrain* ATCC 8739, *S. aureus* Strain ATCC 6539, BaCl2 (barium chloride), H2SO4 (sulfuric acid), MH (Mueller Hinton) Agar, NA (*Nutrient*

*Agar*) Media, distilled water, DMSO (*Dimethyl Sulfoxide*), NaCl (sodium chloride), 70% alcohol, 80% methanol, DPPH powder, ethanol PA, and doxycycline antibiotics.

**2. Tools**

The tools used in this study were BSC (biosafety cabinet) autoclave, oven, microwave, water bath, shaker, 100 mL measuring cup, spatula, 25 mL measuring flask, 5 mL volumetric flask, vortex, funnel, stirring rod, corkborer,ose, cotton sticks, analytical scale, technical scale, 10-100 μLmicropipette, 100-1000 μL micropipette, microtube, blue tip, yellow tip, pasteur pipette, 1 mL and 10 mL measuring pipette, scale paper, 100 mm diameter petri dish, burners (using methylated spirits), calipers, 500 mL erlenmeyer, 100 mL and 500 mL beaker, newspaper, microplate, spectrophotometer, cuvette, mortar, mortil, measuring cup, beaker, separating funnel, matches, and aluminum foil.

**C. Procedure**

This research requires some material preparation by way of working systematically arranged as follows:

**1. Phytochemical testing of the macro-algasimplicia of *H. triquerta, P. australis* and *S. binderi***

Phytochemical testing of macro-alga simplicia is carried out qualitatively to determine the presence of active substances (Harborne, 1998). Phytochemical testing is carried out in the following manner:

**a. Tannins**

A total of 5 g of macro-algae simplicia was put into a beaker added 20 mL of distilled water,heatfor five minutes, and filtered. The filtrate obtained was put into a test tube and added three drops of 10% FeCl3 (iron (III) chloride). Positive results are obtained if a blackish green color was formed.

**b. Saponins**

A total of 5 g of macroalgaesimplicia was put into a beaker followed 20 mL of distilled water, heat for five minutes, and filtered. The filtrate obtained was put into a test tube and tightly closed then shaken vigorously and observed the formation of an emulsion. Positive results are obtained if the foam was stable.

**c. Flavonoids**

A total of 5 g of macroalgaesimplicia was put into a beaker, added 20 mL of distilled water, heat for five minutes, and filtered to get the filtrate. The filtrate obtained was put into a test tube and added a tablespoon of magnesium powder, HCL (hydrochloric acid): EtOH (ethanol) (1: 1), and amyl alcohol to observe the changes. A positive result is obtained if there was an orange layer of amyl alcohol.

E-ISSN : 2774-4116

**d. Steroids**

A total of 1 g of sample was put into a test tube, added hot EtOH followed the filtration process. The filtrate obtained was heated to dry then added with 1 mL of diethyl ether and homogenized. The filtrate which contained diethyl ether added one drop of H2SO4 and one drop of CH3COOH (acetic acid) concentrated anhydrous and observed the changes. A positive result is obtained if a green or blue color was formed.

**e. Triterpenoids**

A total of 1 g of sample was put into a test tube and added hot EtOH then filtered. The filtrate obtained was heated to dry then added with 1 mL of diethyl ether and homogenized. The filtrate which contained diethyl ether addone drop of H2SO4 and one drop of concentrated CH3COOH and then observed the changes. A positive result is obtained if a red or purple color was formed.

**f. Alkaloids**

A total of 1 g of the sample was put into a mortar and added a few drops of NH3 (ammonia) then puree. The refined sample was added with 5 mL of CHCl3 (trichloromethane) and then filtered. H2SO42M was added to the filtrated sample and shaken regularly, finally left to stand until three layers of acid were formed. Positive results on Dragendrof if an orange sediment layer was formed in the first layer, the second layer was formed white precipitate with Mayer reagent and positive results in Wagner's reagent if a reddish-brown precipitate was formed.

**2. Making extract**

Macro-algae *H. triquerta, P. australis*, and *S. binderi*cleaned and washed using water.Dry ground macro-algae sieved with a mesh 18 sieve. As much as 0.5 kg of macro-algae powder was immersed in 1 L of 80 percent methanol at room temperature for 15 hours. To further dissolve the active substances in the powder, the bath was shaken with a shaker 2 times 24 hours.The liquidwas filtered using filter paper and stored in a special light-tight bottle. The remaining powder was re-immersed in 1 L of methanol at room temperature for 15 hours.Theliquidwas filtered and combined with the extract obtained in the first immersion. Soaking was repeated in the same way until clear then all the extract liquid was collected.Theactive-substances filtered extract was evaporated using a water bath with a temperature $40^0$C. The extract obtained was concentrated dry and thick using a rotary vacuum evaporator.

**3. Sterilization equipment**

Equipment such as petri dishes, measuring pipettes, cotton sticks, and vial bottles was wrapped in paper and then sterilized in an oven at $180^0$C for 2 hours.The tools such as measuring flasks were sterilized in an autoclave $121^0$Candother tools such as pipettes and ose were sterilized by using Bunsen fire.

**4. Median bacterial growth**

NA medium was used as a base culture. Firstly, NA media was made from 2.8 grams NApowder,dissolved in 100 mL of distilled water, then heated and stirred until homogeneous.The dissolved NA media was put into the test tube as needed and closed tightly.Secondly, the NA media was sterilized in an autoclave at $121^0$Cfor 15 minutes. After the sterilization process finished, the test tube containing NA was tilted to the desired size until the NA media solidified, finally, the NA media was ready to use.

**5. Bacterial test media**

MH powder was measured as 38 grams,dissolved in 1 L of distilled water then heated until boiling and dissolved completely. Dissolved MH agar was sterilized using an autoclave for 15 minutes at a temperature of $121^0$C. The MH medium for sterile agar was poured into 20 mL petri dishes with a diameter of 100 mm and cooled at room temperature with the closed beaker and the MH agar media ready for use.

**6. Preparation of test bacteria**

One ose of *S. aureus* and *E. coli* bacteria test were dissolved in a physiological 0.85% NaCl solution separately and the number of bacteria used was adapted using *Mcfarlan* 0.5 (bacterial density $1{,}5 \times 10^8$) on a black background and bright light.The standard of turbidity *Mcfarlan*0,5 was made by mixing 0.5 mL of 1% BaCl2 solution with 9.5 mL of 1% H2SO4.

**7.Antimicrobial (antibacterial) testing**

**a. Sample preparation, positive control, and negative control**

The sample was made into a concentration of 5%, 10%, 15%, and 20% by making a 20% concentration solution (weighed 5 grams of macro-algae extract and dissolved in a 25 mL volumetric flask) as a mainlsolution. Furthermore the concentration of 20% diluted to 5%, 10%, and 15%. The 5% concentration was formed by pipetting 250 $\mu$L of macro-algae extract into a microtube which added 750 $\mu$L of DMSO solution.The 10% concentration was made by pipetting 500 $\mu$L of 20% macro-algae extract into the microtubeadded500$\mu$L of DMSO solution. The 15% concentration was prepared by pipetting 750 $\mu$L of 20% macro-algae extract into the microtubeadded250$\mu$L of DMSO solution.

A positive control dose of 30 $\mu$g / 50 $\mu$L was prepared using 3mg of doxycycline which had been crushed previously, and dissolving in a 5 mL volumetric flask. Meanwhile, the negative control only uses the DMSO solution.

**b. Sample testing**

The design used in this research was a completely randomizedfactorialdesignof three types of Macro-algae *Phaeophyceae*, using four concentrations and four repetitions.Randomization at both concentration and control was carried out before labeling on the petri dish containing MH media. The prepared MH media was divided into four parts by making a line and labeled on the bottom of the petri dish. In addition, the *S. aureus* and *E. coli* bacteria were leveled on different petri dishes using a cotton stick. 4 holes or wel made using a cork borer. Furthermore, each pipette 50 $\mu$L of macro-algae extract solution 5%, 10%, 15%, and 20% which had been diluted with DMSO solution in a petri dish,added the

bacteria based on the label. DMSO as a negative control and doxycycline at a dose of 30 $\mu$g / 50 $\mu$L were used as positive controls for *S. aureus*and*E. coli*. Incubated for 1 × 24 hours at 37$^0$C. The inhibition of the extract against bacteria was measured using a caliper. The unit of bacterial inhibition was measured in mm. Antibacterial testing steps were shown in Figure 7 in the appendix.

**8. Antioxidant testing**

The test extract activity was determined by measuring *radical scavenging activity*usingthe DPPH method (Salazar-Aranda*et al*., 2011).

**a. Preparation of 125 $\mu$M DPPH stock solution**

DPPH was measured as 2.5 mg and dissolved with ethanol p.a (pro analysis) into a volumetric flask. Measured to 50 mL then coated aluminum foil. Finally,theDPPH solution was ready for use.

**b. Sample preparation and vitamin C**

Measured the sample and vitamin C, each as much as 10 mg, then dissolved in 1 mL of DMSO. Disonified until late, then vortexed. Sample and vitamin C was ready for use.

**c. Sample testing and vitamin C.**

Sample and vitamin C were put into the microplate as 100 $\mu$L. For replication samples 1 and 2, 100 $\mu$L of DPPH solution was added for 125 $\mu$M, while for negative control only 100 $\mu$L of p.a ethanol was added. Furthermore, the sample was incubated in a dark room for 30 minutes and measured using an ELISA (*Enzyme-linked immunosorbent assay*) at a wavelength of 517 nm.

The blank solution was made of 100 $\mu$L of PA ethanol solution in replications 1 and 2 andadded 100 $\mu$L of DPPH 125 $\mu$M solution. Meanwhile, the negative control only contained 200 $\mu$L of PA ethanol.

The ability of antioxidants was measured as a decrease in DPPH solution absorption due to the presence of the sample.The absorbance value of the DPPH solution before and after adding the extract was calculated by the percentage of inhibition.In addition, the calculation result was implied into the regression equation with the extract concentration (ppm) as the axis (x-axis) and the inhibition value as the ordinate (y-axis).The $IC_{50}$ value from the calculation when the inhibition percentage was 50% $Y = a + bx$.

**D. Data analysis**

Primary data from methanol extract test results of *H. triquerta, P. australis,* and *S. binder* on the growth of *S. aureus*and *E. colis*, the results of antioxidant-activity testing as ratio data were analyzed using *IBM SPSS statistic 22* software and statistical tests were

carried out with linear regression and *One Way*Anova and followed by a *Post Hoc* follow-up test, namely LSD (*Least Significant Different*) and *Tukey*.

## Results and Discussion

### A. Phytochemical content of macro-algae *Phaeophyceae*

Phytochemical tests carried out on three types of macro-algae *Phaeophycea* showed the following results (Table 1):

**Table 1. Phytochemical test results on three types of macro-algae *Phaeophyceae***

| Test | Types of Makro-alga | | |
|---|---|---|---|
| | *H. triquerta* | *P. australis* | *S. binderi* |
| Flavonoid | Positive | Positive | Positive |
| Alkaloid | Negatif | Negatif | Negatif |
| Tannin | Negatif | Negatif | Negatif |
| Saponin | Negatif | Negatif | Positive |
| Quinon | Negatif | Negatif | Negatif |
| Steroid | Positive | Positive | Positive |
| Triterpenoid | Negatif | Negatif | Negatif |

From table 1, three types of macro-algae produced positive reactions for the flavonoid and steroid tests, while saponin positive reactions were only found in the macro-algae species *S. binderi*. Alkaloid, tannin, quinone, and triterpenoid tests produced negative reactions in the three types of macro-algae tested.

Flavonoids are one of the natural plant products (Romeo, 2003) formed C6-C3-C6 carbon framework and more specifically of phenylbenzopyranform (Figure 1 attachment) (Grotewold, 2006). The positive flavonoid reaction occurs because of the reaction of magnesium with hydrochloric acid to form H2 gas bubbles, and concentrated magnesium and HCL metals act as a reduction for benzopyrone core contained in the flavonoid structure turn to a red or orange formed color (Tiwari*et al*., 2011). If macro-algae has flavonoid compounds, the addition of Mg and HCl will form flavilium salts lead to red or orange (Figure 2 attachment).

Positive steroid reactions in the sample with the Liebermann-Burchard reaction (Figure 3 attachment), namely the addition of anhydrous acetate and H2SO4 produce a blue-green color (Banu and Cathrine, 2015; Harborne, 1998).This mechanism is based on the dimerization of cholesterol and sulfuric acid. Steroid compounds are composed of four

core hydrocarbons, the basic structure of steroids is 1,2-Cylopentenophenanthrene (Ahmed, 2007).

The positive saponin reaction (Figure 4 attachment) is formed due to the presence of foam and can last no less than 10 minutes. The foam on the test process is formed glycosides which spumy in water and hydrolyzed into glucose and other compounds (Marliana*et al*., 2005). Saponins are glycosides found in several plants and have a high molecular weight with a sugar- molecules combination of triterpenes and steroid aglycones (Singh, 2002).

This study is in line with research conducted by Sarojini*et al*. (2012) that *Phaeophycea*macro-algae contain flavonoids which is higher than *Chlorophyceaema* and *Rhodophyceae*. In contrast to the results obtained by Heiba*et al*. (1990) stated that the macro-algae *Phaeophyceae* (*H. Triquerta* and *S. binderi*Sonder) produced negative flavonoids.According to Jannah *et al*. (2014) in a study on another type of brown algae, namely *S. vulgare*, showed negative flavonoid results, while in this study, the macro-algae extract of *S. binderi* produced positive flavonoids, both of which came from the same genus. Positive flavonoid results were also presented by Kanagarajjeevitha*et al*. (2014) on *Padinaboergeseni*, and in this study, one of the test materials came from *P. australis*.

The positive steroid results in this study are in line with research conducted by Jannah e*t al*. (2014) which samples derived from macro-algae *S. vulgare* are from the same genus as *S. binderi*. In contrast, results showed steroid negative for *S. ilicifolium* by Basha and Mutukhumar (2014). According to Kanagarajjeevitha*et al*. (2014), positive steroid results were obtained from *P. boergeseni* extract.

Several studies of the *Sargassum* type produced positive saponins, namely methanolic extracts, *S duplicatum, S. tenerrium, S. angustifolium, S. oligocystum,* and *S. boveanum*(Mehdinezhad*et al*., 2016; Raj*et al*., 2015; Santi*et al*., 2014). In contrast to the results obtained by Heiba *et al*. (1990) stated that the macro-algae *Phaeophyceae* (*H. Triquerta*and*S. binderi*Sonder) produced negative saponins. Other different results were also shown by Basha and Mutukhumar (2014) which produced negative saponins on *S. ilicifolium*.

The differences in the content of flavonoids, steroids, and saponins for each type of macro-algae can be found, even though the macro-algae come from the same genus because of different types of area and environmental conditions macro-algae grows. Other factors are different types of test samples, there is a possibility of different phytochemical compounds found between samples derived from simplicia and samples extracts with organic solvents (Karthick *et al*., 2014; Melpha *et al*., 2014) and inthis study used simplicia of the macro-algae *Phaeophyceae*.

**B. Antibacterial activity of three types of macro-algae *Phaeophyceae***

The inhibition zones formed in *S. aureus* and *E. coli* bacteria and positive controls were shown in Figures 9, 10, and 11 in the appendix. The results of graphical data from the mean of three types of *Phaeophycea*macro-algae were shown in Table 2 with the following observation data (Table 1 attachment). Positive controls used doxycycline

antibiotics concerning the M 100-S25 CLSI (*Clinical And Laboratory Standards Institute*) (Patel*et al*., 2015).

**Table 2.Doxycycline inhibition zone standards against *S. aureus* and *E. coli***

| Types of bacteria | Interpretation of inhibition zone diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* | | | *E. coli* | | |
| | R | I | S | R | I | S |
| Doksisiklin | ≤ 12 | 13-15 | ≥ 16 | ≤ 10 | 11-13 | ≥ 14 |

Note: R (resistant), I (intermediate) and S (sensitive)

**Table 3.Average Macro-algae*Phaeophycea*values against inhibition zone *E. coli* and *S.aureus***

| concentration | *S. aureus*(mm) | | | *E.coli*(mm) | | |
|---|---|---|---|---|---|---|
| | *H. triquerta* | *P.australis* | *S. binderi* | *H. triquerta* | *P.australis* | *S. binderi* |
| 5% | 6,85 ± 0,59 | 8,06 ± 1,12 | 6,89 ± 0,56 | 6,40 ± 0,00 | 6,40 ± 0,00 | 6,40 ± 0,00 |
| 10% | 8,23 ± 1,29 | 6,76 ± 0,73 | 7,04 ± 0,87 | 6,40 ± 0,00 | 6,40 ± 0,00 | 6,40 ± 0,00 |
| 15% | 8,56 ± 1,24 | 8,57 ± 1,55 | 6,97 ± 1,15 | 7,16 ± 0,91 | 6,40 ± 0,00 | 6,55 ± 0,30 |
| 20% | 10,09 ± 0,89 | 11,46 ± 1,09 | 8,00 ± 1,29 | 6,90 ± 0,74 | 6,40 ± 0,00 | 6,40 ± 0,00 |
| doksisiklin | 35,89 ± 2,37 | 30,79 ± 1,58 | 35,11 ± 3,33 | 21,67 ± 2,74 | 19,95±1,38 | 19,71 ± 2,63 |
| DMSO | 6,40 ± 0,00 | 6,40 ± 0,00 | 6,40 ± 0,00 | 6,40 ± 0,00 | 6,40 ± 0,00 | 6,40 ± 0,00 |

Diameter wel 6,40 mm

Table 2 showed that the zone of inhibition produced by *S. aureus* and *E. coli* classified as a resistant category for doxycycline standards. *S. aureus* and *E. coli* were still sensitive to doxycycline antibiotics.The three macro-algae *Phaeophyceae* tested were more resistant to *S. aureus* than *E. coli*. *P. australis* had a larger average inhibition zone than *H. triquera* and *S. binderi*. This study was in line with the research of Khoiriyah *et al*. (2014) stated that the methanol extract of *S. vulgare* brown algae on *S. aureus* bacteria produced an inhibition zone that was larger than that of *E. coli*.

The difference in inhibition zone diameter was due to differences in cell wall structure in bacteria *S. aureus* included in gram-positive only had one peptidoglycan wall composed of *teuchuronic acid, teichoic acid* and some contain poly saccharides, while *E. coli*was a gram-negative group having four layers consisting of the outer membrane, lipopolysaccharide, lipoprotein, and peptidoglycan. In the outer membrane layer of *E. coli* contained protein molecule namely porin and functions as passive diffusion of low molecules such as sugars, amino acids, and certain ions so that large molecules such as antibiotics and antibacterial agents were relatively slow to penetrate the outer membrane. This made*E.coli* bacteria more resistant to antibacterials (Broooks*et al*., 2013).

To simplify reading the average inhibition zone value of the three macro-algae *Phaeophyceae* against *S, aureus* bacteria, the following graph was displayed:

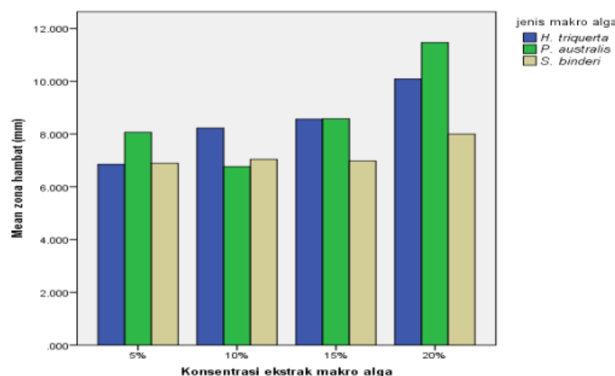


**Figure 1. Graph of the average inhibition zone value of three types of macro-algae*Phaeophyceae* against *S. aureus* bacteria**

The graphic data in Figure 5, there was an increase in the inhibition zone area at per5% increase in the concentration of macro-algae extract.Thiswas following the existing hypothesis, that every increase in the concentration of macro-algae extract was followed by a widening of the inhibition zone.The largest inhibition zone in the three types of macro-algae was produced at a concentration of 20% for *S. aureus* bacteria. However, there was a decrease in the concentration of 5% to 10% in *P. australis* macro-algae this was probably due to natural variations in antibacterial testing.

**Table 4.Anovaunivariate test of macro-algae types, the concentration of macro-algae extracts, and bacteria species against the inhibition zone and their interactions**

| Treatment Level | *p-value* |
|---|---|
| Types of Macro-algae | 0,000 |
| Concentration | 0,000 |
| Types of Bacteria | 0,000 |
| Types of Macro-algae * Concentration | 0,016 |
| Types of Macro algae * Types of Bacteria | 0,001 |
| Concentration * Type of Bacteria | 0,000 |
| JM * Concentration * jb | 0,025 |

The results of the Anova univariate test table 3 showed that the concentration of macro-algae, types of macro-algae, types of bacteria, the interactions of macro-algae types with macro-algae concentrations, interactions between types of macro-algae and types of bacteria, interaction of macro-algae concentrations with types of bacteria, and the interaction of the three (types of macro-algae, the concentration of macro-algae, and types of bacteria) had a significant effect on the results of the observation (bacterial inhibition zone) at the 95% confidence interval ($p \leq 0.05$), which means that ho was rejected.

Anova univariate test results (table 3) showed significant results between the concentration of macro-algae extracts and the bacterial inhibition zone, therefore a further test was carried out to see the level of significance at each existing concentration. In this case, the LSD advanced test was used (table 4).

**Table 5. Results of further LSD test on concentration**

| Konsentrasi | 5% | 10% | 15% | 20% |
|---|---|---|---|---|
| 5% | - | 0,375 | 0,5375 | 1,375 |
| 10% | - | - | 0,5 | 1,3375 |
| 15% | - | - | - | 0,8375 |
| 20% | - | - | - | - |

The results showed that there was a significant difference in the concentration of 20% against the concentration of 5%, 10%, and 15% (green). Significant results were also obtained at a concentration of 15% against a concentration of 10% and 5%. While a concentration of 10% to 5% did not show a significant result at the 95% confidence interval (yellow color).

Anova univariate test results (table 3) showed significant results on the macro-algae species against the bacterial inhibition zone, therefore the LSD test was carried out to see the significance level of the macro-algae species (table 5).

**Table 6. An advanced test of LSD against types of macro-algae**

| Type | *S. binderi* | *P. australis* | *H. triquerta* |
|---|---|---|---|

| | | | |
|---|---|---|---|
| *S. binderi* | - | 0,72656 | 0,74219 |
| *P. australis* | - | - | 0,01562 |
| *H. triquerta* | - | - | - |

From the test, macro-algae species *H. triquerta* against *S. binderi* showed significant results with a 95% confidence interval as well as macro-algae species *P. australis* against *S. binderi* (green color).

The Anova univariate test in Table 3 showed an interaction between the types of macro-algae, the type of bacteria, and the concentration so that further tests were carried out to see the significance level in each combination.The combination of *H. triquerta, S. aureus*, a concentration of 20% (combination 114) and a combination of *P. australis, S. aureus*, a concentration of 15% (combination 313) gave significant results with a 95% confidence interval ($p \leq 0.05$) all types of combinations of macro-algae types, types of bacteria and the concentration of macro-algae extracts (Table 7 appendix).

According to Alghazeer*et al*. (2013),*Phaeophyceae* macro-algae extracts have effective antibacterial activity, both on gram-negative and gram-positive.Active substances such as flavonoids, triterpenoids, and steroids are also effective in inhibiting the growth of gram-negative and gram-positive bacteria (Alghazeer*et al*., 2017; Winarno*et al*., 2012).Another study conducted by Taleb-Contini*et al*. (2003), showed that flavonoids and steroids isolated from the Chromolaena species have antimicrobial activity against Gram-positive bacteria.

There are several mechanisms for the formation of inhibition zones in bacterial cells, including interfering with cell wall synthesis by blocking peptidoglycan synthesis and inhibit bacterial growth. Another mechanism is to inhibit bacterial protein synthesis through ribosomal binding of the 30S sub-unit, for example, doxycycline by blocking the attachment of tRNA so that amino acids cannot be added to form protein chains and ultimately protein synthesis is inhibited.Antibacterial mechanisms also occur in the presence of inhibition of metabolic pathways such as inhibition of folic acid synthesis by sulfonamides and trimethoprim (Cavalier *et al*., 2005).

The difference in polarity of the bacterial cell wall with the properties of the active compound also affects the formation of the inhibition zone (Mambang *et al*., 2014).The flavonoids in the macro-algae *Phaeophyceae* are polar so that it is easier to penetrate the polar peptidoglycan layer such as the peptidoglycan wall layer of gram-positive bacteria than the nonpolar gram-negative peptidoglycan lipid layer (Dewi, 2010).

Based on research conducted by Dzoyem *et al*. (2013) flavonoid compounds (*6,8-diprenyleriodictyol, isobavachalcone and 4-hydroxylonchocarpin*) are bactericidal compounds which cause cell membrane damage and cause inhibition of macromolecular synthesis. Besides that, flavonoid compounds as antibacterial agents significantly fight bacteria*S. aureus* by depolarizing the membrane and inhibiting protein, DNA, and RNA synthesis. Types of flavonoids derived from *chalcones,* namely *pyrazolicchalcones* and *alicchalcones*, have antibacterial activity against *S. aureus* but not *E. coli* (Doan and Tran,

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2011). This is in line with the research conducted, because of the presence of flavonoids in the three types of macro-algae can inhibit *S. aureus*bacteria but not *E. coli*.

According to Epand*et al*. (2007), steroids have antibacterial properties, the correlation mechanism between membrane lipids and the sensitivity of steroid compounds causes liposome leakage and can cause death in bacteria. Steroids can interact with membrane phospholipids which are permeable to lipophilic compounds, causing decreased membrane integrity and altered cell membrane morphology and affects cell fragility and lysis (Ahmed, 2007).

Saponin compounds have antibacterial activity by reducing the surface tension of bacterial cell walls because of active-aglycone compounds which are membranolytic (Hoffmann, 2003). The decrease in the surface tension of the bacterial walls triggers saponins to form complex bonds with sterols and causes the formation of single ion channels. Then the presence of a single ion channel causes instability of the cell membrane that it inhibits the activity of enzymes as anion transport role effect to the inhibitedbacterial growth (Zahro and Agustini, 2013)

## C. Antioxidant activity of three types of macroalgae *Phaeophyceae*

The data on the percent as the antioxidant activityresults of three types of *Phaeophycea* macroalgae following (Table 2 attachment) and the average percentage of inhibition was shown in Table 7.

Table 7. Mean percentage of inhibition of antioxidant activity

| Percentage of Antioxidant Inhibition (%) | | | |
|---|---|---|---|
| Macroalgae extract concentration (ppm) | *H. triquerta* | *P. australis* | *S. binderi* |
| 700 | 5,7055 | 12,023 | 15,8115 |
| 500 | 4,2045 | 13,05 | 14,672 |
| 300 | 6,6065 | 6,745 | 13,105 |
| 200 | 5,5555 | 6,598 | 13,533 |
| 100 | 1,652 | 6,0115 | 8,262 |

Table 7 showed that the three types of macroalgae*Phaeophycea* had antioxidant activity. *S. binder* macroalgae had the strongest antioxidant activity with a DPPH free radical inhibition value of 15% at a concentration of 700 ppm followed by *P. australis*(12%) and *H. triquerta* ( 6%) at a concentration of 300 ppm.The vitamin C as a positive control for

antioxidant activity test had a 94% resistance at a concentration of 20 ppm (table 2). This result was in line with Chia *et al*. (2015) that the IC50 value in the methanol extract*Turbinaria ornate* (brown algae) was lower than the control value. positive*quercetin*.The difference in inhibition rate in macroalgaewas shown in figure 2.

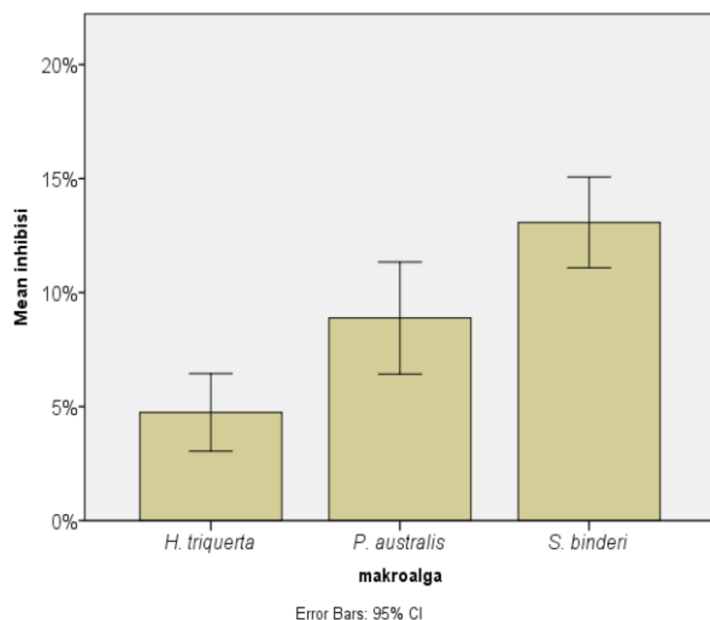


Figure 2. Bar diagram of macroalgae mean on antioxidant activity

The results of the linear regression test for the concentration of macro-algae extracts on the inhibition percentage were shown in table 8.

Table 8.The results of the regression equation and the IC50 value and the significance value of three types of macro-algae

| Types of macroalgae | Y = a+bx | $IC_{50}$ | *p-value* |
|---|---|---|---|
| *H. triquerta* | Y = 3,482 + 0,004x | 13.290 ppm | 0,34 |
| *P. australis* | Y = 4,399 + 0,012x | 3.800 ppm | 0,03 |
| *S. binderi* | Y = 9,461 + 0,010x | 4.053 ppm | 0,04 |

Table 8 showed that the extract of three types of macroalgae did not get $IC_{50}$results at a concentration of 100 ppm, 200 ppm, 300 ppm, 500 ppm, and 700 ppm, which meant that the resulting antioxidant activity was still weak. The results also showed that the p-value of

the macroalgae*H. triquerta*was greater than 0.05, so the regression equation coefficient was not linear or there was no influence between concentration and inhibition percentage.The p-value of *P. australis* and *S. binderi* at a confidence interval of 95% was less than the value of 0.05showed thatusinglinear regression equation coefficient, there was a significant effect between concentration and the percentage of inhibition.

*Phaeophyceae*are known to consist of antioxidant compounds (Demirel*et al*., 2009; Vinayak*et al*., 2011).*Phaeophyceae* have better antioxidant activity than *Rhodophyceae* and *Chlorophyceae* (Indu and Seenivasan, 2013). Several studies have shown that *Phaeophyta* or brown algae have antioxidant activity (Zubia*et al*., 2009).Previous research conducted by Nursid*et al*. (2013) showed that the macro-algae type *H. triquerta* had an inhibition value of 45% at a concentration of 50 ppm and *S. binderi* (below 10%). The difference in the activity of the resulting antioxidant compounds can occur due to environmental factors such as macro-algae, sea influx, temperature tides, and sunlight (Kuncoro, 2004).Temperatures close to $37^0$C make the formation of more reactive free radicals such as chlorine atoms and hydroxyl radicals (Pryor, 2012). Sunlight affects the photosynthetic process, the result of photosynthesis is oxygen which plays a role in the formation of ROS (*Reactive Oxygen Species*) (Asada, 2006).With environmental factors, the possibility of forming active compounds as a response mechanism to free radicals also varies in each macro-algae.

Free radicals are molecules that lose electrons so that these molecules become unstable and always try to take electrons from other molecules or cells (Subandi, 2010). This study used the DPPH method test. DPPH compounds are classified as stable free radicals and experience good delocalization from the reserve of electron molecules as a whole, therefore the molecules do not undergo dimerization.The principle of the test is based on the capture of hydrogen from antioxidants by DPPH free radicals. Antioxidants will donate protons or hydrogen to DPPH which in turn will break down free radical compounds to form non-radical compounds (Kedare and Singh, 2011).

Phenolic compounds are known to play a role in free radical scavenging (Chigayo*et al*., 2016). One of the phenolic compounds that have the potential as antioxidants in macro-algae is flavonoids. Flavonoids are polyphenols, therefore their antioxidant activity is based on the reactivity of hydroxyl substitution in the general reaction of hydrogen atoms (Denisov and Afanas'ev, 2005).

Flavonoids scavenge free radicals by donating a hydrogen atom from the hydroxyl group thereby catching free radicals.This reaction provides a flavonoid radical oxidizer so that the molecule becomes stable, then flavonoid radical oxidation changes in the resonance structure by distributing unpaired electrons in the aromatic nucleus (Amic*et al*., 2003). According to Doan and Tran (2011), pyrazolicchalcone which is a derivative of chalcones, has strong antioxidant activity with the DPPH test.

Other compounds that allow for antioxidant activity are saponins (Ashraf*et al*., 2013). According to Hemalatha and Hari (2013) saponin compounds from Tribulusterrestris extract

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have a strong antioxidant activity, namely 90.30% inhibition at a concentration of 1000 ppm and an IC50 value of 250 ppm.

## Conclusions and Recommendations

### A. Conclusion

From the results and discussion, it can be concluded:

1. *H. triquerta* and *P. australism*hadphytochemicals, flavonoids, and steroids compound while phytochemicals' content of *S. binderi* were flavonoids, steroids, and saponins.
2. The three types of *Phaeophycea* macro-algae had the inhibitory power against *S. aureus*in contrast with *E. coli* and *P. australism* had the greatest inhibitory power.
3. The higher the concentration of *Phaeophyceae* macro-algae extract tested, the greater the inhibition zone produced in *S. aureus* bacteria. The highest concentration in providing resistance for this study was at a concentration of 20%.
4. The three types of macro-algae *Phaeophyceae* tested have antioxidant activity. The best antioxidant activity for this study was *S. binderi*, followed by *P. australis* and *H. triquerta*.

### B. Suggestions

Suggestions that can be submitted from this research are as follows:

1. For further research, it is necessary to test the phytochemical content with a different type of solvent compared to the macro-algae simplicia tested.
2. Further research is suggested to compare several extract solvents in antibacterial testing such as ethanol, acetic acid, and chloroform.
3. The concentration of macro-algae extracts should be increased in observing the antioxidant activity to obtain the $IC_{50}$ value.

## Acknowledgments

We would like to thank LPPM UniversitasNasional for such support and the Laboratory of Botany Faculty of Biology UniversitasNasional for the facilities that have been provided, so that research can be carried out well and smoothly.

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