ЕКОЛОГІЧНА ФІЗІОЛОГІЯ І БІОХІМІЯ ВОДНИХ РОСЛИН

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ФІТОХІМІЧНИЙ СКЛАД ТА АНТИМІКРОБНА АКТИВНІСТЬ РІЗНИХ МОРСЬКИХ МІКРОВОДОРОСТЕЙ ТА ЦІАНОБАКТЕРІЙ

Порівняльне дослідження біологічно активних речовин, виділених з восьми видів морських мікроводоростей і ціанобактерій, проводили на дев'ятий і дванадцятий день їхнього культивування на F/2 середовищі з морською водою. Найбільший вміст вуглеводів, білків та хлорофілу a зареєстровано у клітинах Spirulina platensis, тоді як максимальний вміст хлорофілу b (34,12 мкг/г сирої маси) — у Nannochloropsis oceanica. Anabaena circinalis характеризувалась максимальним вмістом каротиноїдів (6,66 мкг/г сирої маси). Найвищий вміст ліпідів та фенолів визначено в клітинах Chlorella marina, тоді як максимальний вміст флавоноїдів і танінів — у S. platensis. Екстракти з восьми видів водоростей відрізнялися за своєю антимікробною активністю по відношенню до різних патогенних для людини видів мікроорганізмів, що було обумовлено впливом біологічно активних сполук. Phormidium formosum виявився найбільш активним видом по відношенню до досліджуваних патогенних мікроорганізмів. Екстракти з водоростей вивчали з використанням газової хроматографії мас спектроскопії — GC—MS. Ідентифіковані різні жирні кислоти, алкани та фенольні сполуки, що проявляли антимікробну активність. На основі отриманих даних можна зробити висновок, що морські мікроводорості можуть бути використані як відновлюване джерело цінних біологічно активних сполук у харчовій галузі та медицині.

Ключові слова: *метаболіти водоростей, антимікробна активність, Cyanobacteria, діатомові водорості, зелені водорості.*

Algae are the primary producers and classified into prokaryotic Cyanobacteria and eukaryotic microorganisms capable of using solar energy plus water with carbon dioxide to create biomass, including bioactive compounds [33]. Their growths are 100 times faster than those of terrestrial plants and they have the ability to double their biomass in less than one day [49]. They are considered as good sources of vital compounds like pigments, proteins, polysaccharides, phenol compounds, lipids, minerals, and vitamins utilized in many econo-

mical applications like nutrition, medical, and agricultural products, biofuel, etc. [3, 21]. These biochemical compounds are divided into primary and secondary metabolites according to their biosynthetic origins and chemical functional groups [24]. Carbohydrates of algae improve human health in the form of dietary fibers, anti-obesity drugs, anticoagulants, antimicrobial and antiviral drugs, and antioxidants [52]. Protein from algae is the most important constituent contributing to the food's nutritional value [20]. In addition, lipids of microalgae represent an essential source for fish feeding in the aquaculture industry [1]. Marine microalgae have a wide range of pigments. They are used as natural coloring agents instead of synthetic ones [3]. Carotenoids and phenol compounds are the main antioxidant compounds in microalgae, which are used as nutritional and pharmaceutical products [44]. Moreover, flavonoids and non-flavonoids are important antioxidant and antimicrobial compounds [27]. The biochemical composition of marine microalgae and cyanobacteria depends on algal species taxonomic affiliation, nutrient concentration, culture conditions, and growth stage [53].

Marine microalgal and cyanobacterial extracts exhibited ultimately beneficial effect on human health, including their antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, antihelmintic, and antioxidant activities [4]. Various studies demonstrated the antimicrobial activity of microalgae and cyanobacteria [29, 43].

Recently, the pharmaceutical industries pay attention to the natural compounds, which are extracted from renewable sources, especially from marine organisms. Thus, the objective of the present work was to study the biochemical profile of eight marine microalgal and cyanobacterial species isolated from the Eastern Harbor, Alexandria, Egypt, and also to elucidate antibacterial and antifungal activity of their crude methanol extracts.

Material and Methods

Algal species and cultural conditions.

One marine green microalga *Chlorella marina* Butcher, one golden-brown microalga *Nannochloropsis oceanica* Suda & Miyashita, one diatom *Navicula delicatula* Cleve, and five marine cyanobacterial species, including *Anabaena circinalis* Rabenhorst ex Bornet & Flahault, *Oscillatoria acutissima* Kufferath, *Oscillatoria simplicissima* Gomont, *Phormidium formosum* (Bory et Gomont) Anagnostidis & Komarek, and *Spirulina platensis* (Gomont) Geitler, were isolated from the Eastern Harbour, Alexandria, Egypt and inoculated on the F/2 marine enrichment medium [15]. The culture flasks were aerated by air pumps and incubated at 25±1 °C for axenic green and diatom cultures and at 28±2 °C for cyanobacterial cultures under 16 : 8 light/dark with light intensity of 120 μmol $m^{-2} \cdot s^{-1}$. The microalgal and cyanobacterial cells were taken after 9 and 12 days. The cultivated algae were freeze-dried and grounded into a fine powder. The isolated algae were identified according to the following manuals [13, 16, 37].

Phytochemical characteristics.

Extraction of secondary metabolites.

Totally, ten grams of dried samples were packed in the Soxhlet apparatus and extracted with 100 ml of methanol for 8 hr, then the filtrate (crude extracts) was collected.

Preparation of algal methanol extracts.

About 1 g from each dried algal biomass was homogenized separately in methanol, and was sonicated for 20 min using ultrasonic micro tip probe of 400 watts (ULTRASONIC Get 750) then centrifuged at 4500 rpm for 10 min. Supernatants were collected separately and the pellets were re-extracted twice as mentioned before. Combined supernatants were collected and stored in the refrigerator for analysis. Other supernatants were evaporated to dryness at 40 °C using a rotary evaporator. Dried extracts were kept in labeled sterile vials in a deep freezer at 20 °C for antimicrobial assay [9].

Qualitative analysis.

Phenols, flavonoids, tannins, saponins, triterpenes, phytosterols, coumarins, anthraquinones, and cardiac glycosides were determined in the algal methanol extracts according to standard procedures [41].

Quantitative analysis.

Carbohydrate content in the studied algae was determined by the phenol method [14] using glucose as standard. Protein content was quantified following the Lowry method [28] using bovine serum albumin as standard. Lipid content of the tested algae was extracted and determined by the Daneshvar et al. technique [11].

The microalgal chlorophylls *a* and *b* and carotenoids were detected in the methanol extracts according to [23]. The Ritchie method [39] was used to estimate chlorophyll *a* and carotenoid content in the isolated cyanobacterial species. The pigment content in the tested microalgal species was calculated using the MacKinney equations [30].

$$\textbf{Chl}_{\textbf{a}}\ \textbf{(Chlorophyta spp.)} = \frac{10.3\,\mathrm{E665} - 0.918\,\mathrm{E650}}{\mathrm{wt.}}\ \mathrm{mg/g};$$

$$\textbf{Chl}_{\textbf{b}}\ \textbf{(Chlorophyta spp.)} = \frac{19.\,\mathrm{E650} - 3.87\,\mathrm{E665}}{\mathrm{wt.}}\ \mathrm{mg/g};$$

$$\textbf{Carotenoids (Chlorophyta spp.)} = \frac{4.2\,\mathrm{E452} - (0.0246\,\mathrm{Chl.\ a} + 0.426\,\mathrm{Chl.\ b})}{\mathrm{wt.}}\ \mathrm{mg/g}.$$

The equations of Wellburn [51] were used for estimating the pigment content in the tested cyanobacterial species.

$$\textbf{Chl}_{\textbf{a}}\ \textbf{(Cyanobacteria spp.)} = \frac{12.9447(\mathrm{A665} - \mathrm{A720})}{\mathrm{wt.}}\ \mathrm{mg/g};$$

$$\textbf{Carotenoids (Cyanobacteria)} = \frac{1.000(\mathrm{A}_{470} - \mathrm{A}_{720}) - 2.86(\mathrm{Chl}_{\mathrm{a}})}{\mathrm{wt.}}\ \mathrm{mg/g}.$$

The total content of phenols in algal methanol extracts was evaluated using the Folin-Ciocalteu method [17]. The result was expressed as gallic acid equivalent (GAE/g of dry weight). Total flavonoid content was estimated as described in [17]. The content of tannins was determined according to [8]. The total concentration of flavonoids and tannins was expressed in mg of catechin equivalents (CE)/g of dry weight.

Antimicrobial activity assay.

The bioactivity of the methanol crude algal extracts was examined against four gram negative bacteria *Aeromonas hydrophila*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*, two gram positive bacteria *Enterococcus faecalis* and *Staphylococcus aureus*, and one fungal species *Candida albicans*, using the disc diffusion method of the Kirby-Bauer technique [32]. Nutrient agar plates were uniformly inoculated with bacterial cultures 150 CFU/ml using cotton swabs. About $0.75 \cdot 10^6$ fungal spores/ml were aseptically swabbed on the Czapex-Dox plates. Sterilized discs (6 mm) made of the Whatman N 1 filter paper were loaded by the extracts (10 mg/ml) and dried completely under sterile conditions and then incubated at 37 °C for 24 hr for bacterial and at 28 °C for 48—72 hr for fungal species, respectively. Dimethyl sulfoxide (DMSO) and ampicillin (10 mg/ml) represented negative control and positive control, respectively. The antimicrobial activity was measured as the diameter of the clear zone including the diameter of the paper disc (mm). All estimated antimicrobial results are the average of triplicate analyses.

GC-ISQ mass spectrometer analysis of algal methanol extracts.

The GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) was used to identify the bioactive compounds in different methanol extracts. The GC- ISQ analysis was performed using a capillary column TG—5MS (30 m × 0.25 mm × 0.25 μm film thickness). The column temperature was initially held at 55 °C and then increased by 5 °C/min to 250 °C, withhold 2 min, and then increased by 25 °C/min to 300°C. The injector temperature was kept at 270 °C. Helium was used as gas carrier at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 μl were injected automatically using the Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of 50—650 m/z in full scan mode. The components were identified by comparison of their retention times and mass spectra with those of the WILEY 09 and NIST14 mass spectral database [6].

Statistical analysis.

All results are expressed as means±standard deviation (SD) in % of dry weight (DW) basis. The differences between the algal bioactive compounds were detected by Anova: two-factor analysis with replication using Microsoft Excel office 2016. A significant difference was considered at the level of $p<0.05$.

Results and Discussion

Qualitative analysis of phytochemicals.

Marine microalgae are among the most natural sources of food, drugs, and cosmetic industry due to their phytochemical composition. As shown in Tab-

Table 1

Qualitative determination of phytochemical components in the tested algae and Cyanobacteria

Phytochemicals	Cyanobacteria					Microalgae		
	Anabaena circinalis	*Oscillatoria simplicissima*	*Oscillatoria acutissima*	*Phormidium formosum*	*Spirulina platensis*	*Chlorella marina*	*Nannochloropsis oceanica*	*Navicula delicatula*
Phenols	++	+	++	+	++	++	++	+
Flavonoids	+	+	+	+	++	+	++	+
Tannins	+	+	++	++	++	++	+	+
Saponins	—	—	+	—	+	+	—	—
Triterpenes	+	+	+	+	+	+	+	+
Phytosterols	+	+ +	+	+	+ +	+	+	—
Coumarins	+	+	+	+	+ +	+ +	+	+
Anthraquinones	+	—	—	—	—	—	—	—
Cardiac glycosides	—	+	—	—	+	+	—	—

N o t e. High (++), moderate (+) or nil (—) based on the intensity of the color produced from the reactions.

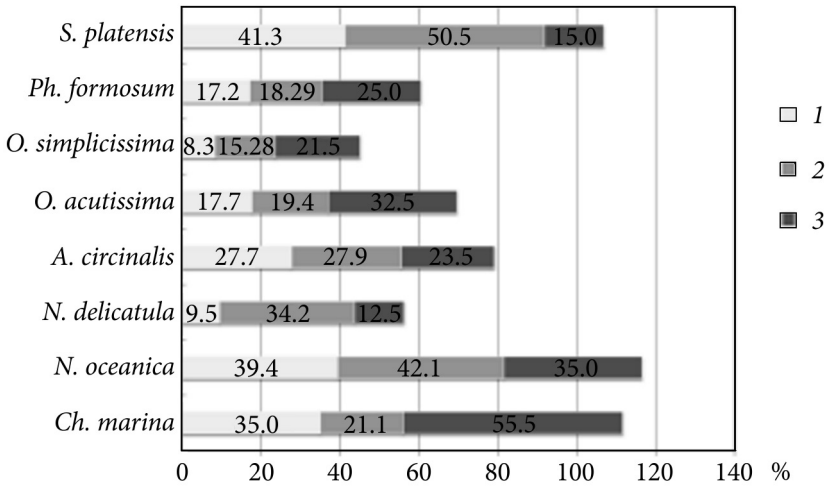

Fig. 1. The proximate composition of the tested algae, %: *1* — carbohydrates; *2* — proteins; *3* — lipids.

le 1, the preliminary phytochemical constituents of methanol extracts showed a variation between algal species. These variations can be related to algal species, cultural conditions, extraction method, and the used solvent [40, 53]. Phenols, flavonoids, tannins, terpenes, phytosterols and coumarins were present in all tested microalgae. These secondary metabolites have a wide range of antimicrobial, antioxidant, and anti-inflammatory activities [27]. Saponin was detected in *Ch. marina*, *O. acutissima*, and *S. platensis* used in animal feeding. Glycosides can be used in the treatment of some heart diseases, e.g. cardiac arrhythmia [5]. It was observed in the previous three species.

Quantitative composition.

The proximate composition of the eight identified microalgae and cyanobacteria is shown in fig. 1. The maximum value of carbohydrate and protein content was detected in *S. platensis* (41.3 % and 50.5 %) and the lowest value was determined in *O. simplicissima* (8.3 and 19.4 %). The present results suggest that protein was the major biochemical component in all tested algae. Similar result was reported by Costard et al. [10]. Santhosh et al. [42] recommended the protein content of *S. platensis* and *Ch. marina* as functional foods preventing damage of tissue and diseases. The highest content of lipids was registered in *Ch. marina* (55.5 %) and their lowest content — in *N. delicatula* (12.5 %). The concentration of photosynthetic pigments is presented in fig. 2. Among cyanobacterial species, the highest chlorophyll *a* content (31.25 μg/g of fresh weight) was in *S. platensis*, whereas the highest content of carotenoids (6.66 μg/g of fresh weight) — in *A. circinalis*. *N. oceanica* had the highest content of chlorophyll *a* and *b* (23.75 and 34.12 μg/g of fresh weight) and *N. delicatula* contained the maximum amount of carotenoids (1.8 μg/g of fresh weight) among the tested microalgae. The difference in chlorophyll content may be due

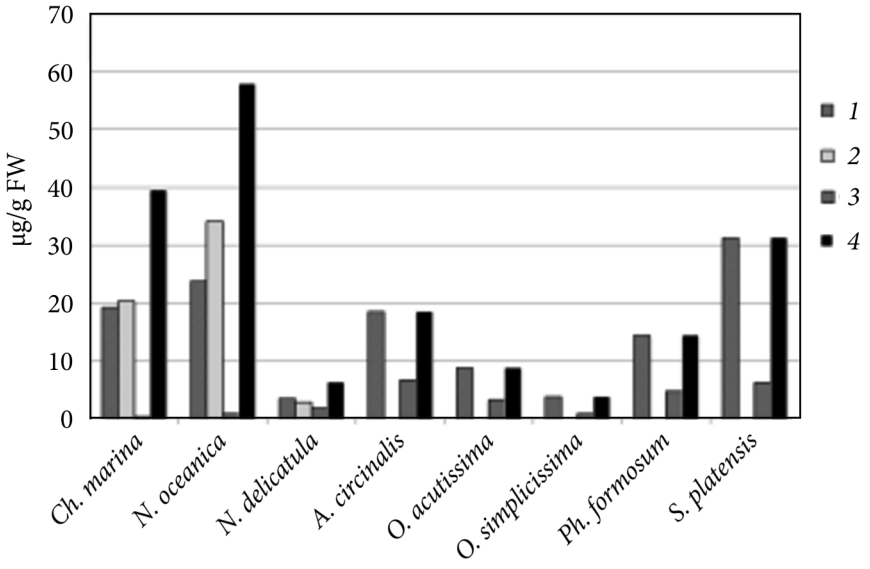

Fig. 2. The content of photosynthetic pigments in the tested algae, µg/g of fresh weight: *1* — chlorophyll *a*; *2* — chlorophyll *b*; *3* — carotenoids; *4* — the total content of chlorophylls.

to cell density as demonstrated by Valenzuela-Espinoza et al. [50], who reported that high chlorophyll *a* content can be accounted for by high cell density. Moreover, the determined chlorophyll values were lower than those estimated by Talero et al. [48], who stated that the content of carotenoids in marine microalgae was 0.2 %.

Figure 3 shows a variety of bioactive compounds such as phenols, flavonoids and tannins in the purified algae. Significant difference in the content of bioactive compounds in the tested algae was detected at $p<0.05$ (F value 13.90). Among the eight tested species, the highest concentration of phenols was found in *Ch. marina* (129.533 mg GAE/g of dry weight), and also in *S. platensis* (74.9 mg GAE/g of dry weight). The highest content of tannins was recorded for the marine cyanobacterial species *S. platensis* (45.8 mg GAE/g of dry weight), which was followed by *Ch. marina* (38.2 mg GAE/g of dry weight). In this case, their lowest content was registered in *O. simplicissima* (5.1 mg GAE/g of dry weight). Generally the detected phenol, flavonoid and tannin values were lower than those detected by Stephen et al. [46]. The significant variation in the biochemical constituents of the collected algae may be related to algal species and their physiological state [22].

Antimicrobial activity.

Microalgae and cyanobacteria are a renewable source of novel active metabolites with different biological activities such as antibacterial, antifungal, antioxidant, and antitumor properties [26, 34]. The crude methanol extracts of the cultured algal species were screened against various pathogens and the obtained results are illustrated in Table 4. The methanol extract of *Ph. formosum*

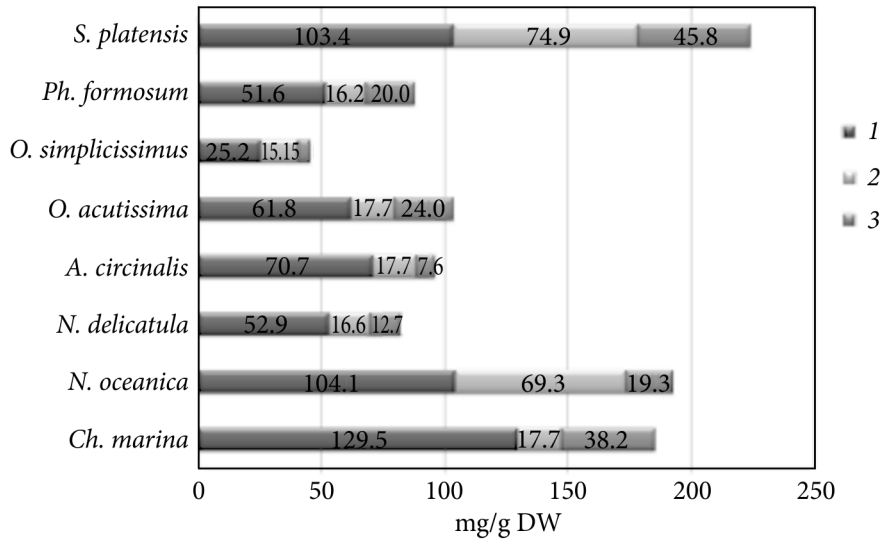

Fig. 3. The total content of phenols, flavonoids, and tannins in the tested algae, mg/g of dry weight: *1* — phenols, mg GAE/g DW; *2* — flavonoids, mg CE/g DW; *3* — tannins, mg CE/g DW.

(18—22 mm) exhibited a broad spectrum of antimicrobial activities inhibiting the growth of most tested microorganisms (fig. 4). It was followed by *O. simplicissima* (13—19 mm), *O. acutissima* (10—16 mm), and *N. delicatula* (7—14 mm). The estimated activity of most tested algae was higher than antimicrobial activity of *Ph. fragile* (12—13 mm) [25]. While, *Ch. marina* and *N. oceanica* showed relatively moderate activity toward *S. aureus* and *P. aeruginosa*, *E. coli* and were less effective in relation to *A. hydrophila* and *Vibrio cholera*. Similarly, Srinivasakumar and Rajashekhar [45] demonstrated that *Ch. marina* methanol extracts possessed the maximum inhibitory activity in a study against *P. aeruginosa*, *P. fluorescens*, and *S. typhi*.

The antimicrobial activities of the tested algae could be attributed to their functional compounds such as phenols, terpenes, carbohydrates, and fatty acids as illustrated in tables 1, 2, and 3. It has been found that these substances are responsible for cellular membrane disruption, interference with some microbial metabolic processes, and also for the variation of gene expression plus signal transduction. In particular, algal fatty acids can initiate peroxidative processes and prevent bacterial fatty acids synthesis [12]. Also, free fatty acids and fatty acid methyl ester (FAME) caused leakage of molecules from microbial cells via interaction with cellular membranes of microbial cells and reduction of their nutrient uptake or inhibition of their respiration [47]. These compounds probably act together, either in an independent or synergistic manner. Methanol extracts of all tested algae, with the exception of that of *N. oceanica*, showed antifungal activity against *C. albicans*. The maximum antifungal inhibition zone was observed with *Ph. formosum* and *O. simplicissima* (20—

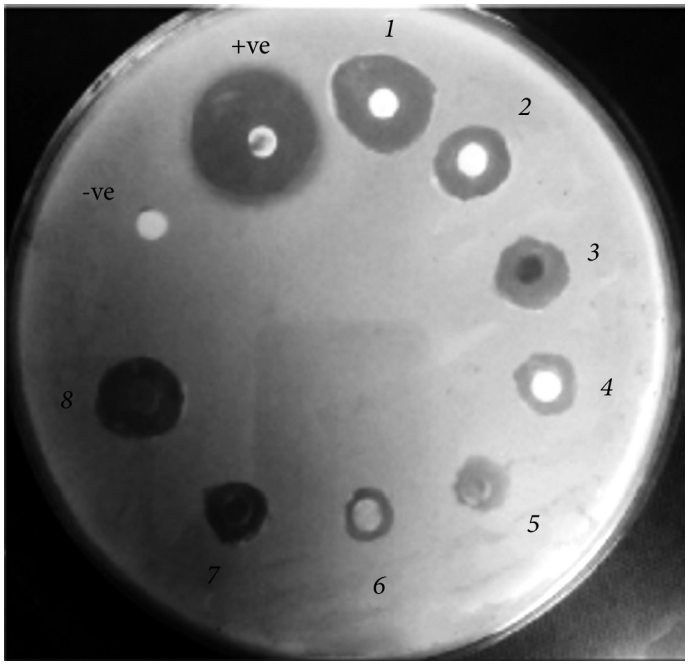

Fig. 4. Inhibition zone of the methanol extract of *Phormidium formosum* (*1*); *Oscillatoria simplicissima* (*2*); *Oscillatoria acutissima* (*3*); *Navicula delicatula* (*4*); *Spirulina platensis* (*5*); *Chlorella marina* (*6*); *Nannochloropsis oceanica* (*7*), and *Anabaena circinalis* (*8*) against the pathogenic bacterium *Pseudomonas aeruginosa*. Positive control (ciprofloxacin 10 mg) and negative control (dimethyl sulfoxide — DMSO).

19 mm) against *C. albicans*. This observation is in agreement with Kumar et al. [25]. The marine microalga *Oscillatoria* sp. showed better antifungal than antibacterial activity [38].

Gas chromatography-mass spectrometry (GC-MS).

A comparative study of the compounds present in the tested algal methanol extracts was performed through GC-MS (tabl. 2). The name, retention time, molecular weight, and biological activity of the components were reported. Totally 49 biochemical compounds were detected and many of them were present in trace ratio. The maximum number of bioactive compounds was found in *A. circinalis* (31 compounds) followed by *N. oceanica* (30 compounds), *N. delicatula* (28 compounds) and *Ph. formosum* (28 compounds), *Ch. marina* (24 compounds), *O. simplicissima* (21 compounds), *S. platensis* (15 compounds) and *O. acutissima* (10 compounds). However a few of them were predominant. Commonly major peaks appeared at 7.03, 8.82, 10.83, 27.09, 28.87, 32.08, 32.32, and 32.7 retention time in all algal extracts (tabl. 3), which corresponded to the prevailing compounds, including eucalyptol, levomenthol, 2,6,10-trimethyl,14-ethylene-14-pentadecene, palmitic acid and 9-octadecenoic acid (Z)-, methyl ester respectively. There are many reports documented the biological properties of these compounds such as antibacterial, antifungal,

Table 2

GC-MS analysis of algal methanol extracts

№	Biochemical composition	RT	Cyanobacteria					Microalgae		
			A. circinalis	*O. simplicissima*	*O. acutissima*	*Ph. formosum*	*S. platensis*	*Ch. marina*	*N. oceanica*	*N. delicatula*
			Peak %							
1.	à-Pinene	4.79			0.81					
2.	1-Tetradecanol	6.22				0.49		0.55	0.45	0.79
3.	Phenol	6.28	0.88		0.66					1.07
4.	Benzothieno[2,3-c]quinolin-6(5h)-one, 2-methoxy	6.44						0.54		
5.	6,9,12-Octadecatrienoic acid, methyl ester	6.62							0.48	
6.	Eucalyptol	7.03	8.42	45.18	21.02	8.83			10.58	12.39
7.	Undecane	8.82	2.19		1.4	2.36	1.11	2.15	2.55	3.24
8.	4H-1,3-Benzodioxin	10.13				0.28				
9.	9,12,15-Octadecatrienoic acid, 2-Phenyl-1,3-Dioxolan-4-yl) Methyl	10.49								0.34
10.	Cyclopentasiloxane, decamethyl-	10.51	0.68			0.5		0.46	0.69	
11.	Levomenthol	10.83	4.06	6.43	6.4	4.24		4.31	5.09	5.82
12.	1-DODECENE	11.33								0.24

Table 2 (continued)

№	Biochemical composition	RT	Cyanobacteria					Microalgae		
			A. circinalis	*O. simplicissima*	*O. acutissima*	*Ph. formosum*	*S. platensis*	*Ch. marina*	*N. oceanica*	*N. delicatula*
13.	(-)-Carvone	12.75					0.81			
14.	Cyclohexasiloxane, dodecamethyl-	15.18	0.7			1.29		0.84	2.32	
15.	Cyclohexane, 1,4-dimethyl-2-octadecyl-	16.02				0.37		0.28	0.9	
16.	Hexadecenoic acid, Z-11-	16.72	0.18						0.2	0.33
17.	Cycloheptasiloxane, tetradecamethyl-	19.57	1.67			2.32			2.77	0.24
18.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	20.06	0.47					0.56	0.4	
19.	1-Nonadecene	21.65	0.22			3.94		0.36		0.45
20.	Tetradecanal	22.11	0.32						0.9	
21.	Cyclooctasiloxane, hexadecamethyl-	23.55	1.62			1.87		1.39	1.73	
22.	Eicosane	24.11	1.84	0.99			17.15	0.96	0.3	
23.	Methyl tetradecanoate	24.67	0.39		0.33	0.39	0.63	0.4	0.59	1.16
24.	Heneicosane	25.91			0.64					
25.	Dodecane, 5,8-diethyl-	26.11			0.45					

Table 2 (continued)

№	Biochemical composition	RT	Cyanobacteria					Microalgae		
			A. circinalis	*O. simplicissima*	*O. acutissima*	*Ph. formosum*	*S. platensis*	*Ch. marina*	*N. oceanica*	*N. delicatula*
26.	13,16-Octadecadienoic acid, methyl ester	26.82								0.28
27.	2,6,10-Trimethyl,14-ethylene-14-pentadec-6E-ene	27.09	9.21	0.79	2.8	2.87		16.23	2.85	2.27
28.	2-Pentadecanone, 6,10,14-trimethyl-	27.21	0.52					2.39	0.72	
29.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	27.6	2.59		0.95	1.22		6.24	1.05	1.1
30.	1-Octadecyne	27.96	3.93		0.95	1.9		10.48	4.17	1.76
31.	(Z)-Methyl hexadec-11-enoate	28.42	2.3		1.61	1.57		0.67	1.83	2.48
32.	Cyclopropanebutanoic acid	28.65	0.4			0.39		0.67	0.43	0.45
33.	Hexadecanoic acid, methyl ester	28.87	9.8	6.04	8.03	13.68	14.41	6.08	12.38	11.77
34.	6-Ethyl-5-Hydroxy-2,3,7-Trimethoxynaphthoquinone	29.18	0.71	1.79	1.65	1.91	3.21	0.69	0.77	1.61
35.	Oxiraneundecanoic acid, 3-pentyl-, methyl	30.83	0.32			1.32	0.53			
36.	Ethyl 9,12-Octadecadienoate	31.86	0.72		0.6	0.51	0.59	0.52	0.42	1.14
37.	9-Octadecenoic acid (Z)-, methyl ester	31.98	0.22		0.33	0.39	0.67	0.28	0.25	0.6

Table 2 (continued)

№	Biochemical composition	RT	Cyanobacteria					Microalgae		
			A. circinalis	*O. simplicissima*	*O. acutissima*	*Ph. formosum*	*S. platensis*	*Ch. marina*	*N. oceanica*	*N. delicatula*
38.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	32.08	7.73	7.93	8.08	10.49	21.77	5.3	7.8	9.29
39.	9-Octadecenoic acid (Z)-, methyl ester	32.2	17.69	15.22	17.81	21.22	23.75		18.2	24.45
40.	10-Octadecenoic acid, methyl ester	32.33	2.72	1.05	1.21	2.32	0.88		2.23	2.82
41.	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	32.43	0.85				2.21		0.44	
42.	Methyl stearate	32.7	2.78	2.2	2.49	3.52	4.7			3.69
43.	9,12-Octadecadienoic acid	32.95	1.43			1.16				
44.	Ethyl 9-Octadecenoate	33.42			0.83					
45.	Heptasiloxane tetradeca methyl-heptasiloxane	35.55				0.79		0.56		0.39
46.	9-Octadecenoic acid (z)-	37.39								0.34
47.	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	38.59					0.72			
48.	Dotriacontane	39								0.37
49.	1,2-Benzenedicarboxylic Acid	39.74							0.68	
	Number of bioactive compounds		31	10	21	28	15	24	30	28

Table 3

Major phytochemical compounds identified in algal methanol extracts

RT	Compound	Molecular formula	Molecular weight	Nature	Biological activity
7.03	Eucalyptol	$C_{10}H_{18}O$	154		Antimicrobial activity [18]
8.82	Undecane	$C_{11}H_{24}$	156		Antibacterial and anti-tumor activities [2]
10.83	Levomenthol	$C_{10}H_{20}O$	156		Antifungal activity [36]
27.09	2,6,10-trimethyl -14-ethylene-14 -pentadecane	$C_{20}H_{38}$	278		Antimicrobial and other biological activities [35]
28.87	Hexadecanoic acid, methyl ester "palmitic acid"	$C_{17}H_{34}O_2$	270		Antimicrobial activity [7]
32.08	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294		Antimicrobial and other biological activities [31]
32.32	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296		Many biological activities [35]
32.7	Methyl stearate	$C_{19}H_{38}O_2$	298		Antifungal and antioxidant activities [36]

Table 4

Antimicrobial activity of algal methanol extracts against different pathogenic microorganisms

Standard antibiotics	Diameter of inhibition zone (mm)						
	Gram (+V) bacteria		Gram (-V) bacteria				Fungal sp.
	S. aureus	*E. faecalis*	*P. aeruginosa*	*A. hydrophila*	*V. cholera*	*E. coli*	*C. albicans*
Ciprofloxacin (+ve)	20±0.01	22±0.02	22±0.02	22±0.02	22±0.02	22±0.02	20±0.01
DMSO (-v)	—	—	—	—	—	—	—
Cyanobacteria							
A. circinalis	9±0.2	—	16±0.2	—	9±0.2	19±3	5±0.1
O. acutissima	12±0.2	10±0.2	14±0.2	16±0.2	13±0.2	15±0.2	10±0.2
O. simplicissima	16±0.3	15±0.3	13±0.2	18±0.3	17±0.3	17±3	19±0.3
Ph. formosum	18±0.3	19±0.3	19±0.3	20±0.3	22±0.3	20±3	20±0.3
S. platensis	6±0.2	8±0.1	10±0.1	5±0.1	—	12±0.02	9±0.2
Microalgae							
Ch. marina	7±0.2	5±0.1	10±0.1	—	—	10±0.1	8±0.2
N. oceanica	5±0.2	—	11±0.1	—	—	13±0.2	—
N. delicatula	9±0.2	10±0.2	12±0.2	12±0.2	14±0.2	13±0.3	7±0.1

anti-inflammatory, anticancer, antihistaminic, antiarthritic, antiandrogenic, and antioxidant properties [35, 36]. In addition, there are many unsaturated fatty acids, alkenes, and esters with antibacterial and antifungal activities [7, 19].

Conclusion

The phytochemical screening of the algal extracts showed the presence of a wide variety of primary and secondary metabolites differing in their concentration. The obtained results suggest that these algae can be recommended as renewable dietary supplements — especially *S. platensis* and *Ch. marina*. Moreover, the methanol extract of *Ph. formosum* exhibited the best antimicrobial efficacy *in vitro* comparing to others selected algae so it might be exploited to isolate novel antimicrobial products. From a practical point of view, more studies are required to detect the suitable cultural conditions to obtain high concentrations of bioactive compounds. In addition, the use of genome modifying tools may improve the productivity of marine microalgae, and also of their bioactive compounds. Further purification and fractionation of algal extracts can gain many active, safe and renewable drugs.

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Abstract

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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF VARIOUS MARINE MICROALGAE AND CYANOBACTERIA

The comparative study of bioactive compounds in eight marine microalgae and cyanobacteria grown on the F/2 marine enrichment medium was performed after 9th and 12th days of exposure. The highest content of carbohydrates, proteins, and chlorophyll a was observed in Spirulina platensis, whereas the highest content of chlorophyll b (34.12 μg/g of fresh weight) — in Nannochloropsis oceanica. Anabaena circinalis was characterized by the maximum content of carotenoids (6.66 μg/g of fresh weight). The highest content of lipids and phenols was determined in Chlorella marina, whereas the maximum content of flavonoids and tannins — in S. platensis. Methanol extracts of the eight algal species exhibited various antimicrobial activities against different human pathogenic microbial species, which were linked to their bioactive compounds. Phormidium formosum was found to be the most active species toward all tested pathogenic microorganisms. The methanol extracts of the algae were chemically characterized by gas chromatography mass spectroscopy — GC—MS. Different fatty acids, alkanes and phenol compounds with antimicrobial activity were identified, such as palmitic acid, octadecenoic acid, 3,7,11,15-tetramethyl-2-hexadecen-1-ol neophytadiene, levomenthol, and eucalyptol. Based on the obtained results, it is possible to conclude that marine microalgae could be a renewable source of valuable bioactive compounds for nutritional and health products.

Keywords: *algal metabolites, antimicrobial activity, Cyanobacteria, diatoms, green microalgae.*