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Antioxidant Activity and Isolation of Bioactive Compounds from Seaweed of Silpau (Dyctyosphaeria sp)

Voulda D. Loupatty[1], Edward J.Dompeipen[2], Mozes S.Y. Radiena[2], Joice P.M Kolanus[2]

[1]Deep Sea Research Center, National Research and Innovation Agency, Jl. Y Syaranamual, Poka, Kec. Tlk. Ambon, Kota Ambon, Maluku

[2]Center for Standardization and Industrial Services in Ambon, Ambon, Indonesia, Jl. Kebun Cengkeh, (Batu Merah Atas) 97128, Ambon-Maluku, Indonesia

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Abstract

Seaweed is the key to developing habitat in deep sea, coastal, and estuarine environments. Seaweed, humans, and animals have been interrelated since the beginning, especially as a food source of fiber, protein, vitamins, antioxidants, and bioactive compounds. Natural antioxidants are seen as a superior and safe method of fighting free radicals. Sources of antioxidants usually come from land plants and rarely from seaweed. Silpau is a seaweed and a local food ingredient in Maluku Province, Indonesia, and has the potential as an antioxidant. Antioxidant and bioactive compounds are needed by humans and animals as antibodies to fight various negative influences from the environment. This research aimed to isolate and characterize active antioxidant compounds and the toxicity of silpau extracts. The extraction of the active compound begins with a single maceration method for 3 x 24 hours with methanol solvent, processing with the partition extraction method for subfraction separation using column chromatography with the silica gel stationary phase and the mobile phase solvent n-hexane: ethyl acetate with a ratio (50: 1 ~ 1: 1) gradient. The sample toxicity was tested using the Brine Shrimp Lethality Test method. The trial activity used the Free Radical Reduction method with 2,2-diphenyl-1-pikrilhidrazil as a reagent. This research succeeded in isolating one bioactive compound from the glare green macroalgae. Based on the phytochemical analysis and FT-IR spectrophotometer, sub-fraction IV was identified as a triterpenoid class of compounds that has an IC50 value of 126,582 µg / ml and is considered an antioxidant.

Keywords: *Silpau, extraction, antioxidants, triterpenoids*

1. Introduction

Seaweed or macroalga are primary producers of the marine food chain. Seaweed plays an important role in the aquatic environment because it contributes to the formation of O2, as a shelter for various marine species, and provides food for various herbivores (Pfister et al.,2019; Poza et al., 2022). Seaweed sustains herbivorous animal communities and provides shelter from Carnivora predators. Seaweed is key in the complex trophic network of water (Butt et al., 2020; Randall et al., 2020). Seaweed or macroalga is one of the most significant marine resources. It usually relates to a group of eukaryotes, composed of a large, photosynthetic, and non-vascular marine organism (Fatin et al,2021; Princely and Dhanaraju, 2017). Seaweed is divided based on its nutrients, pigments, and chemical composition into Rhodophyta, Phaeophyta, and Chlorophyta.

Seaweed is a source of natural sea vegetable products. Seaweed can thrive fast in the ocean. Green seaweed especially Caulerpa sp also known as sea grapes can eaten raw as salad. They have bioactive compounds responsible for the antioxidant and antibacterial effects. (Wing-Fai Yap, et.al., 2019; de Gaillande, at.al., 2017; Naggapan, at.al., 2014). Idalia et.al., (2016), the antioxidants tested consisting of phenolic compounds, flavonoids, and pigments were higher in green alga (Ulva expansa, Codium

isabelae, Rhizoclonium riparium, and Caulerpa sertularioides). Significant efforts should be made to identify and isolate the bioactive compounds that are involved in the antioxidant, antimutagenic, and antiproliferative activities of these seaweed species, particularly in C.sertularioides which showed a high potential for further investigations to propose novel therapeutic biocompounds. Next, Mohamed et. Al.,(2020), shows the results of the present study conclude that macroalgae U. prolifera, collected from the Egyptian Red Sea, have potential bioactive substances with both antioxidant and antibacterial effects and could be used as healthy valuable edible seaweed .

At present, there is a high demand for seaweed as many quarters have begun consuming healthy and 'natural foodstuffs', mainly because seaweed is rich in minerals, vitamins, and proteins. Chlorophyta sp. are richer in nutritional contents than plants that live on land because they utilize only a small amount of energy to form circulatory systems, leaves, roots, stems, and reproductive organs. (Fatin et.al., 2021). There are several bioactive and nutritional compounds reported in the Chlorophyta sp., e.g., natural pigments (NPs), polyunsaturated fatty acids (PUFAs), lipids, proteins, and polysaccharides (Khalid, et.al., 2018)

Glare green macroalgae (Dictyosphaeria sp) is a green algae commonly found attached to coral reefs. Silpau is a green macroalgae that has a dense and hard texture. It has the potential as a natural antioxidant from Maluku waters. It based on previous research has potential as an antioxidant and anticancer. Silpau has been identified to produce terpenoid compounds and many of them are toxic and contain halogens, especially chlorine compounds. The compounds produced from Silpau also contain nitrogen elements in the form of amides or indoles which have antibacterial and antifungal activities (Atta-ur-Rahman and Choudhary, 2001).

Glare alive and attached to coral reefs to a depth of 76 m. Silpau has a thallus with a diameter of 5 cm which can hold high water, around a solid texture consisting of large, bubble-shaped cells that can be seen visually. Silpau has short, generally unbranched rhizoids. Silpau seaweed can be seen in the Figure 1. Silpau was carried out primary and secondary metabolic processes. The primary metabolites produced by macroalgae are generally hydrophilic polysaccharide compounds, called hydrocolloids. Hydrocolloid compounds are used as additives in the pharmaceutical industry with various functions. Secondary metabolites, which are bioactive substances, were developed through various studies to be used as alternative medicines.

Figure-1. Silpau, Green Macro Algae in Leti
Southwest Maluku

The application of extraction technology provides the possibility to isolate secondary metabolites from macroalgae. Pharmacological and microbiological examinations of macroalgae extracts and microbial isolates provide a clearer picture of the benefits of macroalgae. About 500 natural products or natural chemical compounds derived from macroalgae have been identified and the largest percentage of these products are active compounds which are secondary metabolites of various types of macroalgae.
Toxicity is the level of damage to a substance if exposed to organisms. Toxicity can refer to the effects on an entire organism, such as animals, bacteria, or plants, and the effects on an organism's substructure, such as cells (cytotoxicity) or body organs such as the liver (hepatotoxicity). Metaphorically, this word can be used to describe the toxic impact on a larger or more complex group, such as a family or society. The purpose of this study was to isolate and characterize active

antioxidant compounds and toxicities from silpau, green seaweed extract (*Dictyosphaeria* sp).

2. Material and Methods

The materials used in this study were silpau, green seaweed (*Dictyosphaeria* sp) and the chemicals used were methanol p.a, ethyl acetate p.a, n-hexane p.a. DPPH reagent (2,2-diphenyl-1-picrylhydrazyl), sterile distilled water, ascorbic acid, CDCl3, silica gel (70-230 mesh), 2% HCl solution, 1% NaOH solution, and materials used in the cytotoxicity test among others: Artemia salina shrimp eggs. The tools used are a set of glassware, a separating flask, an evaporator, thin layer chromatography, column chromatography, a stain viewer, an FTIR spectrophotometer, a UV-Visible spectrophotometer, and other laboratory equipment. The glare green macroalgae are taken from the coastal waters of Raitawun, Nuwewang village, Letti Island district when the tide is deep (10-15cm).

Extraction was carried out using a single maceration method (Houghton and Raman, 1998). Phytochemical screening was carried out to see the bioactive components in the crude extract of glare green algae (*Dictyosphaeria* sp) concerning Harborne, (2006). Isolation of Glare Green Macroalgae Active Compounds (*Dictyosphaeria* sp). The methanol extract of the glare green macroalgae sample was mixed with zeolite until homogeneous then separated the subfractions using column chromatography with the silica gel stationary phase and the mobile phase used was the n-hexane: ethyl acetate solvent with a gradient (50: 1 ~ 1: 1) ratio. Each fraction obtained was examined for stains by Thin Layer Chromatography (TLC). Fractions that had the same Retention Time (Rf) value were combined to obtain a simpler fraction.

Brine Shrimp Lethality Test (BSLT) Toxicity Test. following the Meyer method (Meyer, et al, 1982).

Antioxidant Activity Test following the Meyer method (Maesaroh et al, 2018).

Antioxidant activity test using free radical reduction method with DPPH reagent (2,2-diphenyl-1-picrylhydrazyl). The absorption was measured at a maximum absorption wavelength of 515 nm using a visible light spectrophotometer.

The percentage of inhibition is calculated by the formula:

Inhibition = (Blank absorbance-Sample absorbance) / (Blank absorbance) × 100%

The IC_{50} value is the concentration of antioxidants (µg / mL) which can inhibit 50% of free radicals. The IC50 value is obtained from the intersection of the line between 50% resistance and the concentration axis, then entered into the equation y = a + bx, where y = 50 and the x value indicates IC50. The extract was said to be active if the IC50 value was less than 100 µg / mL. A compound is said to be a very strong antioxidant if the IC50 value is less than 50, strong (50-100), moderate (100-100), and weak (151-200). The smaller the IC50 value the higher the antioxidant activity. (Badarinath, 2010).

Identification of Active Compounds Using FT-IR Spectrophotometer

Identification of active compounds includes analysis by chemical instrumentation using an FTIR (Fourier Transform Infra-Red) spectrophotometer.

3. Results and Discussion

Extraction of the Chemical Components of the Glare Green Macroalgae (*Dictyosphaeria* sp)

The extraction technique used in the separation of the chemical components of the glare green macroalgae (*Dictyosphaeri*a sp) was maceration. Maceration is the simplest method and is usually used as an initial step in the extraction of natural compounds. This maceration extraction is very beneficial in isolating natural compounds because it is very easy to do. Maceration is carried out by immersing plant samples at room temperature so that the breakdown of cell walls and membranes will occur due to the difference in pressure between inside and outside the cell. Furthermore, secondary metabolites in the cytoplasm of plant samples will be dissolved in organic solvents. Extraction of compounds using maceration techniques will result in perfectly extracted samples because the immersion time can be adjusted. The selection of a specific solvent for the maceration process will provide high effectiveness by paying attention to the solubility of natural compounds with these solvents. In general, methanol solvent is the solvent most widely used in the process of isolating organic compounds from natural materials, because it can dissolve all secondary metabolites (Sofia, 2006).

Extraction of glare components using polar solvents such as methanol. Each extraction step is expected to be able to extract compounds that have polarity following the polarity of the solvent, following the soluble rules. In the first stage, compounds that have high polarity will be extracted. The

next step is the process of partitioning the methanol extract using n-hexane, ethyl acetate, and water to separate each fraction in the methanol extract.
Extraction of a sample of 1 kg of dry glare green macroalgae resulted in a methanol extract that was brownish-yellow in color, solid weighing 380 g (38%). Then partition extraction was carried out on the methanol extract and the weight of the n-hexane fraction was 36.1 g (9.5%), the weight of the ethyl acetate extract was 68.4 g (18%), while the water extract weighed 15.8 g. (4.17%).

Fractionation of the Glare Green Macroalgae EA Fraction (*Dictyosphaeria* sp) Using Column Chromatography

Based on the results of the toxic activity test, the ethyl acetate fraction has a higher potency than the other two fractions, so the ethyl acetate fraction is then separated from its chemical components using column chromatography. The ethyl acetate fraction of the green seaweed sample was mixed with zeolite until it was homogeneous then separated the subfractions using column chromatography with the silica gel stationary phase and the mobile phase used was the n-hexane: ethyl acetate solvent with a ratio (50: 1 ~ 1: 1) gradient. Each fraction obtained was examined for stains by TLC.
Fractions having the same Rf are combined to obtain simpler fractions. The results of combining the fractions based on TLC analysis obtained five fractions, namely Fraction I weights 61.2 mg, Fraction II weights 46.0 mg, Fraction III weights 45.5 mg, Fraction IV weight of 53.7 mg and Fraction V weighs 25.8 mg.

Results of Phytochemical Screening of Green Macroalgae Methanol Extract (*Dictyosphaeria* sp)
Phytochemical screening of methanol extract of green seaweed (*Dectyosphaeria* sp) which contains terpenoids. Phytochemical tests carried out include compounds, among others; alkaloids, flavonoids, steroids, terpenoids, phenols, and saponins. The results of the phytochemical test of the extract of silpau g (*Dictyosphaeria* sp) are shown in Table1.
Table 1. The results of phytochemical screening of methanol extract of green seaweed (*Dictyoshaeria* sp)

Functional Group Compund	Results	Test Method
Alkaloid	+	Deggendorf
Alkaloid	+	Meyer
Flavonoid	+	Amil alcohol
Steroid	+	Lieberman-Burchard
Terpenoid	+	Lieberman-Burchard
Phenol	+	$FeCl_3$
Saponin	+	HCl

Screening of active compounds from Silpau green seaweed was carried out by conducting qualitative phytochemical tests. Phytochemical tests were carried out to determine the type of secondary metabolite compounds or groups of compounds contained in the extract. Identification of secondary metabolite content is an important first step in research to find new bioactive compounds from natural ingredients that can be precursors for the synthesis of new drugs or prototypes of certain active drugs (Harborne, 2006). The group of compounds in the extract can be determined by observing the color change and there is a precipitate after adding a specific reagent for qualitative tests (Sari, 2008).

Toxicity Test Results of Water, Ethyl acetate, and n-Hexane Fractions from Silpau Methanol Extract (*Dictyosphaeria* sp) with the Brine Shrimp Lethality Test (BSLT) Method (Meyer, 1982).
The initial toxicity test using Artemia salina test animals according to the BSLT method was carried out on each fraction of hexane, ethyl acetate, and water. The level of toxicity of the fraction against Artemia salina can be explained as follows, if the fraction is included in the non-toxic group, it is possible that the compounds in this fraction can be developed for wide use, for example as food, supplements, or cosmetic raw materials. If the compounds in the fraction have high toxicity, then the possibility of their use can be developed for medicinal raw materials. The category of toxicity values according to Meyer and colleagues is if the LC_{50} (µg / mL) value of the extract <1000 µg / mL is categorized as toxic and if the LC value is 50 (µg / mL) extract> 1000 µg / mL is categorized as non-

toxic. The results of the toxicity test of the water, ethyl acetate, and n-hexane fractions are shown in Table 2.

Table 2. Toxicity Test Results of Water, Ethyl Acetate, And N-Hexane Fractions from Green Macroalgae Methanol Extract (Dictyoshaeria versluysii) using the Brine Shrimp Lethality Test (BSLT) method

Extract methanol of Silpau after Extraction Partition in solvent:	LC $_{50}$ (µg/ml)
Water	2585,069
Ethyl acetate	126,582
n-Hexane	448,772

In Table 2, showing that the LC_{50} value of each extract after partition extraction, respectively, for the water fraction, ethyl acetate, and n-hexane fraction was 448.772 µg / ml, 126.582 µg / ml, and 2585.069 µg / ml. According to Meyer, extracts of natural ingredients that have an LC_{50} value> 1 000 g / ml are categorized as non-toxic. The LC_{50} value of the Silpau methanol extract water fraction is higher than 1000 g / ml so it is declared non-toxic and can be applied as a food ingredient.

The traditional use of glare as a food ingredient by cooking with water by the people of the Maluku Islands is declared safe from toxic compounds. The LC_{50} values of the ethyl acetate fraction and the n-hexane fraction were higher than the water fraction, this is because the active compounds of secondary metabolites from the methanol extract of Silpau are at a more semi-polar and non-polar polarity level. The polarity of a solvent is chemically determined by its dielectric constant, which can be used as a relative measure of polarity. Water solvent is a polar solvent that has a dielectric constant of 80.10 at 20°C while n-hexane is very non-polar with a value of 1.89 at 20°C and semi-polar ethyl acetate has a value of 6.00 at 20°C.

The presence of bioactive compounds in the n-hexane fraction and ethyl acetate fraction indicates that these compounds have relatively similar polarity to n-hexane and ethyl acetate solvents in accordance with the “like dissolve like” principle and also have toxicity. Ethyl acetate and n-hexane fractions from the methanol extract of Silpau can be directed to be used it for the pharmaceutical and cosmetic industries as pharmaceutical and cosmetic preparations because they have a relatively high toxicity value (LC_{50}). Further research is more directed at ethyl acetate extract because it has higher toxicity than other fractions. (Meyer, 1982: Frengki et al, 2014).

Antioxidant Activity of Silpau Methanol Extract with Free Radical Reduction Method Using DPPH (2,2- diphenyl-1- pikrilhidrazil) reagent (Maesaroh et al, 2018)

The antioxidant activity of a compound can be measured by its ability to reduce free radicals. The free radical that is commonly used as a model in measuring antioxidant reduction is DPPH (2,2-diphenyl-1-picrylhydrazyl) because it is fast, simple and easy to use. DPPH is a free radical that is stable in methanol. The principle of testing for antioxidant activity is the reaction of capturing hydrogen from antioxidants by DPPH free radicals (purple) converted to stable hydrazine (yellow) DPP then the intensity is measured at a wavelength of 515-520 nm (Marxen, et al., 2007). Mechanism of antioxidant reaction shown as Figure 2.

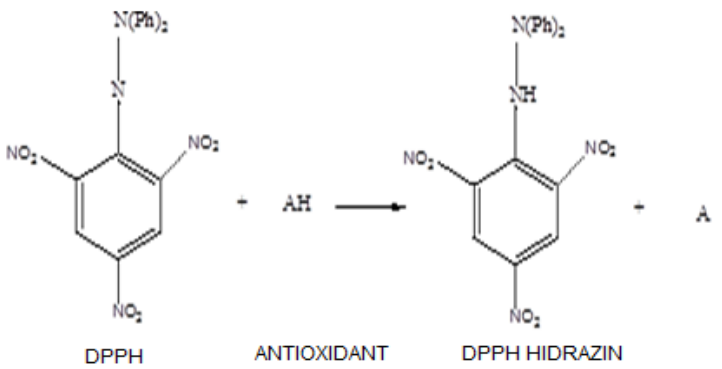

Figure-2. Mechanism of Antioxidant Reaction (Marxen et al, 2007)

Free radical scavenging activity of silpau methanol extract can be observed through color changes from purple to turn yellow and is presented in Table-3. 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-3). Linear regression analysis in order to obtained IC_{50} value based on the data in Table-3, and the equation was: Y = 4.299 + 0.0834x(R2= 0.98). The determination of IC_{50} byentering the response value(Y=50) to the regression equation. Value of xwill be obtainedfrom the regression equationisthe 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-3).

DPPH test for water fraction, ethyl acetate fraction and n-hexane silpau fraction aims to see the activity ability of the three fractions. Measurement of the DPPH test results using a spectrophotometer and obtained the value of the proportion (%) inhibition of each concentration (Inhibition Concentration / IC_{50}). The IC_{50} value of the ethyl acetate fraction (71.36 ppm) was higher than the ethyl acetate fraction (171.36 ppm) and the water fraction (918.70). The antioxidant activity of the three fractions did not exceed the activity of vitamin C as a positive control. The value of the antioxidant activity fraction of the partition extraction fraction of the methanol extract of green algae is presented in Table 3.

Table 3. Free Radical Inhibition Activity of the partition extraction fraction of the silpau methanol extract

Extract methanol of Silpau after Extraction Partition in solvent :	Linear regression equation	% Inhibition (ppm)
Water	Y= 0.0518X + 2.41	918,70
Ethyl acetate	Y= 0.5785X + 8.72	71,36
n-Hexane	Y= 0.2352X + 9.62	171,63
Asccorbat acid (positive control)	Y= 0.5681X + 47.62	4,192

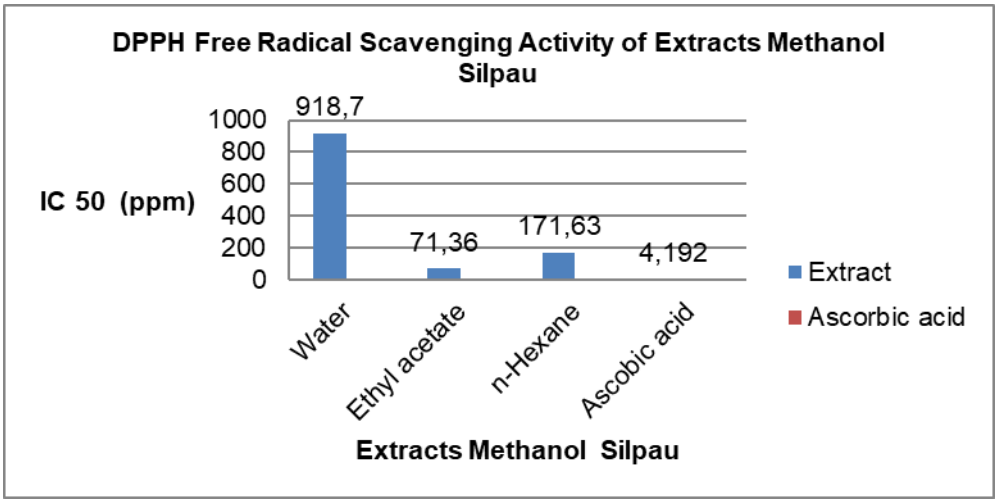

Figure-3. DPPH free radical scavenging activity of silpau methanol extracts

Table-4. DPPH Free radical scavenging activity of sub Fraction of Ethyl acetate Extracts

Sub Fraction	% Inhibition (ppm)
Sub Fraction 1	256,266
Sub Fraction 2	132,874
Sub Fraction 3	115,555
Sub Fraction 4	52,120
Sub Fraction 5	136,826
Ethyl acetate Extract	71,360
Ascorbat acid (positive control)	4,192

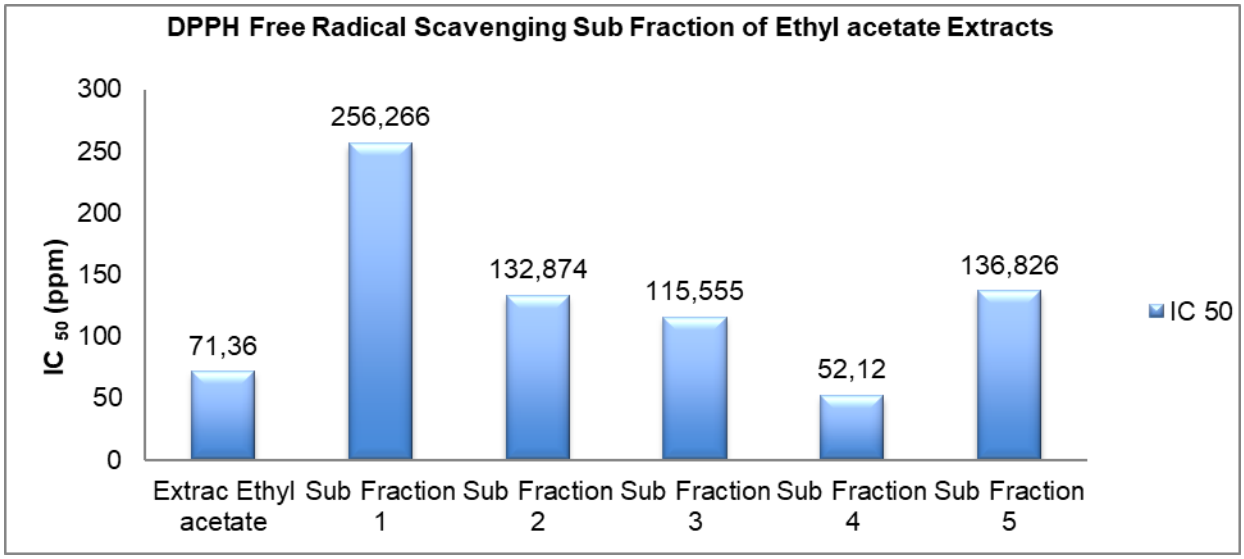

Figure-4. DPPH Free radical scavenging activity of sub-fraction of Ethyl acetate Extracts.

Antioxidant activity test of free radical reduction method from I-V sub-fraction

The DPPH test aims to see the antioxidant activity of the sub-fraction obtained from the separation of the Silpau methanol extract fractions. This test can determine the ability to reduce free radicals from an isolate and also to see the effect of the synergy of each compound in each fraction on its antioxidant activity. The antioxidant activity of isolates from Sub fractions I – V is presented in Figure 4.

The antioxidant activity based on the DPPH test on the methanol extract fraction of the green algae Silpau is shown in Table 3. The free radical reduction activity of the Ethyl acetate fraction determined by IC_{50} was 71.36 ppm and was higher than the IC_{50} Water fraction of 918.70 ppm or IC_{50} of the n-Hexane fraction of 171.63 ppm, but smaller than IC_{50} Vitamin C which acted as a positive control with an IC_{50} of 4.192 ppm. A compound is said to have very strong antioxidants if the IC_{50} value is less than 50 ppm, strong (50-100 ppm), moderate (100-150 ppm), and weak (151-200 ppm). The smaller the IC_{50} value the higher the antioxidant activity. (Badarinath, 2010). Based on the categorization of Badarinath it can be stated that; The antioxidant activity of the ethyl acetate fraction of the extract can be classified as an extract with moderate free radical scavenging activity. However, it can be explained that there is a large difference in determining the antioxidant activity of extracts of natural ingredients when compared to samples of single compounds. If the measurement of antioxidant activity is directed to extracts of natural ingredients, the range of activity is wider, unlike those categorized by Badarinath.

The classification for determining other antioxidant activity is explained by Frankel as follows, the IC_{50} / EC_{50} value (Inhibition Concentration 50 / Effective Concentration 50) is the concentration needed to inhibit free radicals by 50%. In the DPPH test, the smaller the IC_{50} value, the better the antioxidants in reducing free radicals. The following is a classification of antioxidant strength based on the IC_{50} value, according to Frankel IC_{50} <50 µg / ml is classified as very strong, IC_{50} 50-100 µg / ml is classified as strong, IC_{50} 101-250 µg / ml is classified as moderate and IC_{50} 250-500 µg / ml is classified as weak and IC_{50}> 500 µg / ml are classified as inactive. (Frankel, 1991).

Based on the classification of antioxidant activity according to Frankel, the antioxidant activity of Silpau methanol extract which has an IC_{50} value of 71.36 ppm and Sub fraction IV of the ethyl acetate glare fraction which has an IC_{50} value of 52.12 ppm can be classified as a strong antioxidant agent. Likewise, the compounds from the Hexane Fraction and Sub-Fraction II, Sub-Fraction III, and Sub-Fraction V from the Ethyl acetate Fraction were classified as moderate anti-oxidant agents, while the water fraction was inactive as an antioxidant.

Sub-fraction interaction resulted from Silpau Methanol Extract Fractionation and antioxidant activity

According to Gerry S and friends stated that; the interaction of compounds that make up a drug can have an effect on drug activity. The basic concept of pharmacodynamics explains that the interaction of drug components can cause several drug effects, such as additive interactions that will have twice

the effect of the activity of one drug. Synergistic interactions or potentiation will give the effect of one drug to strengthen or enhance the effect of another drug. Antagonistic interactions will have mutually negating effects on each other

Based on the understanding of pharmacodynamics interactions above, it can be examined how the effect of the interaction of a compound with other compounds in a compound extract, its fraction, and its effect on activity. This study was conducted based on the bioassay guidelines, to see how the antioxidant activity of the compound fraction and sub-fraction of the green algae methanol extract of the glare.

Identification of Sub-Fraction-IV Active Compounds with FT-IR Spectrophotometer

The separation of the chemical components of the ethyl acetate fraction then used column chromatography, which began with the Thin Layer Chromatography (TLC) test. The ethyl acetate fraction of the green seaweed sample as much as 68 mg was mixed with zeolite until it was homogeneous then separated the sub-fractions using column chromatography with the silica gel stationary phase and the mobile phase used was the n-hexane: ethyl acetate solvent with a ratio (50: 1 ~ 1: 1) in a gradient and obtained five sub-fractions, namely sub-fraction I, II, III, IV and V, as shown through spot layer chromatography [TLC] in Figure-2

Each sub-fraction obtained was checked for stains with TLC, the sub-fractions having Rf are combined to obtain simpler sub-fractions. The combination of sub-fractions based on TLC analysis obtained five sub-fractions, namely Sub Fraction I had a weight of 61.2 mg, Sub Fraction II had a weight of 46.0 mg, Sub Fraction III had a weight of 45.5 mg, Sub Fraction IV had a weight of 53.7 mg and Sub fraction V has a weight of 25.8 mg as shown in Figure-2. Antioxidant Test Results of the Free Radical Absorption Method (DPPH).

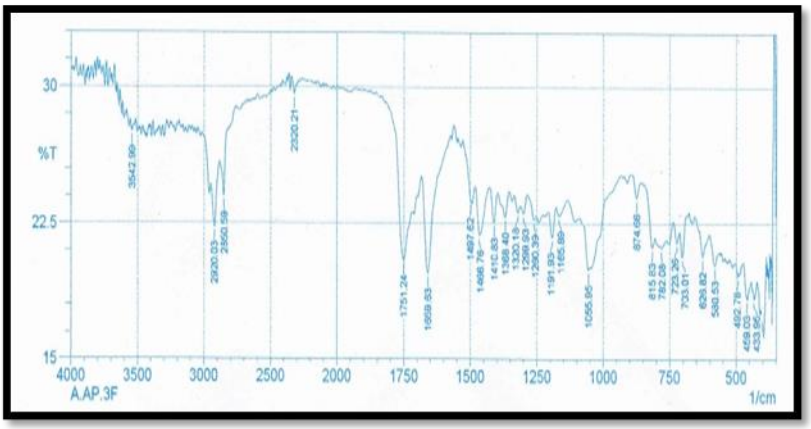

Figure-3. FTIR Spectrum Sub Fraction IV of Silpau Green Macroalgae Extract (*Dictyosphaeria* sp)

FTIR spectrophotometer can be used to determine functional groups contained in a compound, so that the resulting absorption in the FTIR spectrum can strengthen the notion that the isolate is a particular compound. The FTIR spectrum of Sub-fraction IV isolates (Figure-3) shows the presence of triterpenoid compounds. This was supported by the results of the FTIR spectrophotometer analysis which showed that the Sub-fraction IV isolate had a functional group C = O in the area of wave number 1751.24 cm^{-1}.

The results of the FTIR absorption pattern analysis obtained from Sub Fraction IV isolates contained a CH functional group as indicated by the absorption at wave number 2920.03 cm^{-1}, this indicates the possibility of methyl (CH3) and methylene (CH2) groups). This assumption is strengthened by the presence of absorption in the wave numbers 1466.78 cm^{-1} and 1497.62 cm^{-1} which are the absorption of the -CH2 and -CH3 bends which indicate the presence of dimethyl geminal groups as a characteristic of triterpenoid compounds. In the area of wave numbers 874.66 cm^{-1} and 815.83 cm^{-1} are absorption with strong intensity from the C-H range in the alkene group. The carbonyl group can be shown by the presence of absorption at wave number 1751.24 cm^{-1}, which is also strengthened by the presence of CO bonds in the ester group at wave number 1260.33 cm^{-1} and vibrations that occur at wave number 1055.95 cm^{-1}, due to the presence of a primary alcohol group. FT-IR spectrum analysis of Sub-fraction IV isolates of active compounds from green macroalgae Silpau (*Dictyosphaeria* sp) as shown in Table 5.

Table-5. FT-IR Spectrum Analysis of Sub-Fraction IV of Silpau Green Macroalgae

No	Wave lenght (Cm^{-1})	Library Range (Soctares, 1994)	Absorption intensity	Types of vibrations
1	2920,03	3000-2095	M	Stretching C-H Aliphatic
2	2320,21	2500-2000	M	Stretching (X≡Y, X=Y=Z)
3	1751,24	1790-1765	S	Stretching C=O
4	1466,78	1465-1440	M	Bending CH_2
5	1497,62	1490-1150	M	Bending CH_3
6	1260,33	1275-1185	S	Rentangan C-O
7	1055,95	1085-1030	S	Stretching C-OH alcohol
8	874,66	995-675	S	Stretching C-H
9	815,83	995-675	S	Stretching C-H

Information: s= strong; m = medium; w = weak

4. Conclusion

This research has been able to isolate one bioactive compound from green macroalgae glare (Dictyosphhaeria sp). Based on phytochemical analysis data and FT-IR spectrophotometer, sub-fraction IV was identified as a triterpenoid compound that has an IC50 value of 126.582 µg / ml and has the potential to be an antioxidant.

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