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Antioxidant activity of extracted green algae silpau (*Dyctyosphaeria versluysii*)

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Abstract. *Srimariana ES, Apituley DAN. 2019. Antioxidant activity of extracted green algae silpau* (Dyctyosphaeria versluysii). *Nusantara Bioscience 11: 153-156.* Silpau (*Dictyosphaeria versluysii*) is a green algae that is widely available in Southwest Moluccas regency, living on coral reefs and not classified as seasonal plants. Silpau has long been used by local people, generally in the form of processed vegetables or *colo-colo* (traditional food). Except for its nutritional value, comprehensive information about silpau still unknown. Therefore, this study was carried out to determine its potential as an antioxidant. The phytochemical content of silpau was analyzed according to the standard method. The antioxidant activity of green algae silpau extract was carried out by reducing DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals. Phytochemical test of silpau revealed that silpau contain terpenoid compounds. The result of the study showed that the IC_{50} value of methanol extract of silpau was 547.97 ppm, indicated that silpau methanol extract categorized as a weak antioxidant.

Keywords: Antioxidant, *Dictyosphaeria versluysii,* DPPH, IC_{50}, silpau

INTRODUCTION

Marine macroalgae are rich in bioactive compounds that have the potential to be used as functional ingredients in human and animal health (Gupta and Ghannam 2011), as a source of alginate, carrageenan, or agar in various industries, such as the food, pharmaceutical and cosmetics industries and also has the potential as a source of natural antioxidants (Yuan et al. 2005). Antioxidants play an important role in inhibiting and scavenging radicals to protect humans against infections and degenerative diseases. Several marine algae have been reported to possess antioxidant properties (Athukorala et al. 2006). Some previous studies show that some macroalgae have the potential as antioxidants such as *Padina* sp. and others (Setha et al. 2013), *Caulerpa racemosa* var. macrophysa (Yangthong et al. 2009), *Caulerpa lentillifera* (Santoso et al. 2010), *Padina australis* (Santoso et al. 2013), *Martensia fragilis* (Takamatsu et al. 2003), *Ecklonia cava* (Heo et al. 2005), *Eisenia bicyclis* and *Ecklonia kurome* (Shibata et al. 2002), *Ulva rigida* (Mezghani et al. 2016), *Turbinaria ornata* (Deepak et al. 2017). .

Antioxidant activities are attributed to various reactions and mechanisms: prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity and radical scavenging (Huang and Wang 2004). Nowadays, there is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants, for example, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) that are commonly used in lipid-containing food (Li et al. 2007). Natural antioxidants such as α-tocopherol, phenols and β-carotene found in higher plants are being used in the food industry to inhibit lipid peroxidation, and they can protect the human body from free radicals and retard the progress of many chronic diseases (Qi et al. 2005). Antioxidants help to prevent the free radical damage associated with the aging process.

Antioxidant activity can be tested using the DPPH method. This method is simple, easy, fast, sensitive and only requires a small sample. 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a free radical compound that is stable at room temperature and can be used to evaluate the antioxidant activity of several extracts of natural ingredients. Molyneux (2004) stated that DPPH could react with hydrogen atoms derived from an antioxidant to form a diphenyl-picrylhydrazine compound which is non-radical and turn pale yellow.

In Maluku waters, several marine macroalgae species have important economic values, such as *Eucheuma* and *Hypnea* as carrageenan producers, *Gracilaria* and *Gelidium* as agar-agar producers or agarophyte groups, *Sargassum* as alginate producers, and *Porphyra* is found only in the East season around April-August on the South coast of Ambon Island, usually processed into Nori. Microalgae that widely available in all districts of Southwest Maluku is silpau (*Dictyosphaeria versluysii*). Silpau is a green alga commonly found attaching to coral reefs and living in colonies. It is not classified as seasonal plants so that it can be consumed every day. Local communities often use silpau as a vegetable and process it into vegetable called *gudangan* or *colo-colo* (Moluccas traditional food). However, bioactivity evaluation of silpau from Southwest

Moluccas especially as an antioxidant has not been done yet. Therefore, this research was carried out to analyze the chemical compounds and to evaluate the antioxidant activity of silpau extract.

MATERIALS AND METHODS

Study area

Silpau (*Dictyosphaeria versluysii)* were collected from Liti beach, Oirata Village, Kisar Island, Southwest Moluccas district. Kisar Island is one of Indonesia's outer islands located in the Wetar Strait and Timor Leste with coordinates of 8 ° 6 ′ 10″S and 127 ° 8 ′ 36 ″ E (Figure 1).

Procedures

Material

Fresh alga silpau (*Dictyosphaeria versluysii)* collected from Liti beach were put in plastic bags containing seawater to prevent evaporation. In the laboratory, all of the samples were washed with seawater to clean samples from epiphyte, salt, sand, small stones, and other rubbish, then followed by washing under tap water.

Preparation of sample

After draining the existing water for one hour, fresh algae silpau was dried in an oven at 50°C. The dried samples were cut into small pieces (1-2 cm) then ground with an electric mixer. The powder was stored in the refrigerator for further use.

Preparation of silpau extracts

Powder of dried silpau (25 g) was macerated with 250 mL methanol for 24 hours twice. The filtrate was filtered through filter paper Whatman no 41 and placed in a glass container. Then, the filtrate was evaporated at 40°C using a rotary evaporator to obtain the concentrated extract. The extract was subjected to phytochemical screening for terpenoids, tannin, quinone, and anthocyanin test as described by Harborne (1987).

Antioxidant activity (DPPH free radical-scavenging activity)

Antioxidant activity assay was measured through the ability of silpau extracts on reducing the stable free radical DPPH according to the method described by Aranda et al. (2009) with some modifications. DPPH solution in methanol (0,1 M) was prepared. The extract was prepared at the concentrations of 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400ppm, and 500 ppm. Two ml of DPPH solution were added into 0.1 ml of seaweed extract. The mixture was homogenized and incubated at room temperature for 30 minutes. The absorbance of the mixture was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. All reactions were carried out in triplicates, and the degree of decolorization indicated the free radical scavenging activities of silpau extract. The capability of silpau extracts to scavenge the DPPH free radical was calculated by using the following equation:

Radical scavenging activity (%) = 100 x (1-the absorbance of samples / the absorbance of control)

Data analysis

Correlation between sample concentration and the percentage of inhibition of free radical was analyzed using linear regression with the line equation Y = a + bx y is percentage of inhibition, x is concentration of extract silpau, a is intercept and b is slope (Steel and Torie 1989). The activity was expressed as IC_{50} (the inhibition concentration of silpau extract that scavenges 50% of DPPH free radicals). analyzed using linear regression with the line equation.

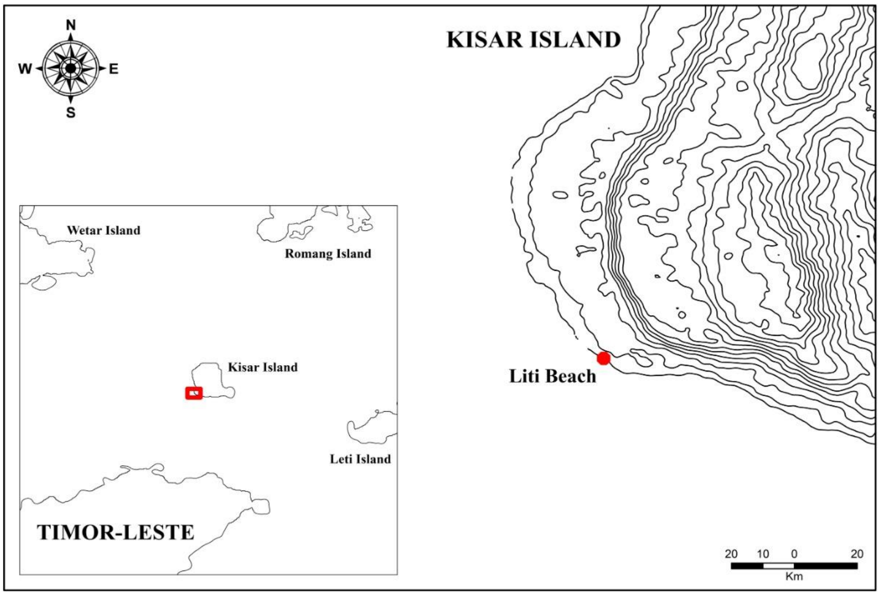

Figure 1. Map of Kisar Island in Southwest Molluccas and sampling location (red circle)

RESULTS AND DISCUSSION

Phytochemical screening of silpau methanol extract

The results of the phytochemical screening of silpau methanol extract were presented in Table 1. It showed that silpau methanol extract contained terpenoids (Table 1).

Result of DPPH radical scavenging activity of extracts silpau

Free radical scavenging activity of silpau methanol extract can be observed through color changes from purple to turn yellow and is presented in Table 2. The highest inhibition percentage (45.99%) was obtained from the highest concentration of silpau extract (500 ppm) while the lowest inhibition percentage (5.87%) obtained from the lowest concentration of silpau extract (50 ppm) (Table 2).

Linear regression analysis in order to obtained IC_{50} value based on the data in Table 1, and the equation was: Y = 4.299 + 0.0834x (R^2 = 0.98). The determination of IC_{50} by entering the response value (Y=50) to the regression equation. The value of x will be obtained from the regression equation is the IC_{50} value. Based on the interpolation of the linear regression equation, the IC_{50} value of silpau methanol extract was 547.97 mg/L (Figure 2).

Table 1. Phytochemical screening of silpau methanol extract

Phytochemical analysis	Results
Terpenoid	+
Tanin	-
Quinon	-
Antosianin	-

Note: +: Present;- : Absent

Table 2. DPPH free radical scavenging activity of silpau methanol extracts

Replication	% Inhibition Concentrations (ppm)					
	50	100	200	300	400	500
1	7.10	12.47	21.55	27.33	37.24	44.59
2	6.05	16.80	21.26	31.53	37.90	48.25
3	4.46	16.24	22.77	28.34	36.31	45.14
Total	17.61	45.51	65.58	87.20	111.45	137.99
Average	5.87	15.17	21.86	29.07	37.15	45.99

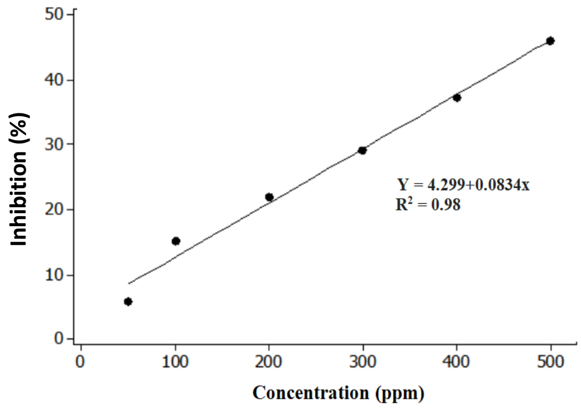

Figure 2. Antioxidant Activity of silpau methanol extracts (*Dictyosphaeria versluysii*)

Discussion

Marine algae are potential sources of secondary metabolites that might be used as a lead compound in the development of new pharmaceutical agents. Secondary metabolites, including terpenes, play important ecological roles in marine organisms, especially for sessile and soft-bodied organisms, they face intense competition for space, reproduction, unclean surface maintenance and predatory prevention (Fusetani 2004). Therefore, marine organisms have developed bioactive secondary metabolites as a potential defensive means against competitors and/or predators. The compound of secondary metabolites of marine algae may contain alkaloids, phenols, flavonoids, saponins, steroids, terpenoids, and active metabolites that have great medicinal value (Abad et al. 2011). Recently, marine algae have been widely used in the pharmaceutical industries (Yuan et al. 2005). Much attention has been focused on the phytoconstituents of marine algal populations in different regions (Khairy and Sheik 2015).

Research on marine algae as sources of bioactive agents for the needs of pharmaceutical industries still little has been done in the eastern part of Indonesia. Most of them are utilized as sources of traditional food material. Such as green algae silpau has high nutritional value. The proximate analysis of fresh silpau conducted by Lewerissa and Srimariana (2012) showed the following results: water 89.17%, ash 2.35%, protein 0.8%, fat 0.52%, 7.09% carbohydrates, iodine 8.79 mg/L, crude fiber 17.23%, Cu 0.048 mg/L, Zn 0.138 mg/L, energy 36.52 kcal/g. A previous study by Trono (1997) showed that silpau contained several minerals, namely Cd, Cu, Hg, Ni, I, Pb and Zn.

The results of phytochemical screening of methanol extract of silpau presented in Table 1. The results showed that terpenoid was found in the silpau extracts. Terpenoids are widely distributed in nature and found abundantly in higher plants. In some marine organisms are also found terpenoids, such as marine sponges and marine algae (Abad et al. 2011). They are known to have special functions associated with other organisms related to reproduction, defense system or symbiosis (Gershenzon and Dudareva 2007; Reis et al. 2013). Terpenoids are also widely used in chemical industries, including many pharmaceuticals, flavors, fragrances, pesticides and disinfectants, and as large-volume feedstocks for chemical industries (Bohlmann and Keeling 2008).

Marine terpenoids from marine sponges show prominent bioactivities, including antimicrobial and antiviral properties (Ebada et al. 2010). These compounds have also been isolated from marine algae, such as red alga *Peyssonnelia* sp. Peyssonoic acid A and B, sesquiterpene hydroquinones, that isolated from the red alga *Peyssonnelia* sp., inhibited growth of *Pseudoalteromonas bacteriolytic*, a bacterial pathogen of marine algae, and *Lindra thalassiae*, a fungal pathogen of marine algae. (Lane et al. 2010). Sesquiterpenoid hydroquinones such as tiomanene and acetyl majapolene A and B isolated from Malaysian Laurencia sp, showed antimicrobial activities (Vairappan et al. 2008).

Antiviral diterpenes have also been isolated from marine algae such as diterpenes 8,10,18trihydroxy-2,6-dolabelladiene and (6R)-6-hydroxydichotoma-4,14-diene-1,17-dial which is isolated from Brazilian brown algae *Dictyota pfaffi* and *Dictyota menstrualis* (Abrantes et al. 2010). Besides that Cirne-Santos et al. 2008 stated that these brown algae also produced dolabellane diterpene dolabelladienetriol as a typical non-competitive inhibitor of HIV RT enzyme. Kamei et al. 2009 suggest that the diterpene sargafuran from the methanolic extract of the marine brown alga *Sargassum macrocarpum*, might be useful as a lead compound to develop new types of anti-*Propionibacterium acnes* substances and new skincare cosmetics to prevent or improve acne.

Antioxidant activity was carried out by the DPPH method (Aranda et al. 2009). The principle of the DPPH method is to capture hydrogen donated by antioxidant compounds from the extracts; form reduced DPPH-H so that it becomes a stable non-radical compound (Chang et al. 2007; Molyneux 2004) or reduce free radicals (Chandini et al. 2008). The reaction is characterized by the change of a purple to pale yellow color detected at a wavelength of 517 nm.

The results of the study in Table 2 showed that the increase of extract concentration also increases the activity of free radical inhibition, which is similar with the results of a study by Kojong et al. (2010); Tanti et al. (2010); Setha et al. (2013); Khairy and Sheik (2015); and Deepak et al. (2017).

The parameters commonly used to interpret the results from DPPH are IC_{50}. The IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity. Molyneux (2004) categorized antioxidant activity into several categories, which are very strong (IC_{50} values are less than 0.05 mg/ ml (<50 ppm), strong (IC_{50} values in the range of 0.005-0.1 mg/ml (50-100 ppm), medium (IC_{50} values in the range 0.1-0.15 mg/ml (100-150 ppm), weak (IC_{50} values between 0.15-0.20 mg/ml (150-200 ppm) (Molyneux 2004). The smaller IC_{50} meaning the higher the activity of antioxidant.

Based on the IC_{50} value of silpau methanol extract (547,97 mg/L), so that it can be concluded that the methanol extract of silpau categorized as a very weak antioxidant. It is probably due to the methanol extract of silpau, was not a pure compound, but still contains other compounds that probably may not have antioxidant activity. In the next study, it needs to determine antioxidant activities using different solvents, and the other bioactive compounds of silpau. It is also needed to study the antimicrobial activity and isolate antioxidant compounds from silpau extract

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