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Antioxidant Activity of Microalgae Extract *Cosmarium* sp. Using 2.2-Azinobis-(3-Ethylbenzothiazoline)-6-Sulfonic Acid (ABTS) Radical Cation Assay

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Abstract. The demand for natural antioxidants has increased due to the harmful effects of synthetic antioxidants, such as toxicity and carcinogenic properties. Microalgae face this requirement as they can produce numerous substances that have the potential to be antioxidants. This study aimed to evaluate the antioxidant activity of *Cosmarium* extracts using ABTS radical cation assay and identify compounds probably responsible for it. Dried biomass was extracted using hexane, ethyl acetate, and ethanol solvents using the maceration method. Extracts were partially purified with TLC and column chromatography. Compound identification was conducted using GC-MS analysis. The result showed that ethanol extract has the best activity with the IC50 at 55.95 ppm, followed by hexane and ethyl acetate extracts with IC50 as much as 104.339 ppm and 180.07 ppm, respectively. Two active fractions were selected fraction after partial purification with chromatographic analysis. Fraction 1 gave IC50 at 99.6 ppm while fraction 2 gave better IC50 at 53.562 ppm, both categorized as strong antioxidants. Compounds identification by GC-MS revealed that both fractions contain fatty acids compounds with 9.12-octadecadienoic acid (linoleic acid) and hexadecanoic acid (palmitic acid) as the dominant compound in fractions 1 and 2, respectively. This study gave insight into the potential of ethanol fraction from *Cosmarium* sp. as a natural antioxidant.

Key words: ABTS, antioxidant, bioactive compounds, *Cosmarium* sp.,GC-MS analysis

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INTRODUCTION

The imbalance condition between the production of reactive oxygen species (ROS) and the antioxidant defenses has caused oxidative damage to tissues and chronic disorders, such as cancer, coronary heart disease, neurodegenerative diseases, and also aging (Assuncao et al., 2017; Goiris et al., 2012; Li et al., 2007). Usually, ROS are produced as a result of cellular metabolism. When ROS contains uncoupled electrons, it becomes reactive and generates free radicals. Our body has a defense mechanism against these unfavorable conditions but needs additional antioxidants from outside (Assuncao et al., 2017). Popular synthetic antioxidants are butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). On the other hand, these antioxidants are reported to negatively impact animal models due to their toxic and carcinogenic properties (Li et al., 2007).

To date, the demand for natural antioxidants has increased due to the harmful effects of synthetic antioxidants (Coulombier et al., 2021). Various natural antioxidants such as vitamin C, tocopherol, and plant extracts are commercializing (Banskota et al., 2019). Many researchers often extract antioxidant compounds from various parts of terrestrial plants. However, there is another source of natural antioxidants, i.e., microalgae (Akar et al., 2019). Microalgae have the unique advantage of producing active compounds in culture. These compounds are difficult to synthesize chemically and have unique structures and functions, which can have the potential as antibacterial, antioxidant, anti-inflammatory, anticancer, antiviral, etc. (Abdo et al., 2012).

Microalgae biotechnology has received significant attention, especially regarding revealing its antioxidant properties. Some cosmetic companies also consider using microalgae extracts to increase their product benefits (Archer et al., 2021). Regarding the potency of antioxidants, microalgae can produce numerous natural antioxidant compounds, such as tocopherols, carotenoids, phenolic compounds, fatty acids, glutathione, peptides, coenzyme Q, ascorbate, polyamines or mycosporine-like amino acids (Assuncao et al., 2017). Besides that, microalgae also secrete enzymes with antioxidant activity, including glutathione reductase, superoxide dismutase, catalase, and peroxidase (Archer et al., 2021). One of the promising microalgae that have not been widely explored for its antioxidant potential is *Cosmarium* sp. This microalga can produce an extracellular polymeric substance (EPS) that plays an essential role in the adhesion and biofilm complex. Based on the research, the substance from *Cosmarium* sp. also showed antibacterial and antioxidant activity (Challouf et al., 2012).

Several studies were conducted on *Cosmarium* application, i.e., as the decolorization of textile dye (Daneshvar et al., 2007), biodiesel/ oil production (Khorshed & Al-Katib, 2021), and antibacterial (Abdo et al., 2012). The latest study by Challouf et al. (2012) revealed this microalga's antibacterial, antioxidant, and cytotoxic activities extracted with methanol, hexane, acetone, and water. However, he mainly focuses on the potency of EPS from *Cosmarium*. More studies about the antioxidant potency from this microalga and the link between that activity with the metabolites involved are still limited. It is a vast opportunity to explore more about it. This study aimed to evaluate the antioxidant activity of different extracts of *Cosmarium* sp. Solvents with different polarities were used to extract the biomass, i.e., hexane, ethyl acetate, and ethanol. Partial purification using chromatography technique and compounds identification using GC-MS were also conducted, which was not mentioned yet in the previous study. The results of this study will be useful in developing natural antioxidants sourced from biodiversity in Indonesia. In addition, using microalgae provides advantages over other sources of antioxidants.

METHODS

This research was initiated with microalgae culture and followed by an extraction process, antioxidant test, partial purification, and identification of compounds with Gas Chromatography-Mass Spectrometry (GC-MS) (Figure 1).

Microalgae Culture

Cosmarium sp. was cultivated in Modified Bristol Medium (MBM), consisting of (g/L):

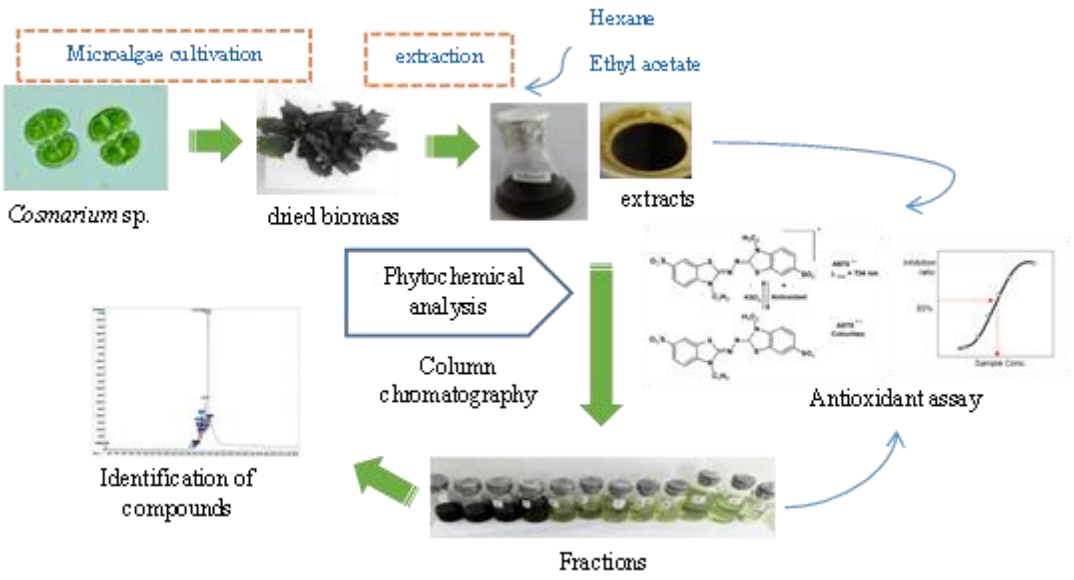

Figure 1. Graphical abstract of research

MgSO4 (Merck) 1.25, KNO3 (Merck) 1.25, KH2PO4 (Merck) 1.25, and micro-nutrient 1.0 ml with final pH adjusted to 7. Microalgae was cultured in continuous aeration and illumination (2000 lux). Microalgae growth was evaluated daily using a UV-Vis spectrophotometer (Hitachi U-3900 H) on λ 680 nm. Microalgae were harvested at the stationary phase by centrifugation technique (6000 rpm; 7 min), and the biomass was oven-dried at 50oC.

Extraction of Bioactive Compounds

Twenty (20) g of dried biomass was extracted with different solvents, i.e., hexane (Merck), ethyl acetate (Merck), and ethanol (Merck), using the maceration method. The extraction process was repeated several times until the solvent was colorless (Ardiles et al., 2020). The extract was collected and concentrated using a rotary evaporator (Stuart RE3022C) at 50oC. The concentrated extract was then dried at 40oC and stored in the refrigerator for further use. The yield of each extract was calculated.

Antioxidant Activity

The antioxidant activity of extracts was evaluated using 2,2-Azinobis-(3-ethybenzodiazoline) 6-sulfonic acid (ABTS) radical cation assay with some modification (Re et al., 1999). 7.4 mM ABTS and 2.6 mM potassium persulfate solutions were mixed in equal volumes and stored at room temperature in dark conditions for 16 hours before use. Distilled water was used to dilute the ABTS solution until it got an absorbance of 0.7 at 734 nm. Extracts (50-150 ppm in final concentration) were reacted with ABTS solution for 15 minutes in the dark at room temperature. Vitamin C was used as a positive control. Absorbance was measured by spectrophotometer (Hitachi U-3900 H) on λ 734 nm. All experiments were conducted in triplicate. The antioxidant activity was expressed as a percentage of inhibition and was calculated using the following equation(Conde et al., 2021) :

$$\text{Inhibition } \% = \frac{(\text{Abs}_{\text{ABTS}} - \text{Abs}_{\text{Sample - control}})}{\text{Abs}_{\text{ABTS}}} \times 100$$

Abs ABTS denotes the initial absorbance of diluted ABTS, and Abs sample denotes the absorbance of the sample after the reaction.

The IC50 was also determined as an indicator of antioxidant capacity. It was calculated by a linear regression equation between the percentage of inhibition and the sample concentration. IC50 was calculated from the equation :

$$IC_{50} = \frac{50 - a}{b}$$

Note: a = intercept ; b = slop (Suryanti et al., 2022).

Phytochemical Analysis

Phytochemical content of extracts was evaluated, such as alkaloid, flavonoid, saponin, tannin, triterpenoid, and sterol. Alkaloid was analyzed using various reagents, i.e., Mayer's, Wagner's, and Dragendorff's. Flavonoid was identified based on the Willstatter cyanidin reaction. Saponin was detected based on the froth production after the solution was shaken vigorously. Tannin was identified by adding a solution of ferric chloride to the extract and will produce a dark blue or blackish-green color. The terpenoid compounds were identified with anisaldehyde sulfuric acid (Abubakar & Haque, 2020; Shaikh & Patil, 2020).

Chromatographic Analysis

Isolation and partial purification of compounds were carried out by chromatographic methods, initiated with TLC analysis. The extract was spotted on the TLC sheets silica gel 60 F254 (Merck). Organic solvents such as hexane (Merck), ethyl acetate (Merck), dichloromethane (Merck) and methanol (Merck) were used as mobile phase. Hexane and ethyl acetate were used in various ratio as follows : 5:1 ; 4:1 ; 7:3 ; 3:2 ; 5:5 ; 1:2 ; 1:3 ; 1:4 ; 1:5 ; 1:10 ; 1:11 ; 1:13 ; 1:15 ; 1:19 while the ratio of dichloromethane and methanol were 1:3 ; 2:1 ; 5:1 ; 10:1 ; 15:1 ; 20:1 ; 30:1. The Rf value of each spot was measured according to Gibbons (2006). A combination of solvents with the best separation was used as a mobile phase in column chromatography. This analysis was developed according to the method by (Coskun, 2016).

Compounds Identification by Gas Chromatography-Mass Spectrophotometer (GC-MS)

The active fraction was diluted in 1 ml ethanol (Merck), and then 5 µl was injected into GC-MS 7890 (Agilent Technologies). The injection and final temperatures were 80°C and 280°C, respectively. This analysis was using helium as carrier gas. The mass spectra of the component were analyzed based on the library.

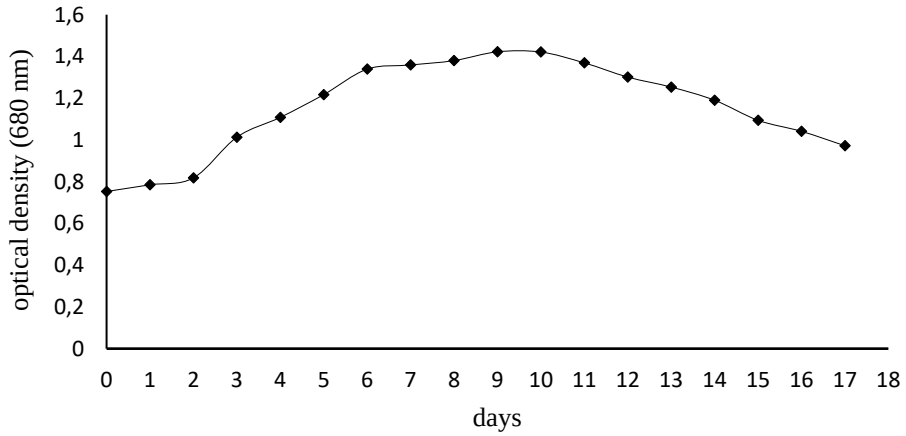

Figure 2. Growth curve of *Cosmarium* sp.

Table 1. The yield of *Cosmarium* sp. extracted with different solvent

Solvent	Algal Biomass (g)	Extract (g)	Yield (%)
Hexane	20	6.5	32.5
Ethyl acetate	20	8.7	43.5
Ethanol	20	6.4	32

RESULTS AND DISCUSSION

The Growth of Microalgae *Cosmarium* sp.

Microalgae were cultivated for 17 days to observe the growth curve. Based on Figure 2 showed that microalgae cells required two days of adaptation (lag phase) before entering the logarithmic (exponential) phase. This phase was indicated by the culture's slow growth. A high optical density was conditioned at the beginning of cultivation. A high optical density allows the culture to adapt quickly and grow faster. The logarithmic phase occurred from day 3 to day 6 and started the stationary phase until day 10. The logarithmic phase was indicated by rapid growth and cell division. The growth requirement for cell division was limited as time passed, and relative growth started declining. It is followed by a slow cell division and enters a stationary phase due to the lack of nutrients for growth. When the lack of resources continues, the cells begin to die (lysis) (Price & Farag, 2013). The culture was harvested in the stationary phase to gain maximum secondary metabolites.

Extraction of metabolites

The maceration method was used in this process, which is an easy and simple way to extract metabolites (Zhang et al., 2018). Solvents with different polarities were used in this process to get metabolites with different characteristics, such as non-polar, semi-polar, and polar compounds. The extraction using hexane produced 6.5 g of extract (yield 32.5%), while ethyl acetate and ethanol produced 8.7 g (yield 43.5 %) and 6.4 g (yield 32 %), respectively (Table 1).

Antioxidant activity of the extract

Antioxidant activity was tested using 2.2-Azinobis-(3-ethyl benzo diazoline)-6-sulfonic acid (ABTS) radical cation assay. This method is a simple and cheap assay to measure total antioxidant capacity (TAC). The 2.2-azinobis (3-ethylbenzthiazoline-6-sulfonate acid) (ABTS) is a stable radical cation with a blue-green chromophore and has maximum absorption at 734 nm. The intensity of ABTS reduced in the presence of antioxidants(Munteanu & Apetrei, 2021).

Table 2. Percentage of inhibition and IC_{50} of *Cosmarium* sp. extracts against ABTS

Samples	**Incubation time & concentration**								
Hexane extract	**1 minute**			**2 minutes**			**30 minutes**		
	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm
	42.30	45.97	50.87	44.23	47.90	52.97	45.62	49.47	53.84
	IC_{50} = 142.240 ppm			IC_{50} = 118.688 ppm			IC_{50} = 104.339 ppm		
Ethyl acetate extract	**1 minute**			**2 minutes**			**30 minutes**		
	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm
	21.50	35.13	38.81	23.60	36.71	39.68	27.44	40.38	43.53
	IC_{50} = 205.064 ppm			IC_{50} = 203.669 ppm			IC_{50} = 180.070 ppm		
Ethanol extract	**1 minute**			**2 minutes**			**30 minutes**		
	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm
	47.02	49.12	52.09	48.95	52.62	54.02	49.47	53.14	55.94
	IC_{50} = 111.637 ppm			IC_{50} = 63.24 ppm			IC_{50} = 55.950 ppm		
Positive control (Vitamin C)	**1 minute**			**2 minutes**			**30 minutes**		
	6 ppm	8 ppm	10 ppm	6 ppm	8 ppm	10 ppm	6 ppm	8 ppm	10 ppm
	45.71	52.35	58.15	47.05	53.44	57.64	49.91	55.63	59.32
	IC_{50} = 7.334 ppm			IC_{50} = 6.976 ppm			IC_{50} = 5.8944 ppm		

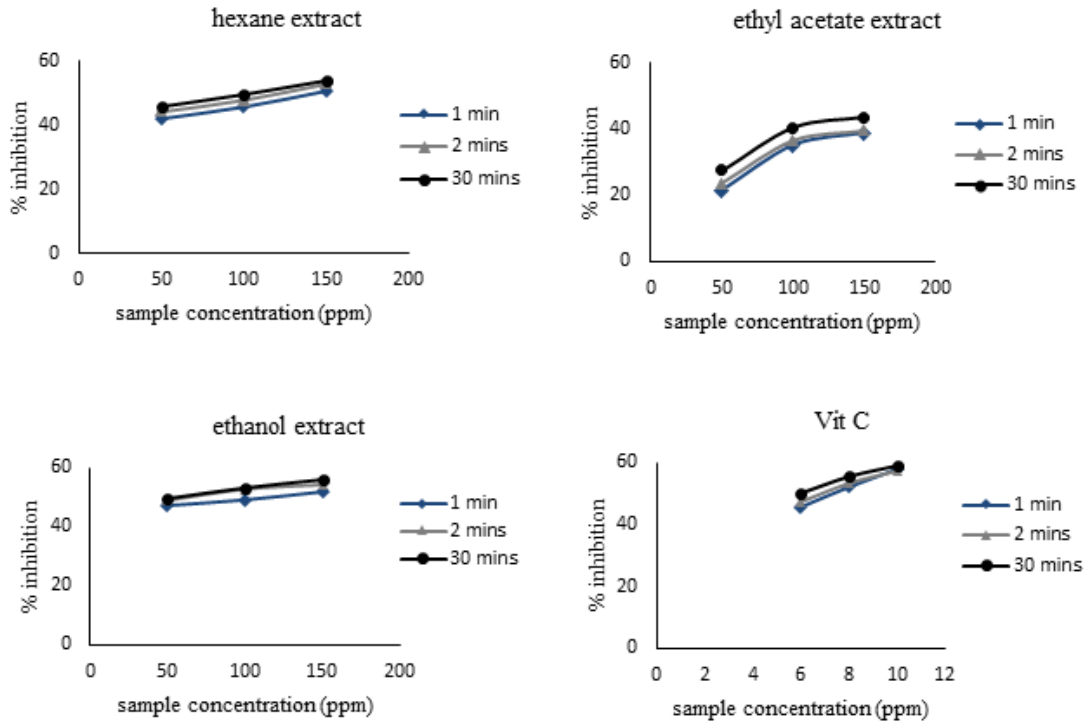

Figure 3. Percentage of inhibition of samples against ABTS during different times of incubation

Table 3. Phytochemical analysis of ethanol extract from *Cosmarium* sp.

Compounds		Phytochemical analysis
Alkaloid	Mayer's reagent	+
	Dragendorff's reagent	-
	Wagner's reagent	-
Flavonoid		-
Saponin		-
Tannin		-
Steroid/Triterpenoid		-

(+) : contain metabolite ; (-) : do not contain metabolite

ABTS scavenging is considered an electron (e) transfer reaction so that it can meet ABTS radical cation's need for an electron (e) to neutralize the positive charge. The effectiveness of antioxidant compounds to scavenge ABTS radicals implied that donating electron (e) may be another approach to scavenging radicals directly. The donating electron (e) mechanism is further supported by the Cu 2+- reducing power assay (Li et al., 2015).

In this study, besides using the different sample concentrations, we also used different incubation times prior to absorbance measurement, i.e., 1 minute, 2 minutes, and 30 minutes. According to Munteanu & Apetrei (2021), the reaction duration and sample concentration play a role in the discoloration of the blue-green color.

The antioxidant activity can be evaluated according to the percentage of inhibition, as shown in Table 2. The result showed that the longer the incubation time (reaction duration), the higher the percentage of inhibition (Figure 2). This result agreed with the previous study by Re et al. (1999). Therefore, 30 minutes of incubation was chosen for further reaction.

The result showed that ethanol extract had the best activity with the IC50 55.95 ppm and was categorized as a strong antioxidant. It is followed by hexane and ethyl acetate extracts with IC50 as much as 104.339 ppm and 180.07 ppm, respectively. Therefore, ethanol extract was chosen for further analysis. According to Hasibuan et al. (2020), a compound with IC50 less than 50 ppm is categorized as a very strong antioxidant, whereas IC50 50-100 ppm is known as a strong antioxidant. In addition, a moderate antioxidant is dedicated to a compound with IC50 100-150 ppm and a weak antioxidant with IC50 150-200 ppm.

Ethanol is a universal solvent with the ability to dissolve broad compounds. It could be the reason for the ethanol extract's highest activity. Based on the principle "like dissolves like", it seems that the compounds from ethanol extract are dominated by polar compounds (Zhang et al., 2018). Vitamin C is generally known as an antioxidant and has vigorous activity. Table 2 showed the activity of vitamin C even at low concentrations. When compared to the control, the result was still low. This indicated that the microalgae extract research still needs optimization to get better activity. However, it can be said that the extract had potency as a natural antioxidant.

Phytochemical Analysis

Phytochemical analysis showed the presence of alkaloids in ethanol extract (Table 3). Alkaloids included polar to semi-polar compounds. These were characterized as low molecular weight compounds with a nitrogen atom in the heterocyclic ring and had alkaline properties. (Suryanti et al., 2022). According to Chung & Woo (2001), alkaloids, especially indole, can efficiently stop free radical chain reactions. Other alkaloids compound, such as quinolone, also show antioxidant properties.

Antioxidant activity from alkaloids could be triggered through the proton transfer mechanism (i.e., H+- switch). The proton switch is related to the H+ ionization of the phenolic-OH, especially in protic solvents. H+ is a reasonably sensitive species. The nitrogen atom (N) in phenolic alkaloids combines with H+ to form amine salts. Therefore, phenolic alkaloids are protonated at the N-atom. The protonated N atom has a strong electron capacity. Extrusion in electron density affects the antioxidant stage since the electron transfer capacity (ET) is a critical component of

Table 4. Fractions purified by column chromatography and TLC

Fraction	Rf Value
1 (fraction no 1)	0.923; 0.830; 0.415; 0.353; 0.323
2 (fraction no 2)	0.923; 0.830; 0.430; 0.384; 0.353; 0.292; 0.261; 0.184; 0.092
3 (fraction no 3)	0.923; 0.384; 0.353; 0.292; 0.261; 0.184; 0.092
4 (fraction no 4-5)	0.323; 0.276; 0.246; 0.184; 0.076
5 (fraction no 6)	0.292; 0.261; 0.215; 0.184; 0.092; 0.061
6 (fraction no 7)	0.261; 0.215; 0.184; 0.092; 0.061
7 (fraction no 8-9)	0.261; 0.215; 0.184; 0.138; 0.092; 0.061
8 (fraction no 10)	0.184; 0.138; 0.092; 0.061
9 (fraction no 11)	0.353; 0.138
10 (fraction no 12-15)	0.123; 0.046
11 (fraction no 16-18)	0.061; 0.030
12 (fraction no 19-20)	0.030

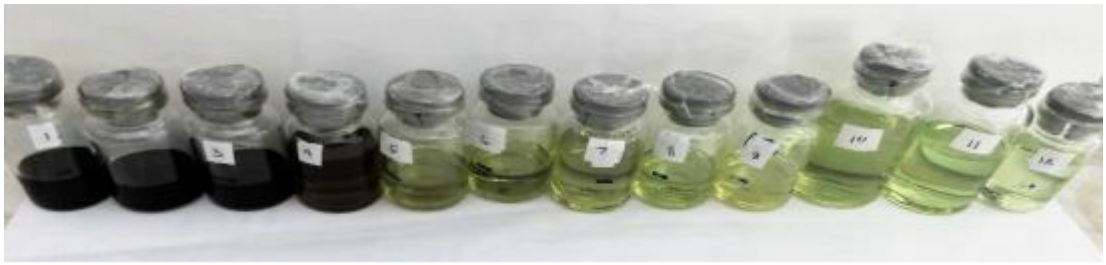

Figure 5. Simplification of purified fractions (from left to right in a row were fractions no 1-12)

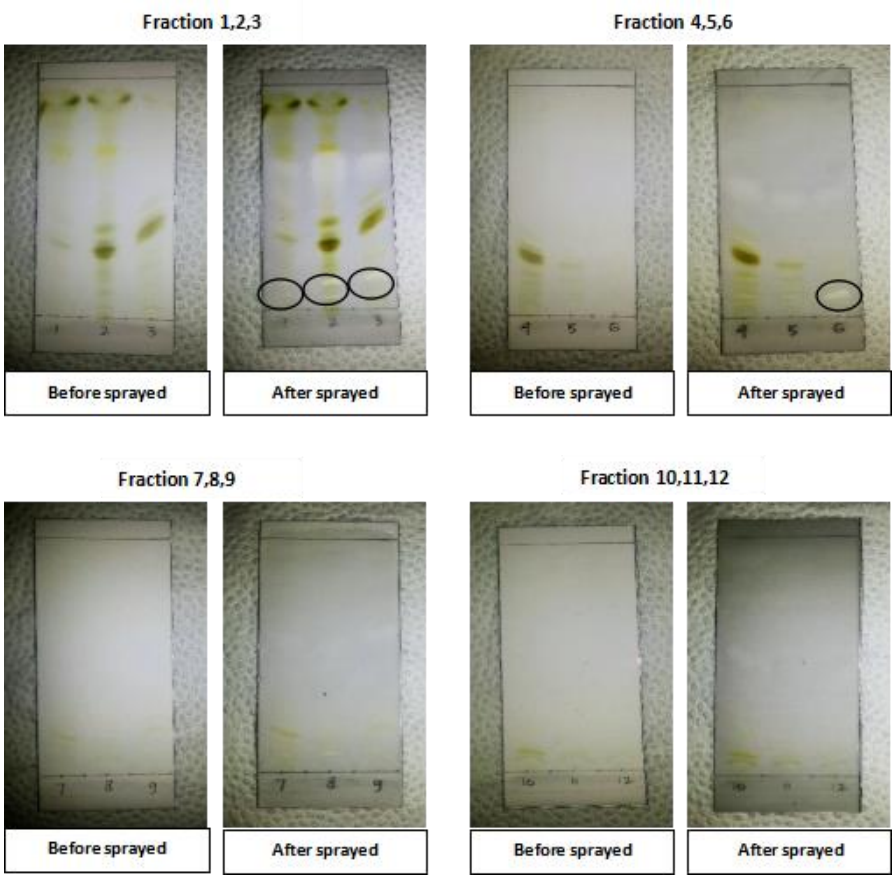

Figure 6. Qualitative test of antioxidant activity of fractions against ABTS

Table 5. Antioxidant activity of fractions according to their percentage of inhibition and IC_{50}

Fractions	Percentage of inhibition from each concentration (%)			IC_{50} (ppm)	Category
	50 ppm	100 ppm	150 ppm		
Fraction 1	20.33	62.18	68.06	99.6	Strong antioxidant
Fraction 2	47.89	62.35	68.57	53.562	Strong antioxidant
Fraction 3	25.21	34.78	42.18	193.95	Weak antioxidant
Fraction 6	30.5	33.41	36.31	385.6	No activity

Table 6. Compounds identification of fraction 1

Compounds	Molecular formula	Retention time (tR)	Area (%)	Quality (%)
Hexadecanoic acid	$C_{16}H_{32}O$	28.893	1.16	94
9.17-octadecadienal	$C_{18}H_{32}O$	29.292	1.90	95
14-methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	29.651	2.00	95
9.12-octadecadienoic acid	$C_{18}H_{32}O_2$	29.885	6.41	95
Triacontyl acetate	$C_{32}H_{64}O_2$	30.616	1.08	92
Dioctyl hexanedioate (Dioctyl adipate)	$C_{22}H_{42}O_4$	30.740	2.99	97
Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	31.244	2.36	90

Table 7. Compounds identification of fraction 2

Compounds	Molecular formula	Retention time (tR)	Area (%)	Quality (%)
Hexadecanoic acid	$C_{16}H_{32}O$	28.803	12.50	98
Methyl (9Z.12Z)-9.12-octadecadienoate	$C_{19}H_{34}O_2$	29.823	3.61	99
(9E.12E)-9.12-octadecadienoic acid	$C_{18}H_{32}O_2$	29.872	8.01	99
Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	30.741	3.60	99

an antioxidant (Suryanti et al., 2022; Xie et al., 2018).

The extract did not contain phenolic compounds, as indicated by negative flavonoid and tannin results. In many plants, those compounds are crucial in antioxidant activity. This result did not contradict a previous study by Li et al. (2007) that investigated the correlation between antioxidant activity and total phenolic content in some microalgae. His result concluded that phenolic compound is less critical as antioxidants in microalgae, as indicated by the poor correlation between the antioxidant activity and phenolic content.

Chromatographic Analysis

Isolation and purification of compounds were initiated with thin-layer chromatography (TLC). This process involved the use of various solvent ratios. Solvent with the best separation will be used for the next chromatography techniques. The result showed that dichloromethane and methanol with a ratio of 20:1 gave the best separation and the most spots (Figure 4).

Twenty fractions were resulted by column chromatography and were simplified to 12 fractions according to the same spot (Table 4 & Figure 5). These fractions were subjected to further analysis.

Antioxidant activity of fractions against ABTS radical cation

Figure 4. TLC separation of extract using dichloromethane and methanol

This analysis was carried out by comparing TLC plates before and after being sprayed with ABTS solution. Positive results are indicated by the presence of white color on the spot.

Based on the result, fractions 1,2,3, and 6 showed white color on the spot (Figure 6). It could be assumed that qualitatively those fractions had antioxidant activity against ABTS radical cation. According to Shivaprasad et al. (2005), ABTS is a radical with a nitrogen center with a characteristic turquoise color which will turn into a colorless non-radical form when reduced by antioxidants. Fractions 1,2,3, and 6 then quantified their activity and calculated their IC50 (Table 5).

Based on IC50 values, fractions 1 and 2 had IC50 below 100 ppm and were categorized as strong antioxidants, followed by fraction 3 with weak activity as much as 193.95 ppm. While fraction 6 did not show antioxidant potency due to its low activity. Fractions 1 and 2 were then subjected to compound identification using a GC-MS instrument.

Identification of compounds

Compounds detected in fraction 1 were dominated by fatty acids, such as hexadecanoic acid (palmitic acid), 9.17-octadecadienoic, and 9.12-octadecadienoic acid (linoleic acid) (Table 6).

This result agrees with a previous study by Khorshed & Al-Katib (2021), which recorded the highest total fatty acids (65.6 %) in *Cosmarium*, and it was distributed as (21.49 %) saturated fatty acids (SFA), (3.36 %) monounsaturated fatty acids (MUFA), and (40.75%) polyunsaturated fatty acids (PUFA). The compound belonging to the fatty alcohol group is triacontyl acetate. While 14-methyl-8-hexadecyl-1-ol belongs to the alcohol group and dioctyl hexanoate and bis(2-Ethylhexyl)phthalate belong to the phthalate group.

Based on Table 7, there were some compounds belonging to fatty acids, such as hexadecanoic acid (palmitic acid), methyl (9Z.12Z)-9.12-octadecadienoate (methyl linoleate), and (9E.12E)-9.12-octadecadienoic acid (linoleic acid). While hexanedioic acid, bis(2-Ethylhexyl) ester belongs to the phthalate group.

Fatty acids are reported as natural antioxidant compounds (Assuncao et al., 2017). Several studies revealed the correlation of antioxidant activities with fatty acid content. Anwer et al. (2022) described antioxidant activity of four algae (*Spirogyra* sp., *Spirulina* sp. *Chlorella* sp. , and *Chara* sp.) related to their total phenols and fatty acids content, especially palmitic acid, stearic acid, oleic acid, and linoleic acid. It could be pre-assumed that the antioxidant activity from *Cosmarium* extract is probably influenced by fatty acid content within the extract. Since the GC-MS can only detect volatile compounds, it cannot be concluded that fatty acid is the only compound responsible for the antioxidant activity.

The previous study with *Cosmarium* particularly focuses on the potency of EPS. More studies about the antioxidant potency of this microalga and the link between that activity with the metabolites involved are still limited. Therefore this study is a bit comprehensive because partial purification and compound identification were also conducted, which was not mentioned before. This result provides preliminary information about the compound involved in the antioxidant activity. However, purification and structural elucidation are needed to determine which compounds are responsible for the bioactivity. The results of this study are expected will be useful in developing natural antioxidants sourced from biodiversity in Indonesia.

So far, exploring antioxidant compounds has mostly used higher plants, whereas microalgae have advantages over other sources. In the future, the use of synthetic antioxidants can be minimized.

CONCLUSION

Negative effects caused by free radical and synthetic antioxidants have led to the use of natural antioxidants. Microalgae *Cosmarium* sp. are potential to be an alternative. Ethanol extract from this microalga showed strong antioxidant activity against ABTS radical cation. Based on the compound identification of selected fractions, it contained fatty acids compounds with 9.12-octadecadienoic acid (linoleic acid) and hexadecanoic acid (palmitic acid) as the dominant compound in fractions 1 and 2, respectively. Further investigation is needed to explore more about the activity of this microalgae. The use of more advanced technology and methods are important to get a comprehensive result.

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