Iran. J. Ichthyol. (Special Issue 2023): 77-84

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

Identification and characterization of bioactive compounds in two algae species of *Haematococcus pulvialis* and *Dunatiella saline* from the waterbodies of Basrah region, Iraq

Mahmood Shakir HASHIM*
Department of Marine Biology, Marine Science Center, University of Basrah, Basrah, Iraq.
Email: mahmood.hashim@uobasrah.edu.iq

Abstract: This study was conducted to identify and characterize bioactive compounds in two algae Chlorophyta species of *Haematococcus pulvialis* and *Dunatiella saline* collected from water bodies in the Karmat Ali area, Basrah Governorate, southern Iraq. The results revealed differences between them in the volatile compounds. A total of 65 peaks of volatile compounds were found in *H. pulvialis*, as the highest percentage was Hexadecanoic acid, ethyl ester, followed by 8-Heptadecene, n-Hexadecanoic acid, and Hexadecanoic acid, methyl ester. In the *D. saline*, 55 peaks of volatile compounds were recorded, such as 2 (4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,(R)-, n-Hexadecanoic acid, Oleic Acid, Vitamin E, and other compounds.

Keywords: Bioactive compounds, Gaschromatography–mass spectrometry, Aquatic ecosystem, Metabolites.

Citation: Hashim, M.S. 2023. Identification and characterization of bioactive compounds in two algae species of *Haematococcus pulvialis* and *Dunatiella saline* from the waterbodies of Basrah region, Iraq. Iranian Journal of Ichthyology (Special Issue 1): 77-84.

Introduction

Algae can produce biologically active substances (Blunt et al. 2008; Cabrite et al. 2010) that are an important source for the production of many bioactive compounds that synthesize them as secondary metabolites playing important roles as antibacterial, antifungal, antiviral (Najdenski et al. 2013; Komatsu et al. 2013), reducing lipid (Panahi et al. 2016), antioxidant and the treatment of cancer cells (Silva et al. 2019). Studies have shown that algae possess high efficiency in producing a group of antibiotics that directly affect resistance to many diseases. They produce two types of effective compounds, the first inside their cells, known as intracellular products, and the second outside their cells, as extracellular products. The algae extracts are different according to their different chemical composition of amino and fatty acid sugars. For example, algae of the genus *Prototheca* produce lactic acids with small amounts of succinic acid, and the genus *Chlorella* can produce acetic and lactic acids (Kim et al. 2006).

Algae have been used in medical and pharmaceutical applications since ancient times, as the first use of algae by the Chinese dates back to about 2700 BC (Hoppe 1979). Considering that specific algal species were used in the treatment of people with Gangrene in Africa because they contain effective substances and anti-growth of some bacteria. Algae constitute a source of about 9% of the biomedical compounds obtained and used in the medical fields (Jha & Zi-Rong 2004). They are also used in cosmetics and foodstuffs (Demirel et al. 2012). Algae are characterized by their ability to live in different environments; they are found in fresh and salty waters. They are either phytoplankton floating on the surface of the water, or they are attached to the animal's bodies as Epizoic. They are also attached to

plants as Epiphytic, sand Epipsamic, or mud Epipelic, or attached to rocks Epilithic. They can withstand a wide range of different environmental factors, some of which live in environments with high temperatures, i.e., thermophilic algae, and some in frozen environments, i.e., karyophylic algae, and a wide range of pH (6.00-10) (Belcher & Swale 1976).

Algae can produce many active substances: antifungal, antibacterial, antiviral, immune-stimulating, enzyme inhibiting, antioxidants, cytotoxic activities, and anti-cancer (Ghasemi et al., 2004). Chlorophyta or green algae are found in different environments, tolerating harsh environmental conditions (Chorus & Bartram 1999; Whitton & Potts 2000; Falconer 2005). They are also found in dry environments in the soil, on rocks, sand, and mud. Correspondingly it can be found in symbiosis with some fungi, plants, and animals (Thajuddin & Subramanian 2005; Baracaldo et al. 2005; Bacher et al. 2015).

Chlorophyta grows in fresh, saltwater, and soil and is similar to blue-green algae, cyanobacteria, in terms of environment, external appearance, and photosynthesis. However, they are prokaryotic bacteria and appear in single cells, filamentous, branched (true or false) or unbranched, or colonies. In the food chain, it has a distinctive feature for them from other algae species (Ling 2000; Bullerjahn & Post 2014). Based on the above-mentioned background, this study aimed to identify and characterize bioactive compounds in two algae Chlorophyta species of *Haematococcus pulvialis* and *Dunatiella saline* collected from water bodies in the Karmat Ali area, southern Iraq.

Materials and methods

Sampling: Samples of *Haematococcus pulvialis* and *Dunatiella saline* were collected from water bodies in the Karmat Ali area in Basrah Governorate, southern Iraq, and were transferred to the laboratory based on Stein (1973), and algae purified from other microorganisms as follow: washing algae with running water several times to remove the mud, then washing it with sterilized distilled water several times. The live mass of the algae was washed according to Weidman et al. (1984) 12 times, then placed on Wattman No. 1 filter papers to dry at room temperature. Then, they were Lyophilization using a lyophilizer, and after drying, the samples were ground with an electric mill, placed in plastic containers, and kept in the refrigerator.

Preparation of the methanolic extract: The methanolic extract was prepared by mixing 50 g of algae powder with 250ml of methanolic alcohol. Then the solution was shaken for 24 hours using a magnetic stirrer. Subsequently, the mixture was filtered using Whattman No. 1 filter papers and the solution was concentrated using a rotary evaporator at 50°C, then kept in the refrigerator until use (Rios et al. 1987).

Bioactive compounds identification using gas chromatography-mass spectrometry:

The bioactive compounds were identified in the extract isolated from algae using the (GC-MS) according to Masayoshi et al. (2014).

Results and discussion

Identification of volatile active compounds: The mass spectrometry results are shown the volatile compounds identified in the studied algae (Figs. 1-2; Tables 1-2). Each peak in the figure shows a compound of the volatile compounds. In Figure 1, a total of 65 peaks of volatile compounds of *H. pulvialis* are represented, and their characterizations are shown in Table 1. Based on the results, the highest value was peak 30, representing Hexadecanoic acid, ethyl ester, followed by peak 15, 8-Heptadecene, peak 29, n-Hexadecanoic acid, and peak 26, Hexadecanoic acid, methyl ester. Other compounds, including Glycerol 1-palmitate and Oxiraneoctanoic acid, 3-octyl-, methyl ester and oleic acid, are explained by peaks 51, 42, and 37, respectively.

Based on the results, a total of 55 peaks of volatile compounds are found in *D. saline*, which is shown in Figure 3 and Table 2. The highest value was found

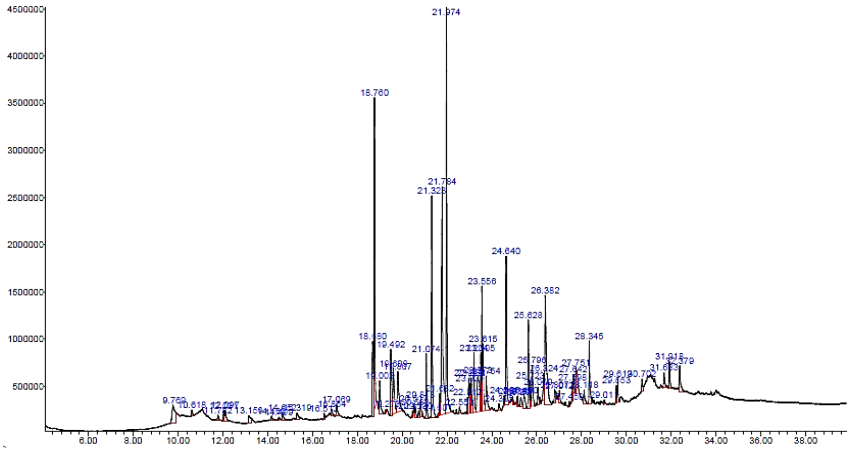

Fig.1. The volatile compounds by GC-MS technology for *Haematococcus pulvialis*.

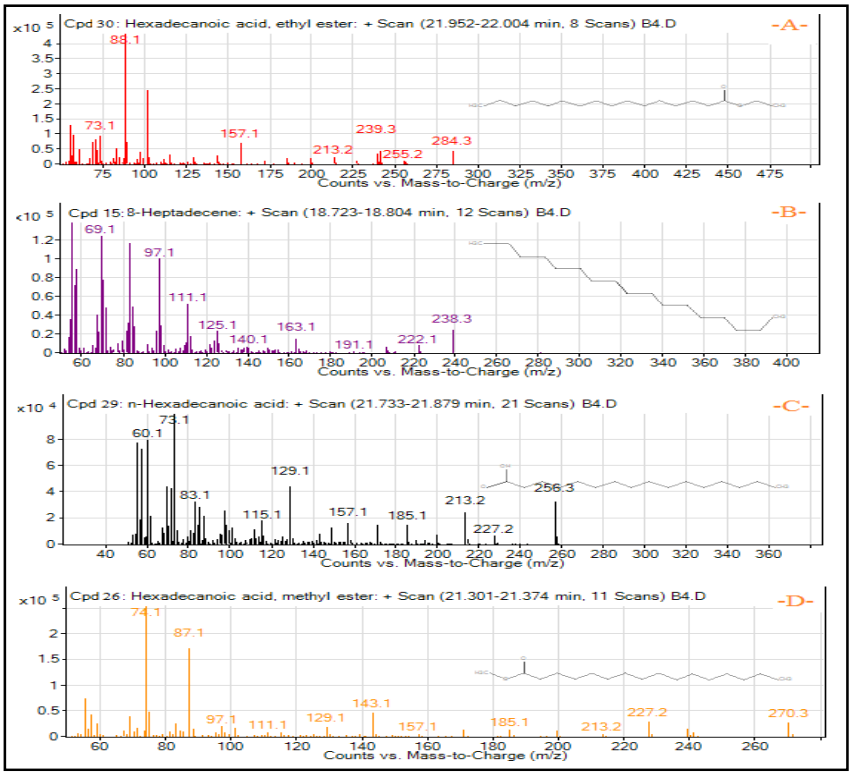

Fig.2. Mass spectrometry that includes the structural formula of the most important compounds separated from the alga *Haematococcus pulvialis*.

for peak 20 represented by 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,(R)-.; followed by peak 36, n-Hexadecanoic acid, then, peak 38 i.e. Oleic acid, peak 50 Vitamin E. Other peaks were 57, 25, and 14 as Phytyl palmitate and 2-Cyclohexen-1-one,2,4,4-trimethyl-3-(3-oxo-1-butenyl)-3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- and Propylamine.N-[9-borabicyclo[3.3.1 [non-9-yl]-respectively. Algae produce many bioactive substances (Ghasemi et al. 2004), such as primary and secondary metabolites (Patel et al. 2015). Because of the huge biodiversity of algae, its presence in open environments, and their exposure to different environmental conditions, they produce a wide range of biologically active compounds that cannot be found in other organisms (Plazaa et al. 2010).

Algae have multiple defense strategies to resist different environmental conditions, which are

Table 1. Retention time and area of volatile compounds separated by GC-MS technology for *Haematococcus pulvialis*.

Peak	R.T.	Area %	Library/ID
1	9.769	1.8756	Hexanoic acid
2	10.618	0.2342	Heptanoic acid
3	11.782	0.2296	Decanoic acid, propyl ester
4	12.031	0.3073	2-Hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone
5	12.097	0.4812	2-Cyclohexen-1-one, 5-methyl-2-(1-methylethyl)-
6	13.159	0.4831	2-Hydroxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione
7	14.169	0.1568	2-Amino-5H-pyrrolo[3,4-d]pyrimidine-4,7(3H,7H)-dione
8	14.499	0.1079	7a-Methyl-3-methylenehexahydrobenzofuran-2-one
9	14.652	0.37	Formamide, N-(4,6-diamino-5-pyrimidinyl)-
10	15.319	0.3488	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-1-methoxy-
11	16.512	0.2088	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-
12	16.864	0.2285	2,4-Di-tert-butylphenol
13	17.069	0.5674	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-
14	18.68	3.1434	2-Cyclohexen-1-one, 2,4,4-trimethyl-3-(3-oxo-1-butenyl)-
15	18.76	7.4468	8-Heptadecene
16	19.002	1.4732	Heptadecane
17	19.273	0.1323	2-Acetamido-d-mannitol
18	19.492	2.8489	2-(1-Cyclohexenyl)cyclohexanone
19	19.609	2.1587	Phenol, 2,3,5-trimethyl-
20	19.807	2.3953	Thymoquinone
21	20.459	0.1357	6-Octen-1-ol, 3,7-dimethyl-, formate
22	20.517	0.2821	2-Pentadecanone, 6,10,14-trimethyl-
23	20.583	0.3927	1-Hexacosanol
24	20.818	0.4817	Z-5-Nonadecene
25	21.074	1.5893	Nonadecane
26	21.323	6.258	Hexadecanoic acid, methyl ester
27	21.601	0.1161	8-Hexadecenal, 14-methyl-, (Z)-
28	21.682	0.9831	Dibutyl phthalate
29	21.784	10.4119	n-Hexadecanoic acid
30	21.974	9.4082	Hexadecanoic acid, ethyl ester
31	22.553	0.2214	Cyclopentane, 2-isopropyl-1,3-dimethyl-
32	22.912	0.365	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
33	22.963	0.9713	11-Octadecenoic acid, methyl ester
34	23.021	0.9414	Palmitoleic acid
35	23.08	1.1266	Phytol
36	23.204	1.7221	Methyl stearate
37	23.373	2.919	Oleic Acid
38	23.505	1.422	9,12-Octadecadienoic acid (Z,Z)-

Table 1. Continued.

Peak	R.T.	Area %	Library/ID
39	23.556	4.7929	(E)-9-Octadecenoic acid ethyl ester
40	23.754	1.5127	Ethyl 5-amino-1-methylpyrazole-4-carboxylate
41	24.325	0.5101	Cyclopropaneoctanal, 2-octyl-
42	24.64	4.7152	Oxiraneoctanoic acid, 3-octyl-, methyl ester
43	24.932	0.2324	Methyl 18-methylnonadecanoate
44	25.145	0.309	4,8,12,16-Tetramethylheptadecan-4-olide
45	25.291	0.4773	Oleic Acid
46	25.43	1.4426	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
47	25.628	2.3898	Octadecanoic acid, 9,10-dihydroxy-, methyl ester
48	25.796	1.5367	Ethyl stearate, 9,12-diepoxy
49	26.06	0.5339	9-Octadecenoic acid
50	26.324	0.9151	Hexanoic acid, dodecyl ester
51	26.382	6.1222	Glycerol 1-palmitate
52	26.807	0.3904	cis-13-Octadecenoic acid
53	27.012	0.4411	Decanoic acid, 2-propenyl ester
54	27.459	0.1702	Isophytol, acetate
55	27.598	1.0222	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide
56	27.751	0.8406	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
57	28.118	0.5324	4-Hydroxy-2-mercapto-5-pyrimidine hydroxamic acid
58	28.345	1.8169	Propyl 5-methyl-3-isoxazolepropanoate
59	29.011	0.1541	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-
60	29.553	0.479	7-Pentadecyne
61	29.619	0.5944	Cyclooctene, 1,2-dimethyl-
62	30.702	0.3166	dl-.alpha.-Tocopherol
63	31.683	0.5099	Carvacrol, TMS derivative
64	31.918	0.9469	Chondrillasterol
65	32.379	1.3502	2-(2-Hydroxyethylamino)-5-nitrobenzonitrile

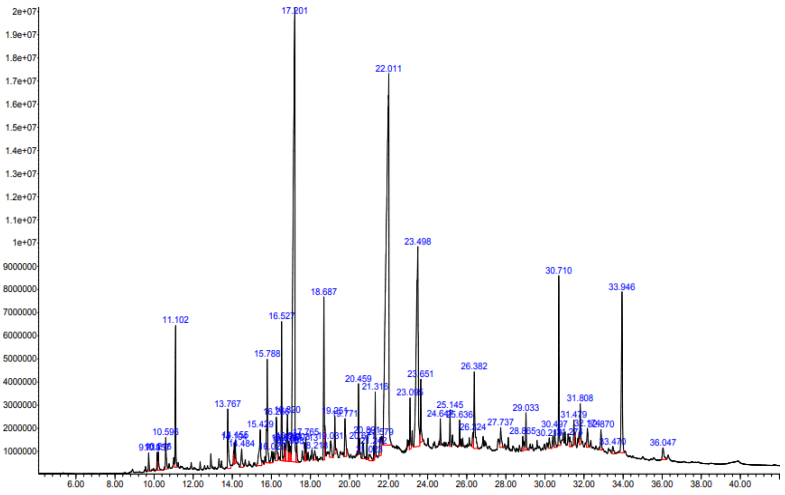

Fig.3. The volatile compounds separated by GC-MS technology for the alga *Dunatiella saline*.

Table 2. Retention time and area of volatile compounds separated by GC-MS technology for the alga *Dunatiella saline*.

Peak	R.T.	Area %	Library/ID
1	9.718	0.3056	Cyclohexanone, 2,2,6-trimethyl-
2	10.157	0.3005	2-Cyclohexen-1-ol, 2,4,4-trimethyl-
3	10.216	0.3902	Isophorone
4	10.596	0.6277	Cyclohexene, 3-methyl-6-(1-methylethyl)-
5	11.102	2.2635	Cyclohexanol, 2,6-dimethyl-
6	13.767	1.3684	1,5-Heptadiene, 3,4-dimethyl-
7	14.104	0.2658	3,5-Dimethoxybenzamide
8	14.155	0.3523	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-3,6-dimethyl-
9	14.484	0.5558	4-Hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-ene-1-carboxaldehyde
10	15.429	1.3906	Citronellal
11	15.788	2.0099	.alpha.-Ionone
12	16.029	0.3388	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-
13	16.256	1.2251	Santolina epoxide
14	16.527	2.1388	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
15	16.674	0.3235	(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene
16	16.71	0.3045	6,10-Dodecadien-1-ol, 3,7,11-trimethyl-
17	16.82	1.369	photocitral A
18	16.901	0.4722	2(3H)-Benzofuranone, 3a,4,5,7a-tetrahydro-3a,6-dimethyl-, cis-(.+-.)-
19	16.988	0.2947	Cyclohexane, 1,1,2-trimethyl-3,5-bis(1-methylethenyl)-, (2.alpha.,3.beta.,5.beta.)-
20	17.201	20.2422	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-
21	17.303	0.3955	2-Pentanone, 4-cyclohexylidene-3,3-diethyl-
22	17.713	0.3066	2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.0.1(1,7)]decan-5-one
23	17.765	0.3529	Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)-
24	18.218	0.3028	Ledol
25	18.687	2.7475	2-Cyclohexen-1-one, 2,4,4-trimethyl-3-(3-oxo-1-butenyl)-
26	19.031	0.5862	7-Hydroxyisotrichodermol
27	19.251	0.6343	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-
28	19.771	1.0381	Tetradecanoic acid
29	20.459	0.8778	Neophytadiene
30	20.671	0.4983	1-Penten-3-one, 4-methyl-1-[2,6,6-trimethyl-2-cyclohexen-1-yl]-
31	20.891	0.3143	1-Hexadecyne
32	21.023	0.3039	5-(Furan-3-yl)-2-methylpent-1-en-3-one
33	21.242	0.2643	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-
34	21.316	0.8415	Hexadecanoic acid, methyl ester
35	21.579	0.6651	Palmitoleic acid
36	22.011	25.6672	n-Hexadecanoic acid
37	23.095	0.8148	Phytol
38	23.498	10.0002	Oleic Acid
39	23.651	1.1647	Octadecanoic acid
40	24.647	0.4161	cis-13-Octadecenoic acid, methyl ester
41	25.145	0.5262	4,8,12,16-Tetramethylheptadecan-4-olide
42	25.636	0.3693	Nonanoic acid, 9-(nonyloxy)-, methyl ester

Table 2. Continued.

Peak	R.T.	Area %	Library/ID
43	26.324	0.3692	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide
44	26.382	1.8555	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
45	27.737	0.5511	9-Octadecenal, (Z)-
46	28.865	0.3199	.alpha.-Tocospiro B
47	29.033	0.6624	1,4-Cyclohexadiene, 6-isopropenyl-1,2,3,4-tetramethyl-
48	30.219	0.2728	1-(2-Methoxyphenyl)-2,5-dihydro-1H-pyrrole-2,5-dione
49	30.497	0.3785	Benzoic acid, 3,5-diformyl-2,4-dihydroxy-6-methyl-, 3-methoxy-2,5,6-trimethylphenyl ester
50	30.71	2.5446	Vitamin E
51	31.274	0.2655	Methyl 2-hydroxy-pentadecanoate
52	31.479	0.438	Anthracene, 9,10-dihydro-9-(3-hydroxypropyl)-
53	31.808	0.773	1,4-naphthalenediol, 5,6,7,8-tetrahydro-2-methyl-
54	32.174	0.4404	Phytyl dodecanoate
55	32.87	0.465	dl-.alpha.-Tocopherol
56	33.47	0.2581	Fumaric acid, pent-4-en-2-yl tridecyl ester
57	33.946	4.3197	Phytyl palmitate
58	36.047	0.4596	1-Eicosene

through this great ability, they produce effective chemical compounds with primary and secondary metabolic pathways (Cardozo et al. 2007). They are a natural resource rich in many compounds such as proteins, sugars, fatty acids, sterols, antioxidants, and dyes. In addition, their presence in open environments and exposure to harsh environmental conditions produce a wide range of secondary metabolites that cannot be found in some other organisms (Plazaa et al. 2010).

References

Bacher, L.; Manassaram-Baptiste, D.; Leprell, R. & Balton, B. 2015. Cyanobacteria and green algae blooms; a review of health and environmental data from the harmful algal bloom related illness surveillance system (HABISS). Toxins 7(4): 1048-1064.

Baracaldo, P.S.; Hayes, P.K. & Blank, C.E. 2005. Morphological and habitat evolution in the cyanobacteria using a compartmentalization approach. Geobiology 3: 145-165.

Belcher, H. & Swale, E. 1976. A beginner guide to freshwater algae. Iondon. Cult. Ceite to Algae and Frotozoa. pp: 1-48.

Blunt, J.W.; Copp, B.R.; Hu, W.P.; Munro, M.H.; Northcote, P.T. & Prinsep, M.R. 2008. Marine natural product. Natural Product Report 25: 35 -94.

Bullerjahn, G.S. & Post, A.F. 2014. Physiology and molecular biology of aquatic cyanobacteria. Frontiers in Microbiology 5: 359.

Cabrite, M.T.; Vale, C. & Rauter, A.P. 2010. Halogenated compounds from marine algae. Marine Drugs 8(8): 2301-2317.

Cardozo, K.H.M.; Guaratini, T.; Barros, M.P.; Falcao, V.R.; Tonon, A.P.; Lopes, C.N.P.; Campos, S.; Torres, M.A.; Souza, A.O.; Colepicolo, P. & Pinto, E. 2007. Metabolites from algae with economic impact. Comparative Biochemistry and Physiology 146: 60-78.

Demirel, Z.; Yildirim, Z.D.; Tuney, I.; Kesici, K. & Sukatar, A. 2012. Biochemical analysis of some brown seaweeds from the Aegean Sea. Botanica Serbica 36(2): 91-95.

Ghasemi, Y.; Yazdi, M.T.; Shafiee, A.; Amini , M.; Shokravi , S.; Zarrini, G. & Parsiguine, H. 2004. A novel antimicrobial substance from Fischerella ambigua. Pharmaceutical Biology 42(4-5): 318-322.

Hoppe, H.A. 1979. Marine algae and product and constituents in pharmacy in marine algae in *pharm.sci* (Hoppe, H.A.; Levering, T. & Tanaka, Y., eds.): Watterde Grugter, Berlin. pp: 215-219.

Jha, R.K. & Zi-rong, X. 2004. Biomedical compounds from marine organisms. Marine Drugs 2(3): 123-146.

Kim, H.; Li, L.; Lee, H.; Park, M.; Bilehal, D.; Li,W. &

Kim, Y. 2009. Protective effects of *Chlorella vulgaris* extract on carbon tetrachloride-induced acuteliver injury in mice. Food Science and Biotechnology 18(5): 1186-1192.

Komatsu, T.; Kido, N.; Sugiyama, T. & Yokochi, T. 2013. Antiviral activity of acidic polysaccharides from *Coccomyxa gloeobotrydiformi,* a green algae, against an in vitro human influenze A virus infection. Immunopharmacology and Immunotoxicology 35(1): 1-7.

Ling, B. 2000. Health impairments arising from drinking water polluted with domestic sewage and excreta in China. Schriftenreihe Verein Wasser Boden Lufthygiene 105: 43-46.

Masayoshi, Y.; Susanne, B.; Keisuke, Y.; Akira, F.; Nobuyuki, M. & Naoharu, W. 2014. Determination of volatile compounds in four commercial samples of Japanese green algae using solid phase microextraction gas chromatography mass spectrometry. The Scientific World Journal ID 289780, 8 p.

Najdenski, H.M.; Gigove, L.G.; Lliev, I.I.; Pilarski, P.S.; Lukavsky, J.; Tsvetkova, I.V.; Ninova, S.M. & Kussovski, V.K. 2013. Antibacterial and antifungal activities of selected microalgae and cyanobacteria. International Journal of Food Science and Technology 48(7): 1533-1540.

Panahi, Y.; Behrad, D.; Narges, J.; Fatemeh, B. & Sahebkar, A.H. 2016. Chlorella vulgaris; amultifunctional dietary supplement with diverse medicinal properties. Current Pharmaceutical Design 22(2): 164-173.

Patel, V.; Berthold, D.; Puranik, P. & Gantar, M. 2015. Screening of cyanobacteria and microalgae for their ability to synthe-size with antibacterial activity. Biotechnology Reports 5: 112-119.

Plazaa, M.; Santoyob, S.; Jaimeb, L.; Garcia-Blairsy Reinac, G.; Herrerob, M.; Senoransb, F.J. & Ibaneza, E. 2010. Screening for bioactive compounds from algae. Journal of Pharmaceutical and Biomedical Analysis 51: 450-455.

Rios, J.; Recio, M. & Villar, A. 1987. Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. Journal of Ethnopharmacology 21(2): 139-152.

Silva, J.P.; Alves, C.; Pinteus, S.; Silva, J.; Valado, A.; Pedrosa, R. & Pereira, L. 2019. Antioxidant and antitumor potential of wild and IMTA-cultivated Osmundea pinnatifida. Journal of Oceanology and Limnology 37(3): 825-835.

Thajuddin, N. & Subramanian, G. 2005. Cyanobacterial biodiversity and potential applications in biotechnology. Current Science 89: 47-57.