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LAntioxidant activity test of ethanol extract of red algae *(Kappaphycus alvarezii)* green variety from north coasts of Jepara district with DPPH method

Arifah Irna Nur Widyastuti[1], Wulandari[1], Ahmad Fuad Masduqi[1]
[1]Department of Pharmacy, Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang, Indonesia

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ABSTRACT

Free radicals are unstable and highly reactive molecules that can cause cells damage. This cell damage can be inhibited by antioxidant compounds. One of the red algae, Kappaphycus alvarezii, is thought to contain phenolics and flavonoids compounds that can b used US antioxidants compounds. this study aims to determine the ethanol extract of red algae *(Kappaphycus alvarezii)* green variety has antioxidants activity using the DPPH method. this study also aims to determine the IC 50 value of the ethanolic extract of red algae *(Kappaphycus alvarezii)* green variety. The antioxidant activity test of red algae ethanol extract was carried out using the DPPH method. The results showed that the ethanolic extract of red algae *(Kappaphycus alvarezii)* green variety had antioxidant activity and the IC 50 value of the ethanolic extract of red algae was 242.28 ppm with a very weakcategory.

1. Introduction

Free radicals are molecules that are unstable and highly reactive because it has no unpaired electrons in its outermost layer so it will attempted take electron from cell body which could causing the damage cell. Cell damage due to the presence of free radicals will cause premature aging and various diseases including cancer, cataracts, liver, coronary heart disease, and diabetes [1]. Cell damage due to free radicals can inhibited by compound antioxidants. Antioxidant is compound which could trigger free radical responses in the body so that body cells can be spared from consequential damage radical reaction free [1].

[1] ***Corresponding Author:***
Wulandari,
Department of Pharmacy,
Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang,
168 University Road, Miscuing Township, Chiayi County 62102, Taiwan, ROC.
Email: wulandari@stifar.ac.id
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Algae is one of the most abundant marine organisms and as wrong one riches natural sea which worth economical [2]. Algae in Indonesia can be used as antioxidants, wrong only one that is Kappaphycus alvarezii, which including type algae red [3]. Kappaphycus alvarezii has a smooth surface with a tubular thallus like a rod that has a variety of colors namely dark brown, greenish brown, yellowish brown, to reddish purple. Kappaphycus alvarezii generally live in daily temperatures with a small range with current and rate salinity which stable. Kappaphycus alvarezii grouped into two varieties, namely the green variety which has more growth both in the dry season and the brown varieties that have the same growth more good on rainy season [4].

According to [5] extract ethanol algae red which extracted use method maceration contain compound phenol, flavonoids, triterpenoids. Flavonoids could utilized as antioxidants with mechanism work donate ion hydrogen so that could neutralize effect toxic from radical free [6]. Compound phenolic could function as antioxidants through ability group phenol for tie radical free with give atom hydrogen through process transfer electron so that phenol changed Becomes radical phenolic (Janeiro, P and Brett, AM, 2004). Work mechanism triterpenoids as an antioxidant that is with method catch reactive species as superoxide, Fe^{2+} , and Cu^{2+} [7].

Antioxidant activity testing can be carried out using the DPPH method (1- 1-difyl-2-picrylhidrazyl), FRAP (Feric Reducing Antioxidants Power), TRAP (Radical Trapping Antioxidants Parameters), and thiocyanate. Method DPPH own excess that is method analysis which fast, simple, easy, and sensitive to small sample concentration. Parameters used to indicate activity antioxidants is Inhibition Concentration (IC_{50}) that is concentration something antioxidant substance that can cause 50% of DPPH to lose its radical character. Substances that have high antioxidant activity will have a low IC_{50} [8]. Quercetin could used as comparison testing activity antioxidants because quercetin is wrong one flavonols from group compound flavonoids which have effect protection membrane cell for inhibit stress oxidative [9].

Based on background behind the on conducted test activity antioxidant ethanol extract of red algae *(Kappaphycus alvarezii)* green variety from North Beach Kec. Jepara with the DPPH method and knowing the value of IC 50 on extract ethanol red algae *(Kappaphycus alvarezii)*.

2. Method

object study this is activity antioxidants in a manner in-vitro extract ethanol red algae *(Kappaphycus alvarezii)* green varieties from North Coast District. Jepara using the DPPH method and the IC_{50} value of the algae ethanol extract red *(Kappaphycus alvarezii)* green variety from North Beach district Jepara.

The tools used in this study were maceration vessels, measuring cups, glass beaker , glass funnel, porcelain cup, rotary evaporator , water bath , vial, tube reaction, rack tube, pipette drops, pipette volume, pipette measuring, pumpkin measure, balance analytic, aluminum foil, spectrophotometer UV-Vis.

Ingredients which used is powder dry extract ethanol algae red *(Kappaphycus alvarezii)* varieties green, ethanol 70%, sulfanilic Acid, $NaNO_2$, acetic anhydrous acid, concentrated

sulfuric acid, 5% $FeCl_3$, concentrated HCl, magnesium powder, amyl alcohol , methanol pa, DPPH, and quercetin.

Extraction conducted with 200 grams powder dry algae red entered into the maceration vessel, 70% ethanol solvent is added by comparison 1:7,5. The powder is soaked for 5x24 hours with solvent replacement every 24 hours while occasionally stirred. Residue separated and filtrate evaporated use rotary evaporators on temperature 60^0 C until formed extract thick. Extract which obtained then concentrated on a water bath at a temperature of 60^0 C until the solvent solution is lost or the amount reduce [10].

Phytochemical screening was carried out by testing the color of phenolic compounds and flavonoids. Test compound phenolic conducted with a number extract added $FeCl_3$ 5%, a positive result is indicated by the formation of a green color, blue, or black [11]. Flavonoid test is done with a number extract added a teaspoon of magnesium powder, 10 drops of concentrated HCl, and 1 ml of amyl alcohol, a positive result is indicated by the formation of a red-orange color to purple red on the amyl alcohol layer [12].

Antioxidant activity testing was carried out using the method DPPH with standard reference quercetin. DPPH was prepared with a concentration of 0.4 mM with method 7.8864 mg DPPH weighed and dissolved in solvent methanol pa as much as 50 ml and covered with aluminum foil. A blank solution is made by means of 1.0 ml of methanol pa plus 1.0 ml of 0.4 mM DPPH, and added with methanol pa up to 5 ml. The maximum wavelength of DPPH is 0.4 mM determined with solution blank entered in cuvette then be measured absorbance using a Visible spectrophotometer at a wavelength of 500- 600nm.

Making solution raw quercetin: raw quercetin 1000 ppm made with 50 mg quercetin weighed and dissolved in methanol pa to 50 ml, homogenized, replicated three times. A standard series of quercetin was prepared with way 5 ml of 1000 ppm solution is put into a 100 ml measuring flask, added with methanol pa until sign limit obtained solution 50 ppm, then 4 ml of 50 ppm solution is put into a measuring flask, added with methanol pa up to 5 ml 40 ppm solution was obtained. 4 ml of 40 ppm solution is put into a measuring flask, added with methanol pa up to 5 ml obtained a solution of 32 ppm, 4 ml of a solution of 32 ppm put into a measuring flask, added with methanol pa until 5 ml is obtained 25.6 ppm solution and 4 ml of 25.6 ppm solution put into a measuring flask, added with methanol pa up to 5 ml obtained 20 ppm solution.

Determination of operating time is determined by means of 1 ml of middle standard solution quercetin was put into a measuring flask, added 1 ml of 0.4 mM DPPH, added with methanol pa up to 5 ml, then the absorbance was measured at length wave max with range time each one minute.

Testing the standard antioxidant activity of quercetin was carried out in the following way ml from respectively-respectively concentration row raw entered to pumpkin measure, 1 ml of 0.4 mM DPPH was added, and added with methanol pa to 5 ml, then allowed to stand according to the operating time . The absorbance was measured at length wave maximum and done which step same on every replication.

Preparation of sample solutions . Sample solution with a concentration of 3,000 ppm prepared by adding 150 mg of ethanol extract of red algae to a measuring flask, dissolved in methanol pa and added until 50 ml, then homogenized, closed aluminum foil, and

conducted replication as much three time. 5 ml solution 3.00 ppm put into a measuring flask, added with methanol pa up to 50 ml obtained solution 300 ppm, 4 ml solution 300 ppm entered to pumpkin measure, added with methanol pa until 5 ml obtained solution 250 ppm, 4 ml solution 250 ppm put into a measuring flask, added with methanol pa until 5 ml is obtained 200 ppm solution, 4 ml of 200 ppm solution put into a measuring flask, added with methanol pa to 5 ml obtained 150 ppm solution. 4 ml of 150 ppm solution put into a measuring flask, added with methanol pa until 5 ml is obtained solution 120 ppm.

Testing the antioxidant activity of the samples was carried out by means of 1 ml of 1 ml of 0.4 mM DPPH was added to each solution concentration with methanol pa until 5 ml, homogenized, hushed up corresponding operating time , and absorbance was measured on maximum wavelength.

Data Analysis: Absorbance obtained from measurements of ethanol extract red algae, 0.4 mM DPPH solution, and reference standard quercetin were used to calculate the percentage of antioxidant activity expressed in percent damping DPPH. Percent damping DPPH counted use formula as following:

$$\% \text{ damping} = \frac{\text{absorbance blank} - \text{absorbance sample}}{\text{absorbance blank}} \times 100\%$$

Then counted score IC_{50} with use equality regression linear Among concentration actually with percent damping. Percent damping DPPH and IC value 50 counted on each replication.

Category IC_{50} could grouped as following: IC 50 < 50 ppm including very strong, IC_{50} between 51 – 100 ppm including strong, IC_{50} between 101 – 150 ppm including currently, IC_{50} Among 151 – 200 ppm including weak, as well as IC_{50} > 200 ppm including very weak [13].

3. Results and Discussion

The ethanol extract of red algae obtained from remaceration was 11.0 grams with a yield of 0.055%. Red algae ethanol extract is already ethanol free with not forming color raspberry red and no there is banana smell.

Results screening phytochemicals on extract ethanol algae red show content compound Flavonoids are presented on table 1.

Table 1. Results screening phytochemicals extract ethanol algae red

Test	Treatment	Library Results	Results Test
Phenolic	Extract + $FeCl_3$ 5%	Green, blue or black[11]	Negative control: (-) yellow colorExtract ethanol: (-) color chocolate
Flavonoids	Extract + Mg powder + 10 drops of HCl (p) + 1.0 ml	orange red topurple red on the coating *amyl alcohol* [12].	Negative control: (-) *amyl alcohol* color white Extract ethanol: (+) *amyl*

amyl alcohol		*alcohol* yellow

Screening results showed that the ethanol extract of red algae did not contain compound phenolic, because possibility in red algae extract ethanol there are no phenolic compounds. Flavonoid test showed that result extract ethanol positive red algae contain compound flavonoids.

Test activity antioxidants conducted with method DPPH and raw comparison quercetin. Stage beginning which conducted that is determination long the maximum DPPH wave is 0.4 mM which aims to determine the length wave on moment compound give absorbance which optimal. Results the measurement of the 0.4 mM DPPH wavelength is 515.8 nm. research that conducted by [14], DPPH provides uptake on long wave 490-540 nm with long wave maximum on 515-517 nm.

Stage next that is determination operating time use raw quercetin which aims to determine the time needed for the sample and DPPH to be able to react stably, when reacting samples with DPPH carried out in dark conditions and cold temperatures in order to capture free radicals Not affected by temperature and light from outside. Obtained the results of operating time raw quercetin is 28 minutes.

Test activity antioxidants raw quercetin conducted with measure absorbance respectively-respectively concentration raw then counted percent damping DPPH and a calibration curve is made between the actual standard series concentration and percent damping DPPH so that obtained equality regression linear raw quercetin. Percent damping DPPH is magnitude activity sample in reduce free radicals. The results obtained that the increasing concentration sample will cause more decrease in absorbance and percent attenuation DPPH is increasing, this shows the greater the concentration then will give damping radical free the more big. IC_{50} counted use equality regression linear and obtained score IC_{50} as on table 2.

Table 2. Results test activity antioxidant raw quercetin

raw Quercetin	IC_{50} (ppm)
Replication I	16.0518
Replication II	17.7026
Replication III	20.7540
Average	**18.1695 ± 2.38**

IC_{50} value is obtained quercetin with an average of 18.1695 ppm, this showed that quercetin has very strong antioxidant activity IC_{50} less than 50 ppm. The smaller the value of IC_{50} the better the activity the antioxidants. The next stage is testing the antioxidant activity of the sample extract ethanol algae red with measure absorbance respectively-respectively. The sample concentration was then calculated as a percentage of DPPH reduction and a curve was made calibration between actual sample concentration and percent DPPH attenuation so that a linear regression equation is obtained which is used to determine IC_{50}. IC_{50} results served on table 3.

Table 3. Test results Activity Antioxidant Extract Ethanol Algae Red

Ethanol Extract Algae Red	IC_{50} (ppm)
Replication I	229,23
Replication II	244.93
Replication III	252.68
Average	**242.28 ± 11.94**

Obtained results score IC_{50} extract ethanol algae red with average 242.28 ppm, Thing this show extract ethanol algae red own activity antioxidants very weak. According to [15], the ethanol extract of algae red which extracted use solvent ethanol 55% own activity antioxidants with score IC_{50} as big 1,179,245 ppm. Extract methanol Kappaphycus alvarezii has very weak antioxidant activity where value The IC_{50} obtained was 163,819.99 ppm. Obtained antioxidant activity very low presumably because the extracts tested were still in the form of a mixture of several compound and exists influence sample which used the dried use hot and light sun which could causing the damage content Kappaphycus alvarezii which function as antioxidants [16].

4. Conclusion

Ethanol extract of green variety red algae *(Kappaphycus alvarezii)* from Pantai North district Jepara own activity antioxidants. Score IC 50 extract ethanol red algae *(Kappaphycus alvarezii)* varieties green as big 242.28 ppm with category very weak. It is necessary to isolate the content of red algae flavonoid compounds *(Kappaphycus alvarezii)* and continued with antioxidant activity testing. Need conducted testing activity antioxidants with method besides DPPH. Need conducted rate determination of flavonoids red algae *(Kappaphycus alvarezii)*.

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