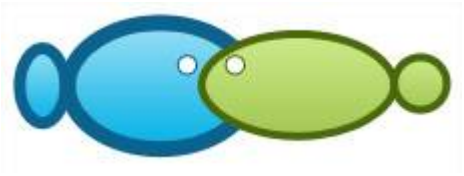

Identification of bioactive compounds in the oil extract from algae *Caulerpa microphysa* at Kien Giang province

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Abstract. Green algae *Caulerpa microphysa* is relatively valuable economically, mostly consumed as food. Polysaccharides contained in *C. microphysa* have anti-tumor, antibacterial, and skin-protective activities. However, there is no scientific study about biological properties and identification of phytoconstituents present in the oil extract from *C. microphysa*. This study aims to assess the potential use of the oil extract from *C. microphysa* collected from Hon Son, Kien Giang, Vietnam in terms of antioxidant, anti-inflammatory, anti-bacterial, and anti-ultraviolet activity. The antioxidant effects of the oil extract from *C. microphysa* were significantly high in DPPH and ABTS radical scavenging activity with EC_{50} values of 8.27% and 0.38% concentration, respectively. The inhibition denaturation of bovine serum albumin for the oil extract showed an EC_{50} value of 12.9% amount. The antibacterial activity of the oil extract from *C. microphysa* is illustrated by the potential inhibitory against *Bacillus subtilis*, *Staphylococcus aureus*, *Listerria innocua*, and *Escherichia coli*. Also, this study identified 26 components in the oil extract, with abundant acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester accounting for 43.87%. This study revealed the significant role of phytochemical substances in the oil extract from *C. microphysa*, which may apply to potential cosmetic production.

Key Words: antioxidant, anti-bacterial, anti-inflammatory, anti-ultraviolet activity, *Caulerpa microphysa*.

Introduction. Environmental factors (ultraviolet radiation (UVR) and pollution) are inducers of the reactive oxygen species (ROS) generation, creating clinical symptoms such as wrinkle formation and skin aging (Berthon et al 2017). Utilizing goods that promote health and beauty is becoming more common in modern life. The synthetic chemicals used for cosmetic purposes are causing many adverse effects on human health. Hydroquinone, arbutin, and kojic acid are widely used as cosmetic agents for skin lightening; however, these ingredients might cause dermatitis and promote tumor growth (Takizawa et al 2004). Hydroquinone also serves as a skin whitening agent between 2 and 10%, but excessive hydroquinone concentration has been linked to causing disorders such as thyroid disorder, leukemia, and liver damage (Enguita & Leitão 2013).

Seaweed is utilized in food, cosmetics, and medicine. It can be grown in aquaculture and agriculture systems. Seaweed contains phytochemical constituents such as pigments, lipids, cellulose, and polysaccharides that have already proven to have significant properties, such as antitumor, skin anti-inflammatory, moisturizing and whitening effects, antioxidant, and anti-aging (De Jesus Raposo et al 2015). Seaweed is now a natural source of significant interest because it is a healthy, sustainable, and environmentally beneficial food source.

The green algae *Caulerpa microphysa* was collected in Hon Son, Kien Hai, Kien Giang province. It has a relatively high economic value and well-developed culture in Taiwan, Japan, China, and the Philippines (Lee et al 2021). Previous research indicated the effects of *C. microphysa* enzyme-digested extracts on ACE-inhibitory activity and *in vitro* anti-tumor properties (Lin et al 2012). The polysaccharides in *C. microphysa* extract exhibited strong wound-healing, moisture absorption, and retention effects, which have potential uses in cosmetics (Lee et al 2021). Other species of the *Caulerpa* genus showed

possible anti-coagulant and antiherpetic activities of sulfated polysaccharides extracted from *C. cupressoides* (Rodrigues et al 2011) and *C. brachypus* (Lee et al 2004). The oil extract from *C. microphysa* in Hon Son, Kien Giang, Vietnam, was investigated for its phytochemical composition and biological properties in the current study. The study gave researchers insight into the oil's antioxidant, anti-inflammatory, anti-bacterial, and anti-ultraviolet properties, which they used to discover potential cosmetic applications.

Material and Method

Plant preparation. The green algae used in this study, *C. microphysa* (Figure 1), was collected in March 2022 from Hon Son, Kien Giang, Vietnam, and taxonomically identified by Dr. Nguyen Thi Kim Hue (Department of Biology, College of Natural Sciences, Can Tho University). The freshly collected seaweeds were thoroughly washed with tap water to remove all the extraneous materials and dried under air shade. The powdered dried seaweed was crushed, pulverized, and kept at room temperature. The powder was treated with 96^{o} EtOH for 48 h by filtration to obtain the EtOH extract (dark green) and oil extract (light yellow).

Figure 1. Green algae *C. microphysa* obtained in Hon Son, Kien Giang province, Vietnam.

Antioxidant assay in vitro. DPPH (2,2-diphenyl-1-picrylhydrazyl): Free radical scavenging capacity of the oil extract from *C. microphysa* was tested as described by Sharma & Bhat (2009) with some alterations. The oil extract was dissolved in EtOH at various concentrations. The mixture included 100 µL extract with 100 µL DPPH reagents, following incubation in darkness for 60 min at room temperature. The mixture was measured spectrophotometrically at 517 nm. The experiment was repeated three times at each concentration. ABTS (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)): Free radical scavenging activity of the oil extract was determined by ABTS radical cation decolorization assay (Nenadis et al 2004). A volume of 10 µL of the oil extract was added to 990 µL $ABTS^{\bullet+}$ solution, the absorbance was measured at 734 nm. All the measurements were carried out at least three times.

DPPH and $ABTS^{\cdot+}$ scavenging effects (%) were calculated using the formula:

$$E\ (\%) = (A_{blank} - A_{sample})/A_{blank} \times 100$$

where: E = antioxidant effectiveness (%);

A_{blank} = the absorbance of the bank;

A_{sample} = the absorbance of sample extract/standard.

In vitro antimicrobial assay. Antibacterial activity of the oil extract from *C. microphysa* was performed by agar well diffusion method, the results were expressed as the zone of inhibition (mm) (CLSI 2015). The antibacterial properties of the lipid extract were tested against 4 bacterial strains as *Bacillus subtilis* ATCC23857, *Staphylococcus aureus*

ATCC25923, *Listeria innocua* ATCC33090 and *Escherichia coli* ATCC25922. Pour approximately 20 mL Luria-Bertani medium into each labeled Petri dish and allow it to solidify for 45 min. The homogeneous suspension (100 µL) of test inoculums 10^6 CFU mL^{-1} was used for inoculation over the respective agar medium plates. Upon solidification, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. The lipid extract was dissolved in 10% DMSO to make a series of 60%, 80% and 100% concentrations. A volume of 50 µL of each extract concentration was added to respective wells. Then, the plates were incubated at 37°C for 24 h. Antimicrobial activity was detected by measuring the zone of inhibition (mm) that appeared after the incubation period (Wiegand et al 2008). DMSO at a concentration of 10% was employed as a negative control.

Protein denaturation assay. The protein denaturation assay was carried out after Dey et al (2011) with a few adjustments. The reaction mixture contained 150 µL of the oil extract and 150 µL of 5% bovine albumin (BSA). The mixture was incubated at 37°C for 15 min, and then heated at 60°C for 10 min. After cooling, the turbidity was measured at 660 nm. Using the following formula, the % inhibition of protein denaturation was determined:

$$\% \text{ inhibition of denaturation} = \frac{1 - A_S}{A_C} \times 100$$

where: A_C = absorption of the control sample;
A_S = absorption of the test sample.

The photoprotection of the oil extract. The sun protection factor is used to measure the oil extract's resistance to UV radiation. In this research, the oil extract was evaluated by UV spectrophotometry by applying Mansur's mathematical equation (Dutra et al 2004). The result was expressed as sun protection factor (SPF) and calculated by the following formula (Mansur et al 1986):

$$SPF = CF \sum_{290}^{320} EE(\lambda)\, I(\lambda)\, A(\lambda)$$

where: EE (λ) = erythrogenic effect of radiation (λ) ;
I (λ) = solar intensity spectrum (λ);
A = spectrophotometric absorbance value;
CF (λ) = correction factor (= 10).

The values of EE x I are constants. The results are shown in Table 1.

Table 1

Normalized product function used in calculation of SPF

Wavelength (nm)	*EE x I (normalized)*
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Gas chromatography-mass spectrometry (GC-MS) analysis. The identification of the components of the oil extract was carried out by gas chromatography equipment (Shimadzu QP-2010 Plus with Thermal Desorption System TD 20, Shimadzu, Kyoto, Japan); AB-INNOWax column (60 m length × 0.25 mm id × 0.25 µm thickness) was used under the conditions: column oven temperature was 50.0°C, injection temperature was 260°C, pressure was 69.0 kPa, total flow was 125.2 mL min^{-1}, and column flow was 1.21 mL min^{-1}. The injected oil sample volume was 0.1 µL, the split ratio was 100, the ion source temperature was 230°C, the interface temperature was 270°C, and the mass

spectrometer was scanned over the 40-650 m/z. Identification of individual oil components was done by comparing the mass data of individual oil component peaks with the standard library database (Wiley 7 NIST 05 mass spectral database).

Statistical analysis. The data are presented as the mean±SD. One-way analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments, followed by Tukey's posthoc test using Minitab 16 software. In all the groups, differences were considered statistically significant with $p < 0.05$. GraphPad Prism5.0 software was used for graphing.

Results

Antioxidant capacities of the oil extract in vitro. DPPH and ABTS radical scavenging activity of the oil extract from *C. microphysa* were expressed in Figures 2 and 3, respectively. The concentration of the oil extract was investigated at 10,000-200,000 ppm and 250-4,000 ppm by DPPH and ABTS assay, respectively. The results showed that there is a positive correlation between the concentration of the oil extract and its antioxidant properties in all testes. The DPPH and ABTS scavenging activity of the oil extract was exhibited with EC_{50} values of 82,673.1 ppm (8.27%) and 3,763.4 ppm (0.38%), respectively.

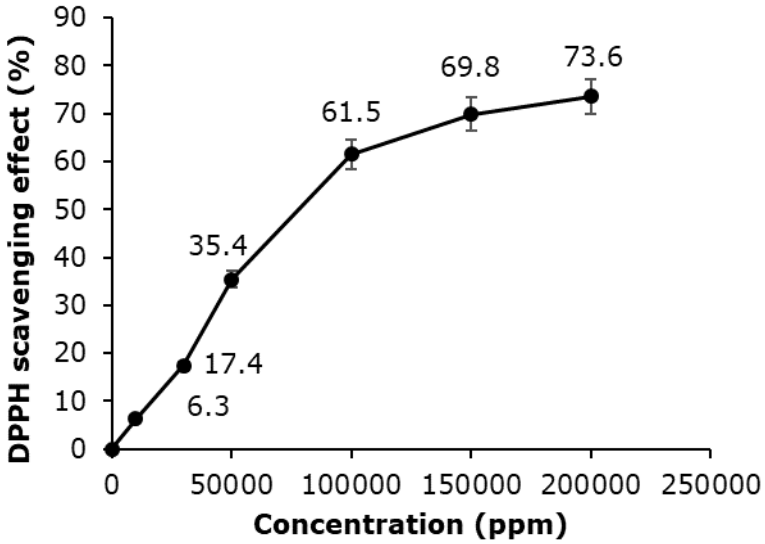

Figure 2. Antioxidant activity of the oil extract by DPPH scavenging racial assay.

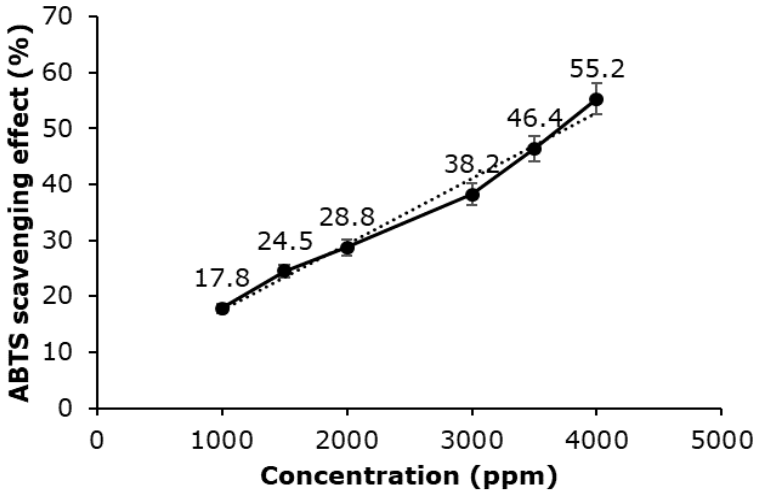

Figure 3. Antioxidant capacity of the oil extract by ABTS scavenging racial assay.

Antibacterial activity of the oil extract. The oil extract was investigated for its antibacterial capacity against 4 bacterial strains including *B. subtilis* ATCC23857, *S. aureus* ATCC25923, *L. innocua* ATCC33090 and *E. coli* ATCC25922 using disc diffusion method. Evaluation of the antibacterial activity of the oil extract was recorded in Table 2. The results revealed that the oil extract was potentially effective in suppressing microbial growth in a dose-dependent manner. At 60% and 80%, the oil extract effectively retarded microbial growth of *B. subtilis*, *S. aureus*, and *E. coli*, while *L. innocua* was inhibited only by 100 % of the oil extract.

Table 2

Antimicrobial screening test of the oil extract against some bacterial strains

Concentration		*Inhibition zone (mm)*			
		B. subtilis (+)	*S. aureus (+)*	*L. innocua (-)*	*E. coli (-)*
60%	600,000 ppm	3.67^{b}±0.67	3.67^{b}±0.67	0	5.00^{c}±1.00
80%	800,000 ppm	5.33^{ab}±1.2	6.00^{ab}±1.00	0	9.67^{b}±0.58
100%	1,000,000 ppm	6.00^{a}±0.00	7.33^{a}±1.20	4.00±0.00	16.0^{a}±3.00

Note: Data are means±standard error of three replicates (n = 3). Values in the same column with different characters are statistically significant differences.

Anti-inflammatory effect of the oil extract in vitro. The anti-inflammatory activity of the oil extract was evaluated against the denaturation of BSA at 50,000-200,000 ppm concentration (Figure 4). There was a significantly higher inhibition capacity of the oil extract at the increasing concentration. The highest inhibition rate was observed at a concentration of 200,000 ppm. The anti-inflammatory activity of the oil extract was exhibited with the EC_{50} value of 129,282.2 ppm (12.9%).

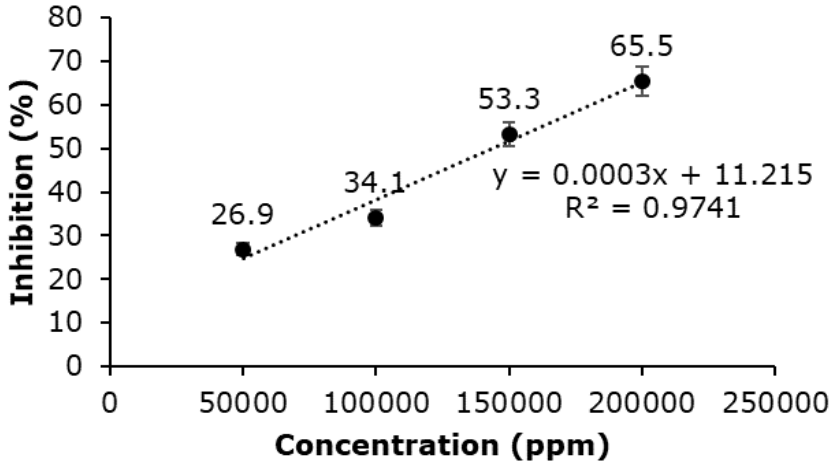

Figure 4. Anti-inflammatory effect of the oil extract against the denaturation of 5% BSA.

UV protection of the oil extract. The oil extract was subjected to absorbance measurement in the UV region which will be the indication of the UV absorption of the extracts. The oil extract has recorded the maximum absorbance in the UV range of 290-230 nm. The extract exhibited significant absorption in the whole UV range. The result suggested a decrease in the UV absorbance of the oil extract from 2.75 to 1.04 with an increase in UV wavelength in the range of 290-320 nm (Figure 5).

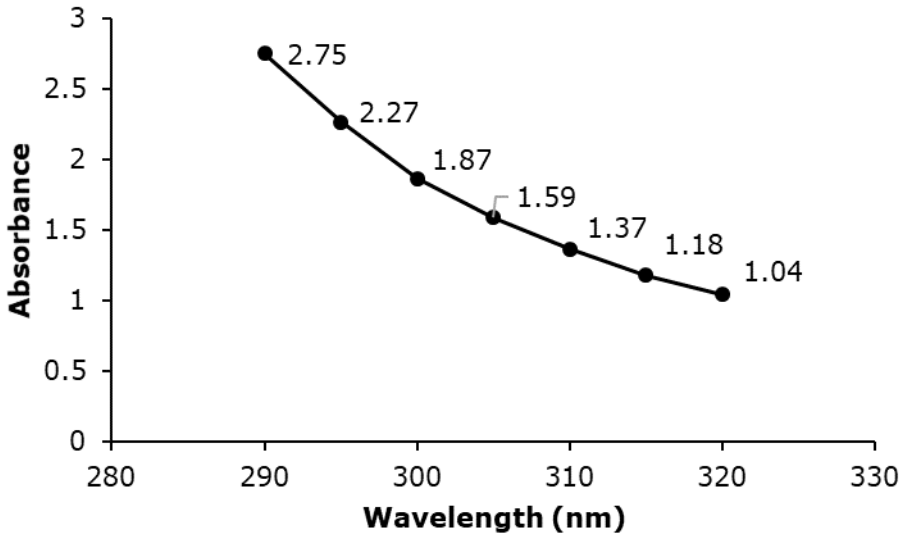

Figure 5. UV absorbance of the oil extract in the UV range (290-320 nm).

GC-MS analysis of the oil extract. Table 3 demonstrates the list of compounds whose GC-MS concentration is not less than 0.1% of the total peak concentration.

Table 3

The identified constituents of the oil extract from *C. microphysa* by GC-MS analysis

No	*Peak area*	*Peak area (%)*	*Compounds*
1	53,681	0.99	4-Ethylbenzoic acid, 2-phenylethyl ester
2	58,410	1.07	Benzenecarboxylic acid
3	66,642	1.23	4-(Ethylamino)-6-hydroxypyrimidine
4	71,531	1.32	Phenylmalonic acid
5	121,536	2.24	3-Methyl-4-propyl-2,5-pyrrolidinedione
6	111,856	2.06	3-cis-Methoxy-5-cis-methyl-1R-cyclohexanol
7	51,796	0.95	3-Hexenoic acid, 3-methyl-, methyl ester
8	43,258	0.8	N-Hydroxymethyl-2-phenylacetamide
9	83,621	1.54	Agaricic acid
10	86,382	1.59	2,4-Di-tert-butylphenol
11	165,454	3.04	Aceteugenol
12	247,323	4.55	6-(3,3-Dimethyl-oxiran-2-ylidene)-5,5-dimethyl-hex-3-en-2-one
13	101,769	1.87	4-Allyl-1,2-diacetoxybenzene
14	41,320	0.76	1,3-Diphenylpropane
15	82,1223	15.1	(3E)-4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-buten-2-one
16	40,822	0.75	Dasycarpidan-1-methanol, acetate (ester)
17	319,489	5.88	(+)-1-Cyano-d-camphidine
18	2,387,111	43.87	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester
19	305,430	5.62	Ether, p-menth-6-en-2-yl methyl
20	221,617	4.08	l-(+)-Ascorbic acid 2,6-dihexadecanoate
21	37,489	0.69	Phthalic acid, butyl (2-chlorocyclohexyl)methyl ester
	Total	100	

GC-MS analysis of the oil extract from *C. microphysa* resulted in 21 isolated compounds (Table 3). The results revealed that the oil extract was mainly composed of three categories of compounds: esters [5 compounds: 4-ethylbenzoic acid, 2-phenylethyl ester

(0.99%); 3-hexenoic acid, 3-methyl-, methyl ester; dasycarpidan-1-methanol, acetate (0.75%); acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester (43.87%) and phthalic acid, butyl (2-chlorocyclohexyl)methyl ester (0.69%)], alcohols [3 compounds: 3-cis-methoxy-5-cis-methyl-1R-cyclohexanol (2.06%); 2,4-di-tert-butylphenol (1.59%) and aceteugenol (3.04%)], and organic acids (3 compounds: benzenecarboxylic acid (1.07%), phenylmalonic acid (1.32%) and agaricic acid (1.54%)]. The major constituent in the oil extract is acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester, representing 43.87%.

Discussion. The skin may become susceptible to various issues due to certain environmental variables such as sun radiation, air pollution, tobacco smoke, and cosmetic products. Sunlight has been shown to cause oxidative stress, photoaging, and photocarcinogenesis (Parrado et al 2019). *Caulerpa* genus is well-known for its strong antioxidant activities (Yap et al 2019). Ethanolic extract from *Ulva rigida* was illustrated to have tumor protection against ROS due to the presence of phenolic, protein, and polysaccharides (Mezghani et al 2013). The current study suggested that the oil extract has DPPH and ABTS scavenging activities with an IC50 value of 8.27% and 0.38%, respectively (Figures 2 and 3).

In addition to ROS, temperature is a crucial element that influences bacterial survival in the presence of antibiotics. Due to an increase in UV radiation, the local temperature is sharply rising and was associated with increasing antibiotic resistance (percent resistant) in common pathogens (Rossati 2017). Therefore, the current project focuses on exploring the potential of the oil extract as a natural antibiotic reversal agent. The antibiotic resistance of the oil extract was the dose-dependence effect against all bacterial strains (Table 1). However, the oil extract at concentrations of 60% and 80% did not exhibit an inhibitory effect against *L. innocua*. The inhibition zones of the oil extract at 80% and 100% against *E. coli* were up to 9.67 and 16.0 mm, respectively. (Nagaraj & Osborne 2014) suggested that the methanolic extract of *Caulerpa racemosa* inhibited *Pseudomonas aeruginosa* and killed the mosquito larvae of *Culex tritaeniorhynchus* (the *Culex* species of mosquitoes transmit the virus with their bite causing Japanese encephalitis). 2-(-3-bromo-1-adamantyl) acetic acid methyl ester and Chola-5, 22-dien-3-ol were identified in *C. racemosa* contributing to their antibiotic resistance by GC-MS analysis.

Three types of solar ultraviolet radiation (UVR) are distinguished: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200–280 nm) (Kaur & Saraf 2010). The ozone layer blocks out UV-C, the radiation that is most harmful to living systems. However, ozone layer depletion increases the amount of UV-C transmission, causing adverse effects on human health. Excess UV-B irradiation is responsible for sunburn via stimulating the metabolism of melatonin. UV-A radiation accounts for up to 97%, this radiation exposure can lead to impaired vision and cataract formation (Dutra et al 2004). The sun protection factor (SPF) is typically used to quantify the effectiveness of UV protection. The higher the SPF, the more effective is the product in preventing sunburn (Kaur & Saraf 2010). The UV protection of *C. microphysa* was exhibited by the value of SPF up to 16.6±0.46, significantly higher than other suncream products and oil plant extractions mentioned in Table 4.

Many oil extracts from plants and vegetables have various biological activities and have been reported to have UV filters. SPF values of the oils of almond, avocado, coconut, and olive were approximately 8 (Kaur & Saraf 2010). Some plant extracts, ie. *Calendula officinalis* and *Pelargonium graveolens* also exhibited potential UV protection with the SPF values of 8.36 and 6.45, respectively (Lohani et al 2019). Research by Nurjanah et al (2019) tested the process for producing sunscreen with the major components from two marine algae, namely *Padina australis* and *Eucheuma cottonii*. The optimum ratio between *P. australis* and *E. cottonii* was found to be (1:1) and (2:1), respectively, with a SPF value of approximately 5. In our study, the SPF value of the oil extract from *C. microphysa* was 16.6, remarkably higher than other plant extracts tested in previous research (Table 4). The oil extract from *C. microphysa* exhibited significant absorption in the UV region (290-320 nm) (Figure 5). According to the absorbance

spectra and the SPF values calculated at 5% of the oil extract in the UV region between 290 and 320 nm, the phytochemical included in the oil extracts can absorb UV light. The current study, which was conducted at lower concentrations, revealed significant SPF values. Consequently, the greater concentration will result in higher sun protection levels.

Table 4

SPF values of the oil extract from *C. microphysa* and other plants

Test sample	*SPF*	*Reference*
The oil extract from *C. microphysa* at 5%	16.6±0.46	Current study
Olive oil extraction	7.55	Kaur & Saraf (2010)
Coconut oil extraction	7.12	Kaur & Saraf (2010)
Essential oil extraction from *Oncosiphon suffruticosum* (L.)	2.30	Adewinogo et al (2021)
Essential oil extraction from *Pelargonium graveolens*	6.45	Lohani et al (2019)
Essential oil extraction from *Calendula officinalis*	8.36	Lohani et al (2019)
Suncream contains components from marine algae *Padina australis* and *Eucheuma cottonii* (1:1)	5.23	Nurjanah et al (2019)
Suncream contains components from marine algae *Padina australis* and *Eucheuma cottonii* (2:1)	4.92	Nurjanah et al (2019)

In the present study, 21 components were identified in the oil extract from *C. microphysa* by GC-MS. Benzenecarboxylic acid (known as benzoic acid) accounts for 1.07% of the oil extract, which is commonly used as antibacterial and antifungal preservative and as flavoring agent in food, cosmetic, hygiene, and pharmaceutical products (del Olmo et al 2017).

Aceteugenol, one of the major components of clove oil (*Syzygium aromaticum*), was found in the oil extract from *C. microphysa* at 3.04%. Musthafa et al (2016) showed that the compound could cause cell damage, resulting in the death of *Candida* spp. cells, and significantly increased the phagocytic activity of macrophages against *Candida* spp.

(+)-1-Cyano-d-camphidine (accounting for 5.84% of the oil extract from *C. microphysa*) was identified in the leaf oil of *Hyptis brevipes* (2.84%) and *Achillea fragrantissima* (0.09%) (Bhuiyan et al 2010). The leaf oil of *H. brevipes* and *A. fragrantissima* were potentially useful in medicines because they exhibited antifungal, antibacterial, antioxidant, and anti-inflammatory activities (Patocka & Navratilova 2019). In the current study, the oil extract from *C. microphysa* contained (+)-1-Cyano-d-camphidine, which may contribute to antibiotic resistance and denaturation of BSA (Table 2 and Figure 4).

Significantly, the major constituent of oil extract from *C. microphysa* was acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester up to 43.87%. This compound accounted for 5.61%, found in the ethanol leaf extract of *Eurycoma longifolia*, having various biological activities such as anti-microbial, anticancer, antimutagenic, antiseptic, antispasmodic, anti-androgenic, anti-inflammatory, and strong antioxidant capacity (Supartini et al 2020).

The content of 4-allyl-1,2-diacetoxybenzene in the oil extract of *E. longifolia* is 1.87%, as detected by the GC-MS analysis. This component is also present in betel essential oil, as reported by Cang et al (2020) (27.391%) and Murugesan et al (2020) (21.24%). 4-allyl-1,2-diacetoxybenzene is known to have various beneficial biological effects such as anti-inflammatory, antibacterial, and antioxidant. Research by Siddique et al (2020) reported that 4-allyl-1,2-diacetoxybenzene rich in *Melaleuca* species play major roles in the anti-bacterial activity. Besides acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester, (+)-1-cyano-d-camphidine and benzenecarboxylic acid, 4-Allyl-1,2-diacetoxybenzene may be a crucial factor impact on the antibacterial properties.

2,4-Di-tert-butylphenol or 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP) is a common toxic secondary metabolite produced by various groups of organisms such as *Microcystis aeruginosa*, microalgal *Phaeodactylum tricornutum*, and *Osmunda regalis*

(Zhao et al 2020). 2,4-DTBP was isolated from thermophilic *Bacillus licheniformis* in Algerian hot spring. It was reported as an antibacterial compound against two multidrug resistance bacteria, namey *P. aeruginosa* and *S. aureus* (Aissaoui et al 2019). Remarkably, 2,4-DTBP exerted a significantly superior anti-inflammatory effect in all three pro-inflammatory genes in macrophage RAW264.7 (Nair et al 2020).

Conclusions. Algae are a rich source of natural antimicrobials and antioxidants. In the present study, the identified constituents and biological activities of the oil extract from *Caulerpa microphysa* were studied. Our results suggested that *C. microphysa* has DPPH and ABTS scavenging radicals, anti-inflammatory, antibacterial resistant capacities, and UV filters. By GC-MS analysis, the oil extract from *C. microphysa* proved to contain high amounts of major antibacterial, antioxidant, and anti-inflammatory components such as acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester (43.87%), aceteugenol (3.04%), (+)-1-Cyano-d-camphidine (5.84%). The oil extract from *C. microphysa* has a potential application in cosmetic products and medicines.

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Conflict of interest. The authors declare that there is no conflict of interest.

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