ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 25 (2) (2022): 41–48

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Bioactive Compounds from Microalgae *Spirulina platensis* as Antibacterial Candidates Against Pathogen Bacteria

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https://doi.org/10.14710/jksa.25.2.41-48

Article Info

Article history:

Received: 6th August 2021
Revised: 31st January 2022
Accepted: 5th February 2022
Online: 28th February 2022

Keywords:
Spirulina platensis; antibacterial; ethyl acetate fraction; purification; active compounds

Abstract

Microbial infection by bacteria has caused severe health problems worldwide. Treatment with antibiotics as the current solution has several drawbacks and triggers the phenomenon of bacterial resistance. Therefore, there is an urgency to look for a natural antimicrobial that is safer and has fewer side effects. One of the most promising antibacterial agents is *Spirulina platensis.* This research was conducted to evaluate the antibacterial activity of microalgae *S. platensis* against *Propionibacterium acne*, *Staphylococcus epidermidis,* and *Enterobacter aerogenes* and identify compounds from the active fraction of microalgae. Biomass was extracted with ethanol 96% using the reflux method then partitioned with immiscible solvents such as hexane, ethyl acetate, and water. Partial purification was carried out by chromatography techniques such as thin-layer chromatography and column chromatography. The compounds of active fractions were identified by GC-MS analysis. The result showed that ethyl acetate extract had vigorous antibacterial activity against all tested bacteria. The highest activity (14.4 ± 0.63 mm and 16.9 ± 1.48 mm) was achieved against *P. acne*; followed by *S. epidermidis* (13.05± 0.14 mm and 13.15 ± 0.0 mm), and *E. aerogenes* (11.7 ± 2.05 mm and 12.6 ± 1.90 mm), at concentrations 20,000 ppm and 30,000 ppm, respectively. The results indicated that the extract is more sensitive to Gram-positive bacteria (*P. acne* and *S. epidermidis*) than Gram-negative bacteria (*E. aerogenes*). Purification of the extract resulted in fraction 2 and fraction 6 as the most potential fractions for further analysis and identification. Based on the antibacterial activity, inhibition zones of fractions are wider than extracts. It could be assumed that the purification process enhances the activity of a sample. GC-MS analysis revealed that the dominant compounds of fractions 2 and 6 were bis (2-ethylhexyl) phthalate (67.76%) and 1,2-Benzendicarboxilic acid, bis (2-ethylhexyl) ester (50,88%), respectively. This result indicated that the ethyl acetate fraction of the microalgae *S. platensis* has the potential as a natural antibacterial.

1. Introduction

Microbial infections have become one of the significant causes of health problems and mortalities globally [1]. Among microbes, most infectious diseases are caused by bacteria. A common skin disease called acne vulgaris can be caused by several skin bacteria, i.e., *Staphylococcus epidermidis* [2] and *Propionibacterium acne* [3]. Acne vulgaris, otherwise known as acne, is a common and chronic inflammatory disorder that affects the pilosebaceous unit, associated with hair follicles [4]. This disease usually affects teenagers and young adults [5], and the causes are associated with increased sebum production, hyperkeratinization, bacterial colonization, and inflammation. The occurrence of skin tissue damage is triggered by enzymes secreted by bacteria *P. acne* [6]. Bacteria *Enterobacter aerogenes* has been reported as

important opportunistic and multiresistant bacterial pathogens for humans. This species is categorized as nosocomial pathogens, which contribute to several infections, such as endocarditis, bacteremia, skin infection, respiratory, urinary, and gastrointestinal tract [7].

The efforts to overcome bacterial infections have been carried out using antibiotics. However, antibiotics have several drawbacks related to toxicity, cost, and the indiscriminate and excessive use of antibiotics may lead to microbial resistance [1]. Therefore, it is necessary to search for natural sources of safer drugs with limited side effects than synthetic ones. Hopefully, this can reduce and replace the synthetic compounds used daily. In the last decades, researchers have been interested in searching for antimicrobial candidates from plants and microalgae such as cyanobacteria [8, 9]. One of the most interesting species from cyanobacteria is *Spirulina platensis,* which produces many metabolites, making them a potential source of medicine [10]. *Spirulina* biomass is rich in metabolites, composed of 60–70% proteins, carbohydrates, fatty acids, chlorophyll *a*, phycocyanin, carotenes, vitamins especially provitamin A, vitamin C, vitamin E, and minerals such as iron, calcium, magnesium, potassium, sodium, zinc, phosphorus [11].

A previous study by Bellahcen (2019) [12] showed that the essential oil of *Spirulina* inhibits the growth *B. antharcis*, *S. epidermidis*, and *E. coli* with the higher inhibition zone of 28 mm. Methanol extract of *S. platensis* also showed inhibition activity against *S. aureus* , *P. acne*, *Streptococcus pyogenes*, *S. epidermidis*, *Bacillus cereus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *E. coli*, and *Vibrio cholerae* [13, 14]. Therefore, the present study was conducted to evaluate the antibacterial activity of microalgae *S. platensis* against bacteria *Propionibacterium acne*, *Staphylococcus epidermidis,* and *Enterobacter aerogenes* and identify compounds from the active fraction of microalgae.

2. Methodology

This research used microalgae *S. platensis* and was conducted in several steps, including cultivation of microalgae, extraction of bioactive compounds, evaluation of the antibacterial activity, partial purification with some chromatography techniques until the identification of compounds with Gas Chromatography-Mass Spectrometry (GC-MS).

2.1. Cultivation of Microalgae *Spirulina platensis*

S. *platensis* was cultivated in modified Zarrouk's medium, consist of (g/L): $MgSO_4$ (Merck) 0.2, $CaCl_2$ (Merck) 0.12, $NaHCO_3$ (Merck) 16.8, EDTA (Merck) 0.64, Urea (Merck) 0.31, TSP (technical media) 0.18, KOH (Merck) 0.5, K_2SO_4 (Merck) 0.5, $FeSO_4$ (Merck) 0.01, and micro-nutrient (Merck) 1.0 ml with final pH adjusted to 8.6. Microalgae was cultured in continuous illumination (2500 lux) and aeration. The growth of microalgae was evaluated daily using a UV-Vis spectrophotometer (Hitachi V-3900 H) on λ 680 nm. Microalgae were harvested by filtration technique, and the biomass was oven-dried at 50℃.

2.2. Extraction of Bioactive Compounds

Sonication (40 Hz for 15 min) was conducted before extraction to break the microalgae cell and optimize the extraction process. Dried biomass was extracted with ethanol 96% (Merck) at 70℃ using the reflux method [15]. The extract was collected and concentrated using a rotary evaporator (Janke & Kunkel RV 05-ST) at 40℃ and stored for further use. Ethanolic extract was subjected to a partition process using immiscible solvents [16], such as hexane (Merck), distilled water, and ethyl acetate (Merck). The yield of each extract was calculated.

2.3. Antibacterial Assay

Antibacterial activity of extracts was evaluated against several skin bacteria, i.e., *P. acne, S. epidermidis*, and *E. aerogenes*, using the paper-disk diffusion method [8] in a double-layer medium. As much as 50 µL of each bacteria suspension 10^7 cell/ml was added to the semi-solid Nutrient Agar (Merck) medium. Extract concentrations were 20,000 ppm and 30,000 ppm. Chloramphenicol 20 ppm was used as a positive control, while each solvent was a negative control. All treatments were incubated at 37℃ for 18–24 hours. Inhibition zone was measured and expressed in mm.

2.4. Phytochemical Analysis

Phytochemical content, including alkaloid, flavonoid, saponin, tannin, triterpenoid, and sterol, were analyzed according to Farnsworth & Harborne [17, 18]. Alkaloid was analyzed using Mayer's, Wagner's, and Dragendorff's reagents. Flavonoid was identified according to the cyanidin reaction of Willstatter, which would detect the y-benzopyrone nucleus in the compound. Saponin was detected with the froth test, by the production of honeycomb froth after vigorous shaking of the solution. Tannin was identified by adding ferric chloride solution to the extract and should result in a dark blue or blackish-green color. In comparison, terpenoid compounds were identified with anisaldehyde sulfuric acid.

2.5. Isolation and Partial Purification of Compounds

Isolation of compounds was carried out by chromatographic techniques. Ethyl acetate was selected for further analysis based on its antimicrobial activity. The extract was spotted on the TLC sheets silica gel 60 F_{254} (Merck). Hexane (Merck), ethyl acetate (Merck), and ethanol (Merck) were used as mobile phase in various ratio (7:2:1; 7:2.2:0.8; 7:2.5:0.5; 6:2:2; 6:3:1; 7.5:1.5:0.5; 7.5:1.5:1 and 7:1.5:1.5). The *Rf* value of each spot was measured as described by Gibbons [19] as a ratio of the movement of the compound to the solvent. Column chromatography was developed using hexane (Merck), ethyl acetate (Merck), and ethanol (Merck) (7.5:1.5:1) as mobile phase, according to the method described by Salituro & Dufresne [20].

2.6. GC-MS Analysis

The active fraction was diluted in 1 ml ethanol (Merck), then 2 µl was injected into GC-MS 5973 N (Agilent Technologies). This instrument was equipped with DB 5 column (60 m in length and 0.25 mm in diameter) at injection temperature 70℃ and final temperature 290℃ and was using helium as carrier gas. The mass spectra of the component were analyzed based on Wiley 10 N.14 Library.

3. Results and Discussion

3.1. The Growth of Microalgae *S. platensis*

The increase in optical density indicates the growth of microalgae. The higher the optical density value, the more cells produced. Based on Fig. 1, the culture did not experience a lag phase (Figure 1). This cultivation was initiated with a high optical density at the logarithmic phase, so the culture grew faster with the suitable medium for growth. A previous study [21] revealed that high cell density at initial cultivation shortens the lag phase and significantly increases the logarithmic phase. Besides that, the biomass produced was also higher. Another factor that influences the absence of the log or adaptation phase is the Zarrouk medium, which is known as the maintenance medium for *Spirulina*. The adaptation phase occurs as a response of cells to the new growth medium. The more different the growth medium, the longer the adaptation phase [22].

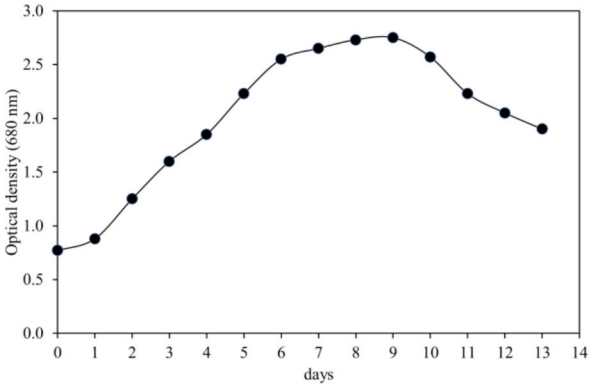

Figure 1. Growth curve of *S. platensis* during cultivation

The logarithmic phase occurred from day 1 to day 6, characterized by the addition of cells due to cells dividing rapidly. Day 7 to day 10 reached a stationary phase characterized by a balance between the rate of cell growth and cell death. In the late cultivation period, the OD starts to decrease as an indication of the death phase. Cell dividing almost does not occur while the death cell occurs massively [23]. The culture was harvested on day 7 at the stationary phase to get maximum compounds. From the experiment, dry biomass yield gained 1.3 ± 0,1 $g.L^{-1}$. This result was relatively low as compared to the many research that reported the maximum biomass dry weight of *Spirulina* in Zarrouk medium ranging from 0.69 to 6.90 $g.L^{-1}$ [11]. This can be explained because, in the stationary phase, the culture is more focused on metabolites rather than growth productivity.

Harvesting *S. platensis* is relatively easy and straightforward because of its enormous size, around 200-300 µm in length and 5–70 µm in width [24]. Therefore filtration method was used in harvesting.

3.2. Extraction of bioactive compounds

The reflux method was used in this process, which involves heat to accelerate and maximize the extraction process. The first extraction used ethanol and produced 6.95 g of extract (yield 27.8%). The next step is partitioning with hexane, ethyl acetate, and water, producing an extract of 8.48%, 2.24%, and 7.92%, respectively (Table 1). The yield of extract indicated that ethanol is quite effective in extracting biomass. Wang (2010) [25] explained that ethanol has an excellent ability to penetrate the cellular membrane to extract intracellular materials. In addition, the yield of the extract also depends on the type of solvents, temperature, extraction time, and the chemical composition of the sample [26].

Each solvent only dissolves compounds with the same/ similar polarity, as the general principle in solvent extraction is "like dissolve like" [26]. In the partition process, non-polar compounds contained in the ethanol extract, such as alkanes, fatty acids, pigments, terpenoids, and alkaloids, will be extracted into hexane solvent. The semi-polar compounds such as alkaloids and flavonoids are extracted into ethyl acetate solvent. In contrast, polar compounds such as flavonoid glycosides, tannins, and alkaloids will be extracted into water solvents [27].

Table 1. The yield of *S. platensis* extract with different solvent

Solvent	Algal Biomass (g)	Extract (g)	Yield (%)
Hexane	25	2.12	8.48
Ethyl acetate	25	0.56	2.24
Ethanol	25	6.95	27.8
Aqueous	25	1.98	7.92

3.3. Antibacterial activity of the extract

Antibacterial activity of the extract was shown by inhibition zone formation. The diameter of the inhibition zone depends on the type of solvent used and the tested antibacterial activity [10]. The result showed that ethyl acetate extract has antibacterial activity against all tested bacteria and showed the highest activity (14.4 ± 0.63 mm and 16.9 ± 1.48 mm against *P. acne*; 13.05± 0.14 mm and 13.15 ± 0.0 mm against *S. epidermidis*; 11.7 ± 2.05 mm and 12.6 ± 1.90 mm against *E. aerogenes*, at concentrations 20,000 ppm and 30,000 ppm, respectively), followed by ethanol extract in the second place (9.05 ± 1.27 mm and 10.5 ± 0.49 mm against *P. acne*; 10.15 ± 0.0 mm and 9.7 ± 0.63 mm against *S. epidermidis*). Hexane and aqueous extracts showed no activity against all tested bacteria (Table 2). Extract of ethyl acetate then was chosen for further analysis.

The antibacterial activity of ethyl acetate extract was categorized as strong activity, while ethanol extract was

a medium category. Davis & Stout (1971) [28] stated that extracts with an inhibitory diameter of more than 20 mm were included in the very strong category, the inhibition diameter ranged from 10–20 mm was included in the strong category, the inhibition diameter ranged from 5–10 mm was included in the medium category. The inhibition diameter was less than 5 mm was included in the weak category. Negative control did not show activity, while positive control (chloramphenicol) showed activity against all tested bacteria.

Compared to the positive control, the antibacterial activity of the extract is lower. Nevertheless, the activity showed the potential of the extract as a natural antibacterial. It is indicated that optimizing processes such as extraction methods, solvent selection, and other strategies still need to be evaluated to maximize the activity. Chloramphenicol as positive control is a broad-spectrum antibiotic that can inhibit Gram-positive and Gram-negative bacteria [29].

Table 2 . Inhibition zone of *S. platensis* extract against tested bacteria

Extracts	Concentration (ppm)	Inhibition zone (mm)		
		P. acne	*S. epidermidis*	*E. aerogenes*
Hexane	20,000	-	-	-
	30,000	-	-	-
Positive control	20	11.85 ± 0.0	9.15 ± 0.0	11.25± 0.0
Negative control		-	-	-
Ethyl acetate	20,000	14.4 ± 0. 63	13.05± 0.14	11.7 ± 2.05
	30,000	16.9 ± 1.48	13.15 ± 0.0	12.6 ± 1.90
Positive control	20	10.15± 0.0	12.25 ± 0.0	9.15 ± 0.0
Negative control		-	-	-
Ethanol	20,000	9.05 ± 1.27	10.15 ± 0.0	-
	30,000	10.5 ± 0.49	9.7 ± 0.63	-
Positive control	20	15.15± 0.0	10.95± 0.0	8.95± 0.0
Negative control		-	-	-
Aqueous extract	20,000	-	-	-
	30,000	-	-	-
Positive control	20	9.15± 0.0	8.95± 0.0	8.15± 0.0
Negative control		-	-	-

Inhibition zones are expressed as mean ± standard deviation. Positive control: chloramphenicol. Negative control: each extraction solvent. (-): no inhibition zones

The results indicated that the extract is more sensitive to Gram-positive bacteria (*P. acne* and *S. epidermidis*) than Gram-negative bacteria (*E. aerogenes*). Gram-positive bacteria are generally more easily inhibited or killed than Gram-negative bacteria. It is due to differences in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-positive bacteria have a thick cell wall and only have a single layer (monolayer). In contrast, the cell wall of Gram-negative bacteria is thin and has 3 layers (multilayer), making it challenging to absorb antibacterial compounds [30]. Gram-positive bacteria have a hydrophilic side, i.e., carboxyl, amino acids, and hydroxyl so that bacteria Gram-positive are more sensitive to antibacterial compounds. The formation of the inhibition zone is highly dependent on the amount of antibacterial agent that is dropped onto the disc, the solubility of the antibacterial agent in the medium, the diffusion coefficient, and the effectiveness of the antibacterial agent [31].

3.4. Phytochemical Analysis

Phytochemical analysis showed the presence of flavonoid and tannin in ethyl acetate extract (Table 3). These compounds have polar properties to be found in semi-polar ethyl acetate extract. Flavonoids show the antibacterial effect through 3 mechanisms, i.e., inhibiting the cell membrane function [32], inhibiting nucleic acid synthesis, and inhibiting the energy metabolism [33]. Meanwhile, tannin is also able to inhibit microorganisms [34].

Table 3. Phytochemical screening of ethyl acetate extract from *S. platensis*

Compounds/Metabolites		Phytochemical screening
Alkaloid	Mayer	-
	Dragendorff	-
	Wagner	-
Flavonoid		+
Saponin		-
Tannin		+
Steroid/Triterpenoid		-

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

3.5. Isolation and Partial Purification of Compounds

Thin-layer chromatography (TLC) was the first step in purifying compounds. Solvent with the best separation will be used for column chromatography. The result showed that hexane, ethyl acetate, and ethanol with a ratio 7.5:1.5:1 gave the best separation and the greatest number of spots (Figure 2). Rf value of the spots were 0.78; 0.68; 0.52; 0.4; 0.36; 0.32; 0.28; 0.24; 0.2; 0.16. A good eluent can separate compounds in large quantities marked by the appearance of spots. The spots formed are tailless, and the distance between the spots is clear [18].

Table 4. Purification with column chromatography

Fraction	R_f Value
1 (fraction no 1)	0.76; 0.7
2 (fraction no 2)	0.72; 0.68; 0.6
3 (fraction no 3)	0.7; 0.68; 0.58
4 (fraction no 4-5)	0.56; 0.54
5 (fraction no 6-8)	0.54; 0.52
6 (fraction no 9-13)	0.4
7 (fraction no 14-15)	0.3
8 (fraction no 16-35)	0.9

Based on column chromatography, there are 35 fractions in total and 8 combined fractions achieved according to the spots simplification (Table 4). These fractions were subjected to further analysis.

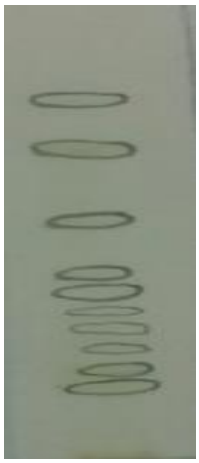

Figure 2. TLC separation of extract using hexane: ethyl acetate: ethanol 7.5:1.5:1

3.6. Antibacterial activity of fractions

The result showed that the inhibition zones of fractions are wider than extracts (Table 5).

Table 5. Inhibition zone of fractions against tested bacteria

Fractions	Inhibition zone (mm)		
	P. acne	*S. epidermidis*	*E.aerogenes*
F1	12.35 ± 3.11	15.5 ± 0.07	12.95 ± 0.77
F2	14 ± 0.91	14.25 ± 0.98	13.75 ± 0.56
Positive control	14.65 ± 0.0	18.35 ± 0.0	18.15± 0.0
Negative control	-	-	-
F3	10.2 ± 0.07	15.1 ± 0.77	12.1 ± 0.21
F4	9 ± 0.21	14.7 ± 0,35	13.35 ± 0.56
Positive control	14.95 ± 0.0	11.95 ± 0.0	19.75 ± 0.0
Negative control	-	-	-
F5	10.25 ± 2.26	16 ± 2.33	14.05 ± 0.35
F6	9.1 ± 0.21	17.65 ± 2.12	12.8 ± 0.49
Positive control	13.85 ± 0.0	16.65 ± 0.0	13.45 ± 0.0
Negative control	-	-	-
F7*	14.1 ± 0.63	18.05 ± 0.14	13.3 ± 0.21
F8*	16.75 ± 0.14	23.1 ± 1.20	8.45 ± 0.14
Positive control	14.25 ± 0.0	19.65 ± 0.0	17.55 ± 0.0
Negative control	-	-	-

*Fraction concentrations were 20,000 ppm, while the other's concentrations were 10,000 ppm

It could be assumed that the purification process enhances the activity of the sample. The strongest fractions activity was achieved against *S. epidermidis* (14.25 ± 0.98 mm and 17.65 ± 2.12 mm for fractions 2 and 6, respectively). Fractions 2 and 6 were subjected for identification with GC-MS according to the activity against all tested bacteria. Both fractions had strong activity because of the inhibition zone at the range of 10-20 mm, except fraction 6 against *P. acne*, which was categorized as a medium category [28]. Fraction 5, even though it had higher activity than fraction 6, also had a high deviation standard. That is why fraction 6 was picked up for the following analysis. Fractions 7 and 8 were not used for further analysis because they had higher concentrations than the others (20,000 ppm).

3.7. Identification of compounds

Identification of compounds with GC-MS showed that fraction 2 has 17 compounds (Table 6), with bis (2-ethylhexyl) phthalate present in minutes 18.85 as the dominant compound (Figure 3). This compound had an antibacterial activity with the mechanism of action by reacting with the cell membrane's hydrophobic side, leading to impaired permeability from the cell membrane [35].

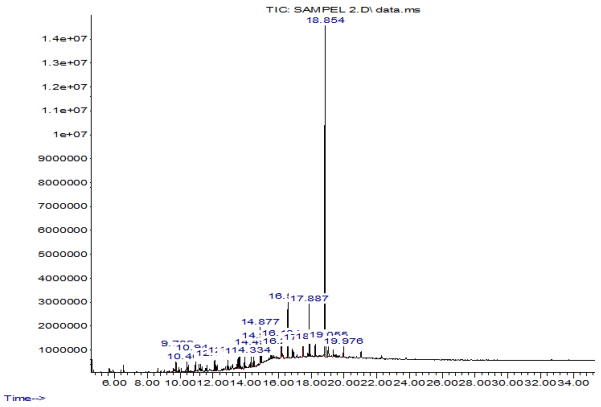

Figure 3. GC-MS chromatogram of fraction 2

Other compounds such as heptadecane, 1-hexadecene, docosane, and 1-octadecene also had antibacterial activity [10, 36, 37]. Eicosane from *Ceratonia siliqua* extract shows antibacterial and antifungal activity, also cytotoxic effect to HeLa and MCF-7 cells [38]. Neophytadiene acts as antifungal, antibacterial, and antimicrobial [39, 40, 41]. Alkane also shows antimicrobial activity, i.e., Octadecane to *S. aureus* and *E. coli* [42], and Nonacosane to *S. aureus* ATCC 6538, *S. aureus* ATCC 29213, *E. coli* ATCC 25923, *E. coli* ATCC 29988, *Proteus mirabilis* ATCC 43071 [43]. Triterpenoid dibutyl phthalate (DBP) has bioactivity as antifungal, antibacterial, and antimalaria [44]. Fatty acids such as hexadecanoic acid and methyl ester also inhibit microbes [45].

Fraction 6 has 13 compounds detected in GC-MS analysis (Table 7), with 1.2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester as the dominant compound (Figure 4). This compound can inhibit *S. aureus* and *B. subtilis* [46], *Aeromonas hydrophila, Edwardsiella tarda*, and *Vibrio ordalli* [47]. Some compounds such as tetradecane, octadecane, eicosane, and nonacosane were also detected in fraction 2. Therefore, those compounds were believed to have antibacterial activity. Phenol compound also detected in this fraction, i.e., 2.6-bis (1.1-dimethylethyl)-4-methyl-phenol and potential as an antibacterial agent. This result correlated with phytochemical analysis, where flavonoids and tannins are phenolic compounds. According to [48], phenol, phenolate, or polyphenol had antimicrobial activity. Phenol was also reported to denature cell proteins (breakdown the protein structure) via hydrogen bonds formed between phenol and protein [49].

Table 6. Identification of compounds from fraction 2

Retention time (tR)	Compounds	Molecular formula	Area (%)	Similarity Index (%)
12.096	1-Hexadecene	$C_{16}H_{34}$	0.74	99
12.145	Hexadecane	$C_{16}H_{32}$	0.80	98
12.907	Heptadecane	$C_{17}H_{36}$	0.82	98
13.586	1-Octadecene	$C_{18}H_{36}$	0.86	99
13.628	Octadecene	$C_{18}H_{38}$	0.80	99
13.929	Neophytadiene	$C_{20}H_{38}$	0.98	94
14.496	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.03	99
14.874	Dibutyl phthalate	$C_{16}H_{22}O_4$	2.25	96
14.930	5-Eicosene	$C_{20}H_{40}$	1.16	97
16.182	1-Nonadecene	$C_{19}H_{38}$	1.03	96
16.210	Docosane	$C_{22}H_{44}$	0.52	91
17.491	1-Nonacosene	$C_{29}H_{58}$	0.88	93
17.519	Tetracosane	$C_{24}H_{55}$	1.01	95
18.247	Eicosane	$C_{20}H_{42}$	1.24	95
18.855	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	67.76	98
19.051	Eicosane	$C_{20}H_{44}$	2.01	95
19.975	Octadecane	$C_{18}H_{38}$	1.11	91

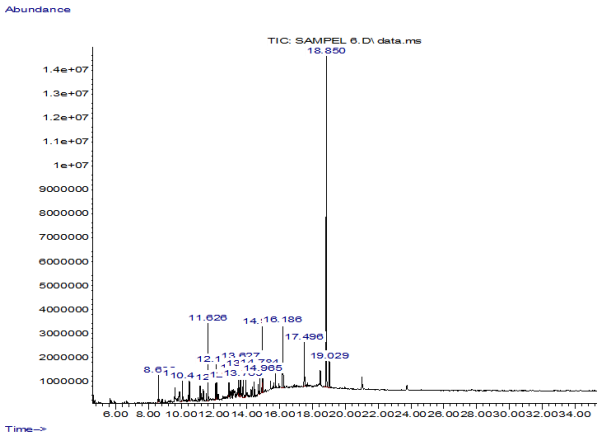

Figure 4. GC-MS chromatogram of fraction 6

Table 7. Identification of compounds from fraction 6

Retention time (tR)	Compounds	Molecular formula	Area (%)	Similarity index (%)
10.500	Tetradecane	$C_{14}H_{30}$	1.55	98
11.627	2.6-bis(1.1-dimethylethyl)-4-methyl-phenol	$C_{15}H_{24}O$	6.21	98
12.095	1-Hexadecane	$C_{16}H_{34}$	1.39	98
12.144	Hexadecane	$C_{16}H_{32}$	2.48	97
12.907	Heptadecane	$C_{17}H_{36}$	1.15	98
13.586	1-Octadecene	$C_{18}H_{36}$	2.47	99
13.628	Octadecene	$C_{18}H_{38}$	2.66	98
13.936	Neophytadiene	$C_{20}H_{38}$	2.07	94
14.965	Eicosene	$C_{20}H_{44}$	1.30	98
16.189	1-Nonadecene	$C_{19}H_{38}$	5.73	99
17.498	Cyclotetracosane	$C_{24}H_{48}$	4.28	99
18.848	1.2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$	50.88	91
19.030	1-Nonacosene	$C_{29}H_{58}$	3.26	93

4. Conclusion

Several drawbacks related to antibiotics to treat bacterial infections have prompted researchers to look for safer natural antimicrobials. *S. platensis* is a promising microalga due to its metabolites content and bioactivity. Extract of ethyl acetate was successfully obtained from *S. platensis* and showed strong antibacterial activity against all tested bacteria, i.e., *P. acne*, *S. epidermidis*, *E. aerogenes*. Based on the result, the extract was more sensitive to Gram-positive bacteria than Gram-negative bacteria, influenced by the structure and component of the bacterial cell wall. Partial purification with the chromatography method showed fraction 2 and fraction 6 as the potential antibacterial fraction for further analysis and identification. The purification process is assumed to enhance the activity of a sample, indicated by the wider inhibition zones formed. GC-MS analysis revealed that the dominant compounds of fractions 2 and 6 were bis (2-ethylhexyl) phthalate (67.76%) and 1.2-Benzendicarboxilic acid, bis (2-ethylhexyl) ester (50.88%), respectively. Further purification is needed to gain a pure compound responsible for the antibacterial activity. Overall, optimizing processes such as extraction methods, solvent selection, and other strategies still need to be evaluated to maximize the activity.

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