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ORIGINAL PAPER

Fatty acid profiling as bioindicator of chemical stress in marine *Pterocladia capillacea, Sargassum hornschuchii* and *Ulva lactuca*

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Abstract Contaminants are increasing in the aquatic ecosystem due to the development of industrial and agricultural activities. Contaminants include organic pollutants such as pharmaceuticals and endocrine-disrupting compounds, which accumulate in the ecosystem's primary producers and propagate through the food chain. The total lipid contents and fatty acid profiles in the marine macroalgae *Pterocladia capillacea*, *Ulva lactuca* and *Sargassum hornschuchii* proved to be a good bioindicator to assess contamination levels. The lowest value of the total lipid content was 1.90% obtained in *S. hornschuchii* under the bisphenol treatment, while the highest value was 4.66% obtained in *U. lactuca* under exposed to clofibric acid. The fatty acid methyl ester profiles were analysed by using gas chromatography. The total fatty acids varied from 5.85% (*U. lactuca* after exposure to bisphenol) to 28.38% (*P. capillacea* treated with clofibric acid). The ratio of saturated to unsaturated fatty acids was significantly higher in *U. lactuca* after exposure to acetyl salicylic acid than in the other macroalgae under different treatments.

Keywords Seaweed · Fatty acids · Pharmaceuticals · Biodiesel

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Introduction

In the last decades, contaminants' discharges are one of the themes that concerned the scientific community and politicians and received special attention from media because of the threat and adverse effects that they may cause in aquatic ecosystems (McKnight et al. 2012). The expansion of industrial, anthropogenic and agricultural activities is the main factor leading to their increase in aquatic ecosystems (Peixoto et al. 2006).

Marine ecosystems play an important role in the most storage for a large variety of organic and inorganic pollutants, which are constantly discharging due to the active anthropogenic activities (Kennish 1996). There are four main pollutant sources of marine ecosystems: (1) landscape level causes; (2) industrial and domestic discharges; (3) inflows from rivers; (4) shipping and precipitation from atmosphere. The great input to the sea pollution gives land-based sources—more than 80% (Kennish 2000). Through them are intense agriculture activities discharging the large variety of chemical stressors for organic and inorganic nature, contaminating the marine aquatic environment.

Energy is the most fundamental necessity for human life. Petroleum is an effective fuel serving all the world to get its requirement of power consumption, where the maximum part of energy used in the world takes from the petroleum and natural gas. However, the whole reliance of mankind on fossil fuels may cause a maximum shortage in the future. Therefore, we must be found an alternative new and renewable source of energy. Biofuels made of bio-products decrease the need for petroleum oil and offer great useful for sustainability and diminish pollutant and greenhouse gas emissions (Hansen et al. 2009). The application of biofuels like biodiesel is extremely promising. Mainly the features of using biodiesel are that it is renewable, non-

toxic, biodegradable, eco-friendly and can be used without changing existent engines because it has similar characteristics to diesel fuel and produces less harmful gas emissions, such as sulphur oxide (Agarwal 2007; Hansen et al. 2009). Also the advantages of biodiesel production are low cost and reducing the environmental contamination by decreasing the net carbon dioxide emissions by 78% on a life cycle basis as compared to traditional diesel fuel (Gunvachai et al. 2007). Biodiesel is composed of many fatty acid methyl esters made from triglycerides by transesterification with methyl alcohol (Gerpen 2005). Through transesterification, the glycerides in fats or oils contents react with an alcohol in the existence of a catalyst and are changed into monoesters, with producing free glycerol as a by-product (Banerjee and Chakraborty 2009; Enweremadu and Mbarawa 2009; Zabeti et al. 2009).

Biodiesel can be created from various feedstocks. Biodiesel is made from renewable biological sources such as vegetable oils (edible and non-edible oil) and animal fats. All originating oil or fat is distinguished by a different fatty acid composition, and the final ester properties differ significantly based on the feedstock, alcohol used in the esterification and the exact chemical process followed (Knothe 2005). Recently, many researchers have been focused on the production of biodiesel from low-cost non-edible sources, such as seed of castor and algae (Komninos and Rakopoulos 2012; Pinzi et al. 2009). The majority of research has concentrated on the marine production of biofuels derived from macroalgae (seaweeds) and microalgae (single-cell plants) (Singh and Cu 2010; Williams and Laurens 2010). Biodiesels derived from the micro- and macroalgae have become known as one of the most encouraged unusual sources of lipids for use in biodiesel production because they are renewable in nature, also can be formed on a huge scale and are friendly to the environments (Carvalho et al. 2011). However, the biggest problem is the cultivating and harvesting microalgae at an industrial scale, while macroalgae are simpler for production, which are more in coastal environments, mostly in nearby-shore coastal waters with favourable substrates for attachment (Murphy et al. 2013). A former study notified that seaweed species have total lipid contents of less than 5% dry weight. By contrast, there are many species with total lipid contents greater than 10% dry weight, and these are interesting candidates for oil-based products (Gosch et al. 2012). Because high prices of fossil fuel are likely to increase and because macroalgal production costs will likely decrease as production is expanded, it is prudent to develop methods to obtain significant quantities of biofuel from marine biomass to meet European energy needs and climate alteration targets (Hughes et al. 2012). The objective of this study was to assess the effect of different pharmaceuticals and endocrine-disrupting compounds on lipid productivity of *Pterocladia capillacea*, *Ulva lactuca* and *Sargassum hornschuchii* as promising macroalgae for biodiesel production.

Materials and methods

Area of study and sampling

Seaweed species belong to different classes, including *P. capillace* (c. Agardh), a genus of red macroalgae, class Florideophyceae and order Gelidiales; *U. lactua* linneals (Gmel) Born, a genus of green macroalgae, class Ulvophyceae and order Ulvales; and *S. hornschuchii* (C. Agardh), class Phaeophyceae and order Fucales, which were collected through the Autumn from Abu Qir Bay in 2015. Healthy samples were identified depending on the morphological features by using the herbarium and the identification scheme of the late Prof. A. H. Nasr (Botany Department, Faculty of Science, Alexandria University). Abu Qir Bay is a semicircular bay along the Egyptian Mediterranean seashore, approximately 30 km east of Alexandria, with an average water depth of 11 m and an area of approximately 360 km^2. This bay is distinguished by the presence of abundant rocks with several petite and fine holes that are excellent domains for the attachment of algae. The samples were placed separately in polythene bags, kept inside an ice box and transported to the laboratory. Samples were washed thoroughly using tap water to remove surface salt and spread on blotting paper to remove excess water.

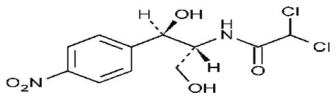
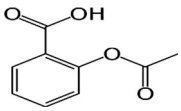
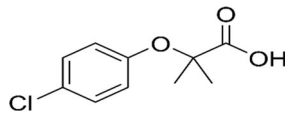
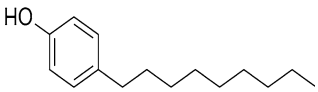
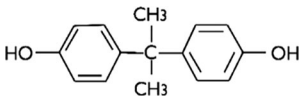
Chemicals

All chemicals reagents employed in this study were purchased from Sigma-Aldrich companies (Table 1). The concentrations of pharmaceutical were quantified using HPLC device equipped with a UV-light spectrophotometer or fluorescence detector. The instrument was initially calibrated before each use with standard solution for each compound. Conditions of HPLC assay for acetyl salicylic acid by Akay et al. (2008), chloramphenicol by Barata et al. (2005), clofibric acid by Lau-Cam et al. (2006), nonylphenol by Wang et al. (2007) and bisphenol by Gattulo et al. (2012), where the chromatographic separation were performed on a C18 reverse phase column by using different solvents.

Experimental design

Pterocladia capillacea, U. lactuca and *S. hornschuchii* were grown in 500-mL Erlenmeyer flasks by mixing 1 gm of fresh algal biomass with 100 mL of pharmaceutical solution of specific concentration. The different

Table 1 The chemical structure and IUPAC name of different pharmaceuticals used in this study

Pharmaceutical name	Chemical structure	IUPAC name
Chloramphenicol		2,2-Dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl] acetamide
Acetyl salicylic acid		2-Acetoxybenzoic acid
Clofibric acid		2-(4-Chlorophenoxy)-2-methylpropionic acid
Nonylphenol		4-(2,4-Dimethylheptan-3-yl) phenol
Bisphenol		4,4′-(Propane-2,2-diyl) diphenol

concentrations of pharmaceuticals were prepared, viz. 5, 10, 15, 20, 25, 30, 35 and 40 mg/L, and were used in case of algae tolerance experiments. The concentration of compound was prepared by adequate dilution of its stock solution using sea water. The stocks of acetyl salicylic acid, clofibric acid, nonylphenol and bisphenol were prepared by dissolving 1000 mg of each pharmaceutical compound in 1 L of methanol, while stock of chloramphenicol was prepared by dissolving 1000 mg of antibiotic in 1 L of deionized distilled water. Following are the biosorption experiment concentrations of pharmaceuticals: acetylsalicylic acid (20 mg/L for *P. capillacea*, *U. lactuca* and *S. hornschuchii*), chloramphenicol (25 mg/L for *P. capillacea* and *S. hornschuchii* and 15 mg/L for *U. lactuca*), clofibric acid (5 mg/L for *P. capillacea*, 20 mg/L for *S. hornschuchii* and 35 mg/L for *U. lactuca*), nonylphenol (5 mg/L for *P. capillacea* ,10 mg/L for *S. hornschuchii* and 20 mg/L for *U. lactuca*) and bisphenol (10 mg/L for *P. capillacea* and *S. hornschuchii* and 20 mg/L for *U. lactuca*). The maximum biosorption was achieved by both algae after 12 h. Moreover, increasing contact time from 12 up to 36 h resulted in a slight decrease in biosorption of all pharmaceuticals.

The experiment was performed at natural light photoperiod (16 h light/8 h dark) and room temperature (29 ± 2 °C) with three replicas. The control was carried out by using the algal biomass in sea water (without addition of pharmaceutical compounds) and was run parallel.

Lipid extraction and fatty acid analysis

The seaweed samples were analysed in triplicate for their proximate. Lipids were extracted with a chloroform–methanol mixture (2:1 v/v). The lipids in chloroform were dried over anhydrous sodium sulphate, after which the solvent was removed by heating at 80 °C under vacuum AOAC (2000). The fatty acids were converted to methyl esters using the method of Christie (1998). The samples were esterified in 1% sulphuric acid in absolute methanol and extracted with hexane to separate the layers. The hexane layer was washed with water containing potassium bicarbonate and dried over anhydrous sodium sulphate. The solvent was evaporated using a rotary evaporator. The fatty acid methyl esters (FAMEs) were analysed by a Shimadzu gas–liquid chromatography equipped with a flame ionization detector with a packing column with Hp-5 material. The carrier gas was nitrogen, and the short speed was 5 mm/min. For the identification and quantification of FAMEs, their retention times were compared with standards. The values are expressed as a percentage of the total fatty acids mixture.

Statistical analyses

Statistical analysis employed SPSS version 10.0 for testing significance of differences between treatments and control at the 0.05 probability level ($P \leq 0.05$). Most of experiments were tested with analysis of variance F test

(ANOVA). The mean value of triplicate data was calculated.

Results and discussion

The effect of different pharmaceuticals on the total lipid contents based on the dry weight of *P. copillacea* is given in Table 2. The highest value of lipid content was 3.71% in the clofibric acid treatment, followed by 3.66% in the acetyl salicylic acid treatment, 2.90% in the nonylphenol treatment and 2.60% in the chloramphenicol treatment and the lowest value was 2.45% in the bisphenol treatment as compared to the control 2.51%. There was a significant effect of both the acetyl salicylic acid and clofibric acid on the total lipid content of *P. copillacea.* The fatty acid methyl esters as a percentage of the total fatty acids mixture are given in Table 2. The total percentage of identified saturated fatty acids was 7.720, 16.232, 10.536, 13.887 and 4.055 and of the unsaturated fatty acids was 4.907, 8.717, 17.855, 9.192 and 6.871 in the chloramphenicol, acetyl salicylic acid, clofibric acid, nonylphenol and bisphenol, respectively, as compared to that of the control 8.612 and 3.641 in saturated and unsaturated fatty acids, respectively. It is clear that all the total unsaturated fatty acids significantly increase with control under all treatments. Also, there was a significant effect of the acetyl salicylic acid, clofibric acid and nonylphenol on the total saturated fatty

Table 2 Total lipid contents and fatty acid profiles of *Pterocladia capillacea* before and after exposure to different pharmaceuticals

Fatty acids[a]	Pharmaceuticals					
	Control	Chl	ASA	Clo	Non	Bis
C6:0	0.139	0.060*	0.025*	0.005*	0.088	0.061*
C8:0	1.784	0.061*	0.065*	3.641*	2.404*	1.327
C10:0	0.448	0.462	0.118*	1.245*	0.463	0.228*
C11:0	0.208	0.155*	0.178	0.634*	0.274	0.141*
C12:0	0.401	0.049*	0.172*	0.852*	0.061*	0.002*
C13:0	0.445	0.044*	0.121*	1.075*	1.130*	0.382
C14:0	0.586	0.911*	4.018*	1.242*	0.995*	0.274*
C15:0	0.986	1.283*	3.302*	0.589*	2.347*	1.342*
C16:0	3.329	4.256*	7.640*	1.139*	5.501*	0.104*
C17:0	–	0.036*	0.062*	0.109*	0.539*	0.133*
C18:0	0.286	0.403*	0.531*	0.005*	0.088*	0.061*
Total saturated	8.612	7.720	16.232*	10.536*	13.887*	4.055*
C14:1	1.097	1.450*	2.032*	1.919*	2.574*	2.148*
C15:1	1.194	1.524*	3.726*	4.290*	2.919*	1.658*
C16:1	0.098	0.579*	1.293*	5.244*	1.357*	0.041*
C17:1	0.224	0.305	0.600*	1.832*	0.435*	1.224*
C18:1	0.189	0.312*	0.244	4.234*	0.552*	0.050*
C22:1	0.636	0.707	0.808	0.235*	0.828*	0.465*
C18:2	0.203	0.030*	0.014*	0.101*	0.353	1.285*
C20:5	–	–	–	–	0.174	–
Total unsaturated	3.641	4.907*	8.717*	17.855*	9.192*	6.871*
Total	12.253	12.627	24.949*	28.391*	23.079*	10.926*
SFA/UFA[b]	2.365	1.573*	1.862	0.590*	1.510*	0.590*
C16:1/C18:1/C14:0	1: 2: 6	6: 3: 9	13: 6: 40	5: 4: 1	14: 6: 10	4: 5: 30
Total lipid[c]	2.51	2.60	3.66*	3.71*	2.90	2.45

Chl chloramphenicol, *ASA* acetyl salicylic acid, *Clo* clofibric acid, *Non* nonylphenol, *Bis* bisphenol

* Significant with control

All data were recorded as the mean of the three replicates

[a] Fatty acid methyl esters as a percentage of the total fatty acids mixture

[b] SFA/UFA: ratio of saturated fatty acids to unsaturated fatty acids

[c] Total lipids as percentage of dry weight

acids. The appearance of new saturated fatty acid, heptadecanoic acid (C17: 0), under all pharmaceutical stressors, was not detected in control.

The ratio between saturated and unsaturated fatty acids was 1.573, 1.862, 0.590, 1.510 and 0.590 as compared to the control 2.365. For the individual fatty acids, the major saturated fatty acids were myristic acid (C14:0), pentadecyclic acid (C15:0) and palmitic acid (C160) in acetyl salicylic acid treatment, whereas octanoic acid (C8:0), decanoic acid (C10:0), tridecanoic acid (C13:0) and myristic acid (C14:0) were the major saturated fatty acids in clofibric acid treatment and octanoic acid (C8:0), tridecanoic acid (C13:0), pentadecyclic acid (C15: 0) and palmitic acid (C16:0) were the major saturated fatty acids in nonylphenol treatment. The myristoleic acid (C14:1), the pentadecanoic acid (C15:1), palmitoleic acid (C16:1) and cis-heptadecanoic acid (C17:1) were the major unsaturated fatty acids under all pharmaceutical treatments.

The total fatty acid significantly increases in the following order: clofibric acid > acetyl salicylic acid > nonylphenol > chloramphenicol > bisphenol with 28.391, 24.949, 23.079, 12.627 and 10.926%, respectively, as compared to the control 12.53%.

Table 3 shows the variation in total lipid content of *U. lactuca* under different pharmaceutical treatments. The highest percentage was 4.66 of dry matter in the clofibric acid treatment followed by 4.42 in acetyl salicylic acid treatment, which significantly increased. Comparable percentages of 3.13 and 3.11 were observed in chloramphenicol and nonylphenol treatments, respectively. By contrast, the total lipid was significantly decreased under bisphenol treatment. Table 3 also shows an overview of the fatty acid profiles of the alga. In this study, the total saturated fatty acid was increased significantly in the treatment of chloramphenicol, acetyl salicylic acid and clofibric acid, while it decreased in the treatment of nonylphenol and bisphenol as compared to the control. The total fatty acid was recorded the maximum values with 21.158 and 22.098% in acetyl salicylic acid and clofibric acid treatments, respectively, as compared to the control 7.781%. We identified several individual fatty acids under various pharmaceutical treatments with different concentrations. The saturated fatty acid, primarily palmitic acid (C16:0), was recorded the highest significant values with 4.823, 13.165, 2.588, 2.386 and 1.453% in chloramphenicol, acetyl salicylic acid, clofibric acid, nonylphenol and bisphenol, respectively. Moreover, a significant increase was observed in the following unsaturated fatty acids: mainly myristoleic acid (C14:1) with 0.832, 0.693, 0.559, 1.141 and 0.956% and pentadecanoic acid (C15:1) with 0.911, 1.970, 3.460, 1.645 and 0.956% in chloramphenicol, acetyl salicylic acid, clofibric acid, nonylphenol and bisphenol, respectively. The appearance of the two saturated fatty acids (C6:0) and (C18:0) was completely absent in control. All values of unsaturated fatty acids under various pharmaceutical treatments were shown higher than in control except palmitoleic acid (C16:1), oleic acid (C18:1) and linolenic acid (C18:2) in the bisphenol treatment.

The total lipid content of *S. hornschuchii* under different pharmaceutical treatments was 2.51, 3.18, 3.38, 3.14 and 1.90% in the chloramphenicol, acetyl salicylic acid, clofibric acid, nonylphenol and bisphenol, respectively, as compared to the control 2.20%. The fatty acid composition varied among the different pharmaceutical treatments (Table 4). Acetyl salicylic acid treatment had the highest significant value of saturated fatty acid content (15.787%), while there was no significant effect of chloramphenicol (6.736%), clofibric acid (6.289%), nonylphenol (5.894%) and bisphenol (7.426%). However, the results were recorded for the unsaturated fatty acid contents that significantly increase in the following order: clofibric acid > nonylphenol > acetyl salicylic acid > chloramphenicol > bisphenol with 16.923, 9.480, 8.925, 4.532 and 4.166%, respectively. The percentages of saturated fatty acid palmitic acid (C16: 0) were 4.559, 12.227, 2.388, 2.510 and 2.868, and the percentages of the unsaturated fatty acid docosahexaenoic acid (C22:6) were 1.612, 2.442, 2.320, 2.772 and 1.884 from chloramphenicol to acetyl salicylic acid to clofibric acid to nonylphenol to bisphenol, respectively, recorded the most values of individual fatty acids under different pharmaceuticals were increased than those of control.

From Tables 2, 3 and 4, we noticed that the ratio of C16:1/C18:1/C14:0 was varied completely in control and under all pharmaceutical treatments except in clofibric acid treatment, which approximately 5:4:1, 5.4:4.3:1.1 and 5.6:4.5:1.1 in *P. copillacea, U. lactuca* and *S. hornschuchii*, respectively.

It is important to known the effects of organic pollutants on the fatty acid profiles of marine species. Organic pollutants are chemical substances that persist in the environment, bioaccumulate through the food web and cause adverse effects to the environment (Filimnova et al.2016).

In the present study, it is a new idea to estimate macroalgae as a feedstock for oil-based products to establish both qualitatively and quantitatively their total lipid content and fatty acid profiles. Subsequently, the extent to which organic pollutants affect the total lipid content and fatty acid profiles of the algae is determined (Juneja et al. 2013). The results in Tables 2, 3 and 4 show that total fatty acid contents of *P. copillacea, U. lactuca* and *S. hornschuchii* increased after treatment with chloramphenicol, acetyl salicylic acid, clofibric acid and nonylphenol. In contrast, total fatty acid contents of all algal species reduced after exposure to bisphenol. The

Table 3 Total lipid contents and fatty acid profiles of *Ulva lactuca* before and after exposure to different pharmaceuticals

Fatty acids[a]	Pharmaceuticals					
	Control	Chl	ASA	Clo	Non	Bis
C6:0	0.0	0.003*	0.027*	0.0	0.007	0.009
C8:0	0.029	0.010	0.044*	0.049*	0.015	0.005*
C10:0	0.168	0.314*	0.343*	0.718*	0.016*	0.116
C11:0	0.069	0.053	0.105*	0.269*	0.025*	0.043*
C12:0	0.152	0.012*	0.054*	0.250*	0.016*	0.026*
C13:0	0.786	0.014*	0.044*	0.097*	0.040*	0.013*
C14:0	0.122	0.214*	0.417*	1.088*	0.185	0.099
C15:0	0.446	0.523	0.227*	0.714*	1.056*	0.828*
C16:0	3.836	4.823*	13.165*	2.588*	2.386*	1.453*
C17:0	0.112	0.092	0.066*	0.516*	0.092	0.065*
C18:0	–	0.145*	0.693*	–	0.117*	0.144*
C20:0	–	–	0.027*	–	–	–
Total saturated	5.720	6.203*	15.212*	6.289*	3.955	2.801
C14:1	0.545	0.832*	0.693	0.559	1.141*	0.914*
C15:1	0.546	0.911*	1.970*	3.460*	1.645*	0.956*
C16:1	0.129	0.154	1.863*	5.413*	1.050*	0.006*
C17:1	0.023	0.085*	0.089*	0.588*	0.670*	0.156*
C18:1	0.342	0.151*	0.381	4.362*	0.358	0.202
C22:1	0.074	0.112	0.195*	1.057*	0.257*	0.495*
C18:2	0.393	0.583	0.693*	0.271	0.326	0.281
C18:3	0.009	0.016*	0.062*	0.099	0.212*	0.042*
Total unsaturated	2.061	2.844	5.946*	15.809*	5.659*	3.052*
Total	7.781	9.047*	21.158*	22.098*	9.614	5.853
SFA/UFA[b]	2.78	2.18	2.56	0.40*	0.71*	0.92*
C16:1/C18:1/C14:0	13: 34: 12	15:15: 21	19: 3.8: 4.2	5.4:4.3:1.1	11: 1: 2	0.6: 20: 9.9
Total lipid[c]	3.10	3.13	4.42*	4.66*	3.11	2.40

Chl chloramphenicol, *ASA* acetyl salicylic acid, *Clo* clofibric acid, *Non* nonylphenol, *Bis* bisphenol

* Significant with control

All data were recorded as the mean of the three replicates

[a] Fatty acid methyl esters as a percentage of the total fatty acids mixture

[b] SFA/UFA: ratio of saturated fatty acids to unsaturated fatty acids

[c] Total lipids as percentage of dry weight

pattern of total fatty acid increase may be due to their vital role in the tolerance to several physiological stressors in a variety of organisms. Moreover, the increase in the amounts of fatty esters was detected ($P \leq 0.05$). These results were in accordance with findings of Yeh and Chang (2011), Sun et al. (2014) and Fakhry and El Maghraby (2015) who reported that nitrogen deficiency could increase the lipid content of various algae. Also, Battah et al. (2014) indicated that manganese chloride, cobalt nitrate and hydrogen peroxide increased the content of lipid in *Chlorella vulgaris*, Yang et al. (2002) revealed that exposure to 2,4-dichlorophenol led to increasing the lipid content in marine diatom.

Tables 2, 3 and 4 show that the total saturated and total unsaturated fatty acids of both algal biomasses increased after different pharmaceutical treatments, except that the total saturated fatty acid after bisphenol treatment decreased in algae studied. Data in this research demonstrated a significant effect of different pharmaceuticals on the proportions of the total saturated and total unsaturated fatty acids. The increase in the total saturated and total unsaturated fatty acids could be explained by the fact that toxicant stress can change the lipid and fatty acid composition of algae (Rocchetta et al. 2006). The mechanisms involve mostly oxidative stress and production of reactive oxygen/nitrogen species that lead to oxidation of lipids

Table 4 Total lipid contents and fatty acid profiles of *Sargassum hornschuchii* before and after exposure to different pharmaceuticals

Fatty acids[a]	Pharmaceuticals					
	Control	Chl	ASA	Clo	Non	Bis
C8:0	0.014	0.039*	0.027	0.049*	0.044*	0.022
C10:0	0.032	0.007*	0.044	0.718*	0.523*	0.010*
C12:0	0.119	0.133	0.155	0.250*	0.211*	0.164
C13:0	0.285	0.508*	0.554*	0.097*	0.210*	0.684*
C14:0	0.449	0.766*	0.439	1.188*	1.254*	0.498
C15:0	0.096	0.292*	0.417*	0.814*	0.214*	0.398*
C16:0	4.461	4.559	12.227*	2.388*	2.510*	2.864*
C17:0	0.070	0.071	0.165*	0.516*	0.541*	0.058
C18:0	0.162	0.267*	1.066*	0.269*	0.247*	0.624*
C20:0	0.176	0.094	0.693*	–	0.140	0.104
Total saturated	5.864	6.736	15.787*	6.289	5.894	5.426
C15:1	1.328	1.385	1.485	1.559	1.085	1.102
C16:1	0.157	0.157	1.187*	5.460*	1.487*	0.234*
C17:1	0.067	0.035	0.095	0.450*	0.045	0.034*
C18:1	0.092	0.428*	2.400*	4.589*	2.422*	0.248*
C20:1	0.003	0.025	0.045*	0.085*	0.045*	0.030*
C22:1	0.011	0.018	0.038*	0.078*	0.037*	0.018
C18:2	0.052	0.570*	0.874*	1.057*	0.845*	0.474*
C18:3	0.037	0.014*	0.214*	0.271*	0.341*	0.022
C20:3	0.016	0.081*	0.081*	0.140*	0.041*	0.054*
C20:4	0.005	0.082*	0.214*	0.442*	0.217*	0.018*
C20:5	0.074	0.125*	0.145*	0.472*	0.143*	0.048*
C22:6	1.599	1.612	2.442*	2.320*	2.772*	1.884
Total unsaturated	3.441	4.532	8.925*	16.923*	9.480*	4.166
Total	9.305	11.168*	24.712*	23.212*	15.374*	9.192
SFA/UFA[b]	1.71	1.60	1.77*	0.37	0.62	1.78*
C16:1/C18:1/C14:0	2: 1: 5	2: 4: 3	12: 24: 5	5.6:4.5:1.1	15: 25: 13	2: 2: 5
Total lipid[c]	2.20	2.51	3.18*	3.38*	3.14*	1.90

Chl chloramphenicol, *ASA* acetyl salicylic acid, *Clo* clofibric acid, *Non* nonylphenol, *Bis* bisphenol

* Significant with control

All data were recorded as the mean of the three replicates

[a] Fatty acid methyl esters as a percentage of the total fatty acids mixture

[b] SFA/UFA: ratio of saturated fatty acids to unsaturated fatty acids

[c] Total lipids as percentage of dry weight

(Pinto et al. 2003). Li et al. (2011) demonstrated that microalgae can change their biosynthetic pathways towards the formation and accumulation of lipids under stress conditions and then serve as energy storage rather than for the formation of structural compounds. Sakthivel et al. (2011) mentioned that many microalgae have the ability to produce substantial amounts of triacylglycerols as a storage lipid under oxidative stress. Skerratt et al. (1998) stated that exposure of *Phaeocystis antarctica* to high UV-B irradiation results in oxidative stress and increases the concentration of total lipids, triacylglycerols and free fatty acids.

It is clear from this study that the selected seaweeds have low lipid content under different pharmaceutical treatments and control. This is consistent with Jensen (1993), who reported that the lipid content is very low in seaweeds, ranging from 1 to 5% of the dry matter, and varies significantly between different algae. In this study, *U. lactuca* had the highest total lipid content, followed by *P. copillacea* and *S. hornschuchii*. Accordingly, a low lipid content of these macroalgae reduces their usefulness for biodiesel production and assures that macroalgae are hopeful species for other industrial products. Murphy et al.

(2013) suggested that the macroalgae contained high amounts of natural sugars and other carbohydrates, suitable for biogas and ethanol production rather than biodiesel. However, Gosch et al. (2012) estimated the lipid contents of macroalgae *Dictyota, Spatoglossum, Derbesia* and *Caulerpa* and reported the range of lipid from 10 to 12% of dry weight that is comparable with the microalgae *Tetraselmis, Rhodomonas, Scenedesmus* and a few strains of *Skeletonema* and *Isochrysis* (Huerlimann et al. 2010; Mata et al. 2010). Many authors indicated that mixing the macroalgae and utilizing a great quantity of raw materials are helpful to get high amounts of lipid, and accordingly, seaweeds can be used as a main source for biodiesel production

The present study showed that the marine algae subjected to different pharmaceuticals exhibit different concentrations of total saturated and unsaturated fatty acids, with a characteristic profile for each. For the ratio of saturated to unsaturated fatty acids in this study, *U. lactuca* exhibited the highest ratios (2.78, 2.18, 2.56, 0.40, 0.71 and 0.92), followed by *P. copillacea* (2.37, 1.57, 1.86, 0.59, 1.51 and 0.59) and *S. hornschuchii* (1.71, 1.60, 1.77, 0.37, 0.62 and 1.78) under different pharmaceuticals as follows: control, chloramphenicol, acetyl salicylic acid, clofibric acid, nonylphenol and bisphenol, respectively. However, quantification of the fatty acid composition and different levels of saturation were important factors in the determination of the appropriateness of these oils as biodiesel feedstock. Ramos et al. (2009) notified that monounsaturated, polyunsaturated and saturated methyl esters are the significant parameters of the European standard for any biodiesel composition. For biodiesel production, algae with a great amount of saturated fatty acids are influenced because they lead to more oxidative stabilization and higher ignition quality (cetane number) and give an overall higher-quality product (Hu et al. 2008; Knothe 2008).

Moreover, the fatty acid methyl ester profile is the main factor which determines the appropriateness of any feedstock for the use in biodiesel fuel production (Knothe 2009) The seaweed biodiesel can be competitive with other types of biodiesel feedstocks, and the perfect blend of the fatty acids C16:1, C18:1 and C14:0 has been proposed to be in the proportion 5:4:1 (Schenk et al. 2008). Tables 2, 3 and 4 show the closest ratio to that idea (5: 4:1), (5.4: 4.3: 1.1) and (5.6: 4.5: 1.1) in *P. copillacea, U. lactuca* and *S. hornschuchii*, respectively, under clofibric acid treatment. According to the requirements of Lithuanian Standard LST EN 14,214, the contents of linolenic acid methyl ester in biodiesel fuel should not exceed than 12%, which was recorded in our study 0.101, 0.271 and 1.057 in *P. copillacea, U. lactuca* and *S. hornschuchii*, respectively, under clofibric acid treatment.

Therefore, it can be forecast that biodiesel fuel produced from the selected species of algae under clofibric acid treatment will meet the requirements of the standard concerning linolenic acid methyl ester contents.

Conclusion

Biodiesel is a good alternative fuel for diesel because it is eco-friendly, biodegradable and renewable in nature. In this work, the total lipid contents and fatty acid profiles of *P. copillacea, U. lactuca* and *S. hornschuchii* under different pharmaceutical treatments were identified. However, these algae displayed distinct variation in the total lipid and fatty acid contents under the pharmaceutical stressors. Generally, the total amounts of total lipids were low, with the highest content of 4.66% of dry weight of *U. lactuca* under clofibric acid treatment, which must be significantly increased for the use in biodiesel production. Furthermore, the quantitative and qualitative characters of the various fatty esters determine the properties of biodiesel and the quality of fatty acid yields of the tested algae under different pharmaceutical treatments making them suitable for other products than biodiesel except the treated algae with clofibric acid, which is appropriate for biodiesel production.

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