Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 450–455

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Screening for bioactive compounds from algae

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ARTICLE INFO

Article history:
Received 28 January 2009
Received in revised form 10 March 2009
Accepted 14 March 2009
Available online 25 March 2009

Keywords:
Functional food
Bioactive
Algae
Antioxidant
Antimicrobial

ABSTRACT

In the present work, a comprehensive methodology to carry out the screening for novel natural functional compounds is presented. To do that, a new strategy has been developed including the use of unexplored natural sources (i.e., algae and microalgae) together with environmentally clean extraction techniques and advanced analytical tools. The developed procedure allows also estimating the functional activities of the different extracts obtained and even more important, to correlate these activities with their particular chemical composition. By applying this methodology it has been possible to carry out the screening for bioactive compounds in the algae *Himanthalia elongata* and the microalgae *Synechocystis sp*. Both algae produced active extracts in terms of both antioxidant and antimicrobial activity. The obtained pressurized liquid extracts were chemically characterized by GC–MS and HPLC-DAD. Different fatty acids and volatile compounds with antimicrobial activity were identified, such as phytol, fucosterol, neophytadiene or palmitic, palmitoleic and oleic acids. Based on the results obtained, ethanol was selected as the most appropriate solvent to extract this kind of compounds from the natural sources studied.
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1. Introduction

Nowadays, there is a marked trend in the food industry towards the development and manufacture of functional products [1]. This new class of food products has found a great success in the market due to the growing interest of consumers for healthy food. Functional food is generally defined as food that contains one or more functional ingredients that provides with an additional health benefit besides the energetic and nutritional aspects that every food must confer [2]. There are wide range of compounds that are used or could be potentially employed as functional ingredients [3]: carotenoids [4], polyphenols [5] and other antioxidant pigments [6], polyunsaturated fatty acids [7], among others [8]. Another important requirement for these functional ingredients is their natural origin, which is always preferred to the synthetic one [7].

These general characteristics have led to the increase on the research of new natural sources for this kind of compounds. Plants such as rosemary [9], oregano [10], soybean [11], tomato [12], green tea [13], and garlic [14] have been described as important sources of bioactives. More recently, some researchers have envisioned the enormous possibilities of algae and microalgae as potential source of bioactive compounds; particularly, some microalgae have been studied as a potential natural source of different functional compounds [4,15–17] while some macroalgae have been suggested as a new and unlimited source of new functional food ingredients [18]. Not only the presence of a particular compound makes these microorganisms interesting, but also their huge diversity and the possibility of not only harvesting them but also of growing them at different conditions, leading to an enrichment of some bioactives.

In our laboratory, we have worked in a comprehensive procedure to obtain and characterize functional compounds from natural sources as well as to demonstrate their activity. This procedure includes the use of environmentally friendly extraction techniques together with advanced analytical tools, biological methods and statistical approaches in order to obtain, test and characterize natural extracts that could be potentially used for the food industry. The aim of the present contribution is to demonstrate the usefulness of our comprehensive approach to perform screenings of different algae species in order to attain bioactive compounds to be used in the industry. Therefore, in this work the general procedure is described and two different algae, *Himanthalia elongata* and *Synechocystis sp*., are studied as examples of the use of this new methodology. This approach includes the study of the extraction conditions using pressurized solvent extraction (PLE) as a green extraction technique (testing different solvents, extraction temperatures and times), the functional characterization of the extracts, measuring both their antimicrobial and antioxidant activ-

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doi:10.1016/j.jpba.2009.03.016

ities, and their chemical characterization by means of advanced analytical techniques such as HPLC-DAD or GC–MS. To the best of our knowledge, this is the first time this comprehensive screening methodology has been used to obtain functional compounds from *Himanthalia elongata* and *Synechocystis sp*.

2. Experimental

2.1. Samples and chemicals

Microalgae (*Synechocystis sp*.) and macroalgae (*Himanthalia elongata*) samples consisted of air-dried algae supplied by Las Palmas de Gran Canarias University (Las Palmas, Spain), stored under dry and dark conditions until use.

n-Hexane and methanol, HPLC grade, were provided by Lab Scan (Dublin, Ireland), while ethanol was purchased from VWR BDH Prolabo (Madrid, Spain), and chloroform from Merck (Darmstadt, Germany). Methanolic base was provided from Supelco (Bellefonte, USA), and sodium sulphate was purchased from Fluka Chemie AG (Buchs, Switzerland). Ammonium acetate was supplied by Panreac (Barcelona, Spain). Carotenoid standards, isolated from phytoplankton, used in LC-DAD (β-cryptoxanthin, canthaxanthin, echinenone, fucoxanthin, lutein, neoxanthin, violaxanthin and zeaxanthin) and chlorophylls (*a* and *b*) were purchased from DHI Water and Environment (Hoersholm, Denmark) whereas β-carotene was obtained from Sigma–Aldrich (Madrid, Spain). The purified water was obtained using a Milli-Q Water system (Millipore, Billerica, MA, USA).

Mixture from n-undecane to n-octacosane used in GC–MS, was from Aldrich (Sigma–Aldrich Chemie, Steinhein, Germany).

2.2. Pressurized liquid extraction (PLE)

Extractions of algae were performed using an accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, CA, USA), equipped with a solvent controller. Three different solvents (i.e., hexane, ethanol, and water) were used to obtain extracts with different compositions. Extractions were performed at four different extraction temperatures (50, 100, 150, and 200 °C), while the extraction time was 20 min. The extraction procedure has been described elsewhere [4]. When water was used, the extraction cell was filled with sand between the sample (6.0 and 2.0 g of sand at the bottom and top, respectively) to prevent the clogging of the system. The extracts obtained were protected from light and stored under refrigeration until dried.

For solvent evaporation, a Rotavapor R-210 (from Büchi Labortechnik AG, Flawil, Switzerland) was used. For water extracts, a freeze-dryer (Labconco Corporation, Missouri, USA) was employed.

2.3. Functional characterization

2.3.1. Antimicrobial activity

The PLE extracts were individually tested against a panel of microorganisms including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 11775, *Candida albicans* ATCC 60193 and *Aspergillus niger* ATCC 16404. A broth microdilution method was used, as recommended by NCCLS (National Committee for Clinical Laboratory Standards), to determine the minimum inhibitory concentration. All tests were performed as previously described [4].

2.3.2. Antioxidant activity

The antioxidant activity of PLE extracts was measured by the improved TEAC (Trolox Equivalent Antioxidant Capacity) assay, performed essentially as previously described [19], considering at least three different concentrations of each extract. Moreover, all analyses were done in triplicate.

2.4. Chemical characterization

2.4.1. HPLC-DAD analysis

Dry extracts were dissolved (concentration equal to 10 mg/ml) in the corresponding solvents (ethanol and water extracts in ethanol and hexane extracts in acetone) to be injected in the HPLC instrument (injection volume was 20 μL).

The pigment composition of the extracts was analyzed by HPLC using an Agilent 1100 Liquid Chromatograph equipped with a DAD (Agilent, Palo Alto, CA). The separation was carried out in a Novapack C_{18} column (150 mm × 3.9 mm, 4 μm particle size) from Waters (Milford, MA). The mobile phase was a mixture of solvent A (methanol/ammonium acetate 0.1 N; 7:3) and solvent B (methanol) at 0.9 mL/min according to the following step gradient: 25% B at initial conditions, changing to 50% in 1 min, and reaching 100% B at minute 10. Then, the mobile phase composition was kept constant until the end of the analysis. The identification of the peaks was performed using standards when possible. When no standards were available, tentative identification was based on UV–vis spectral characteristics and comparison with data appearing in the literature.

2.4.2. GC–MS analysis

Both the volatile fraction and fatty acids of the PLE extracts were analyzed using an Agilent-6890N GC system with a programmed split/splitless injector coupled to an Agilent-5973N quadrupole mass spectrometer (Agilent, Palo Alto, CA). The system was controlled by means of Agilent MSD Chemstation software. The particular separation conditions were adapted from [20]. Volatiles and fatty acids were tentatively identified by MS in SCAN mode, using a mass interval ranging from 35 to 450. Their spectra were compared with those in an MS library (Wiley Registry of Mass Spectral Data), with data found in the literature and with standards when available. Additionally, to identify compounds more precisely, their linear retention indices (RIs) were calculated. To do this, a mixture of hydrocarbons (n-undecane to n-octacosane, Sigma–Aldrich Chemie, Steinheim, Germany) dissolved in n-hexane was employed.

3. Results and discussion

As it has been previously mentioned, the goal of the present work was to demonstrate the usefulness of the comprehensive methodology developed to characterize potential functional ingredients from algae. The first step includes the selection of the raw material and the optimization of the extraction conditions. PLE is based on the extraction at high temperatures and pressures enough to maintain the solvent in the liquid state during the whole extraction procedure [21]. Among the advantages of PLE over the traditional extraction techniques, it is worth to mention that PLE is faster, automated and frequently does not require large amounts of toxic organic solvents [22]. Furthermore, PLE implies the extraction of a sample in an oxygen and light-free environment, which is of importance when working with bioactive compounds. When the extraction solvent used is water, the technique is named as subcritical water extraction (SWE) [22].

Following this methodology, two different algae species have been studied for the first time as possible natural sources for bioactive compounds, i.e., *Himanthalia elongata*, a brown algae, and microalgae *Synechocystis sp*. belonging to Cyanophyceae family.

Typically the screening starts with the selection of different solvents (covering a wide range of dielectric constants) and therefore, able to extract bioactives of different polarity. Besides, different temperatures are studied (namely, 50, 100, 150 and 200 °C). This

Table 1
Extraction conditions, yield produced and antioxidant and antimicrobial activities of the *Himanthalia elongata* PLE extracts.

Solvent (dielectric constant, ε)	Extraction temp (°C)[a]	Yield (%)	Antioxidant activity[b]	Antimicrobial activity			
				Escherichia coli MBC[c]	*Staphylococcus aureus* MBC	*Candida albicans* MFC[d]	*Aspergillus niger* MFC
Hexane (1.9)	50	3.41	0.060 ± 0.001	7.50	8.25	12.00	14.00
	100	3.50	0.118 ± 0.004	7.50	8.25	12.00	14.00
	150	4.72	0.143 ± 0.003	7.00	8.25	10.00	12.25
	200	7.59	0.140 ± 0.001	7.25	8.25	10.00	12.25
Ethanol (24.3)	50	8.29	1.036 ± 0.010	7.00	7.00	8.50	13.00
	100	10.56	1.067 ± 0.003	6.50	7.00	8.25	13.00
	150	19.23	0.480 ± 0.007	6.00	6.25	8.00	12.25
	200	36.91	0.286 ± 0.006	6.00	6.25	8.00	12.00
Water (78.5)	50	9.51	0.246 ± 0.002	13.50	13.00	12.50	14.25
	100	15.08	0.273 ± 0.002	13.00	13.00	12.50	14.00
	150	46.43	0.350 ± 0.006	12.50	12.50	12.25	13.00
	200	51.56	0.565 ± 0.001	13.00	12.50	12.25	13.00
Reference antibiotic				10.00	10.00	100.00	150.00

[a] Extraction time always 20 min.
[b] Antioxidant activity expressed as TEAC mmol of Trolox/g of extract.
[c] MBC, minimum bactericidal concentration.
[d] MFC, minimum fungicidal concentration. MBC and MFC values given as mg/mL for samples and as μg/mL for antibiotic.

is of particular importance when using water as extraction solvent since it has been previously demonstrated that the use of liquid water at high temperatures is a valuable tool for the extraction of medium-low polarity compounds [7]. In fact, subcritical water has been suggested as a valuable technique to obtain antioxidants from natural sources [10]. Extraction time was kept constant and equal to 20 min since the influence of this parameter has demonstrated to be low in previous works [15]. As it can be observed in Tables 1 and 2, the extraction yield significantly increases with the extraction temperature for both algae. This behavior can be explained by an increase on the solubility of the sample compounds with temperature along with an improved mass transfer from the sample to the pressurized solvent. It is also interesting to note the differences among the tested solvents, which can give an idea of the main compounds found in the extracts (according to the different solvent polarities tested). In both cases, the higher the polarity of the solvent employed, the higher the yield produced. This trend is even more marked when considering *Synechocystis sp*. being, nevertheless, water in both cases the solvent which produced higher yields. This fact can led to deduce that the main compounds in the chemical compositions of these two algae would be medium-highly polar compounds, as it would corresponds with the decrease in the water dielectric constant at high temperatures.

The next step in the methodology would be to measure their functional activities. Thus, *in vitro* assays provide the first screening of bioactivities and, therefore, the first selection of active extracts. Tables 1 and 2 show the antioxidant activities obtained for the produced extracts using the TEAC assay for each algae. Interestingly, the behavior of this parameter was different depending on the solvent used. While higher antioxidant values were obtained increasing water temperature, ethanol extracts presented their maximum at medium extraction temperatures. Besides, these extracts were for both algae those which provided the highest antioxidant capacities followed by water and hexane, suggesting the presence of medium polarity antioxidants.

Regarding the antimicrobial activity, results obtained are also shown in Tables 1 and 2 for *Himanthalia elongata* and *Synechocystis sp*., respectively.

As it can be observed, bacteria were the most sensible microorganisms considering both algae whereas their activity against the

Table 2
Extraction conditions, yield produced and antioxidant and antimicrobial activities of the *Synechocystis sp*. PLE extracts.

Solvent (dielectric constant, ε)	Extraction temp (°C)[a]	Yield (%)	Antioxidant activity[b]	Antimicrobial activity			
				Escherichia coli MBC[c]	*Staphylococcus aureus* MBC	*Candida albicans* MFC[d]	*Aspergillus niger* MFC
Hexane (1.9)	50	1.10	0.008 ± 0.000	8.00	9.00	13.50	15.00
	100	0.90	0.015 ± 0.001	8.00	9.00	13.50	15.00
	150	1.74	0.018 ± 0.001	7.00	8.50	13.50	14.00
	200	3.27	0.035 ± 0.000	8.00	8.50	13.50	14.00
Ethanol (24.3)	50	6.00	0.263 ± 0.010	7.00	7.00	12.50	14.00
	100	13.20	0.407 ± 0.008	7.00	7.00	12.00	14.00
	150	12.83	0.466 ± 0.002	7.00	6.50	12.00	14.00
	200	23.98	0.092 ± 0.001	7.00	5.60	12.00	14.00
Water (78.5)	50	11.00	0.015 ± 0.001	13.50	13.50	13.25	16.50
	100	16.45	0.017 ± 0.001	13.50	13.50	13.25	16.50
	150	33.10	0.037 ± 0.001	13.00	13.50	13.50	16.00
	200	63.41	0.330 ± 0.006	13.00	13.50	13.50	16.00
Reference antibiotic				10.00	10.00	100.00	150.00

[a] Extraction time always 20 min.
[b] Antioxidant activity expressed as TEAC mmol of Trolox/g of extract.
[c] MBC, minimum bactericidal concentration.
[d] MFC, minimum fungicidal concentration. MBC and MFC values given as mg/mL for samples and as μg/mL for antibiotic.

Table 3
Volatile compounds identified in the *Himanthalia elongata* and *Synechocystis sp.* PLE extracts obtained at 200 °C and 20 min using ethanol and their relative abundance.

ID	RT (min)	RI	Compound	*Himanthalia elongata* Relative abundance (%)	*Synechocystis sp.* Relative abundance (%)
1	23.15	1431	Tetradecane	–	–
2	24.23	1477	2-Pyrrolidinone, 5 (-cyclohexylmethil)-	–	6.3
3	24.48	1487	2-Hexenedioic acid, bis (trimethylsilyl)ester	–	4.8
4	25.40	1538	Pentadecane	–	28.3
5	25.64	1553	Phenol,2,4-bis (1,1-dimethylethyl)-	6.5	1.4
6	25.68	1555	Phenol,2,6-bis (1,1-dimethylethyl)-4-methyl	3.3	0.8
7	26.87	1645	1,2-Benzenedicarboxylic acid, diethyl ester	–	1.0
8	27.90	1745	Cyclododecane	8.3	–
9	28.56	1768	Tetradecanoic acid	3.8	–
10	28.92	1857	Tetradecanoic acid, ethyl ester	–	10.8
11	29.32	1906	Neophytadiene	–	4.7
12	29.52	1932	Neophytadiene derivative	–	1.4
13	29.67	1950	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (Fitol)	–	2.8
14	29.75	1960	Hexadecanoic acid, ethyl ester	–	1.0
15	30.36	2045	Ethyl 9-hexadecenoate	–	11.0
16	30.20	2021	Hexadecanoic acid	35.6	–
17	30.49	2065	Hexadecanoic acid, ethyl ester	–	12.4
18	30.51	2069	Neophytadiene derivative	–	–
19	31.35	2197	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl, derivative (Fitol-derivative)	–	13.3
20	40.64	>3600	Stigmasta-5,24(28)dien-3-ol (Fucosterol)	42.6	–

fungi was low. Significant differences in the antimicrobial activity were found depending on the solvent used. In general, extracts obtained using ethanol were more active than those obtained with hexane. The analysis of the antimicrobial activity of these extracts as a function of extraction temperature indicated that an increase in the extraction temperature normally did not produce a significant change in their antimicrobial activity.

However, the characterization of the new bioactives from algae goes farther. Once some bioactivity is found, the extracts selected are chemically characterized in order to determine the compounds responsible for the biological activity observed. *Himanthalia elongata* and *Synechocystis* extracts were characterized by HPLC-DAD in order to determine their pigments and other non-volatile antioxidants composition and by GC–MS to study their volatiles and fatty acids.

Different natural antimicrobial compounds have been described in algae belonging to a wide range of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons [4,20,23]. Thus, two different GC–MS methods were used to analyze both, fatty acids and volatile compounds, in the ethanol extracts that showed higher antimicrobial activity from the two studied algae (that is, 200 °C). As can be seen in Table 3, several volatile compounds were identified in ethanol extracts from *Synechocystis sp.* and *Himanthalia elongata*, mainly fatty acids, alkanes, phenols and compounds such as phytol (2-hexadecen-1-ol, 3,7,11,15-tretramethyl) and neophytadiene. These compounds have been already proposed to have certain antimicrobial activity [24]. Others, like fucosterol, have been also described as active in other brown algae [25]. Results on the fatty acid composition of ethanol extracts of both algae at 200 °C are shown in Table 4. The antimicrobial activity has been usually attributed to long-chain unsaturated fatty acids (C_{16}–C_{20}), including palmitoleic, oleic, linoleic and linolenic acids, while long chain saturated fatty acids, including palmitic and stearic acids were less effective [26]. Therefore, the activity found in these algae could be related with the amount of palmitoleic and oleic acids existing in the extracts. In fact, it has been observed that *Staphylococcus aureus* was particularly sensible to palmitoleic and oleic acids [26]. Interestingly, *Synechocystis sp.* extract, which was the richest in terms of palmitoleic acid (see Table 4), was also the most active against this bacteria. Also, the most active extracts (those obtained with ethanol at 200 °C) contained significantly higher amount of fatty acids compared to the others (data not shown). Besides, according also to this theory, the analysis of the water extracts (the less active) revealed that they did not contained significant amounts of neither volatiles nor fatty acids, which is agreement with the analytical data obtained.

On the other hand, it is well-known that a high carotenoid content could be found in *Synechocystis* [27]. For this reason, an HPLC-DAD method was used with the aim to identify, at least tentatively, the carotenoids present in the extracts and that could be considered as responsible for the antioxidant activity observed. Some of these carotenoids were characterized using standards, whereas the rest were assigned tentatively on the basis of their specific elution and UV–vis spectra. While in the *Synechocystis sp.* extract, the main carotenoid was β-carotene, followed by zeaxanthin, myxoxanthophyll and echinenone, fucoxanthin and zeaxanthin were the most abundant in the *H. elongata*

Table 4
Fatty acids identified in the more active PLE extracts of *Synechocystis sp.* and *Himanthalia elongata*. Extraction conditions: ethanol at 200 °C for 20 min.

ID	RT (min)	Common name	Fatty acids (methyl esters)	*Himanthalia elongata* Relative abundance (%)	*Synechocystis sp.* Relative abundance (%)
1	6.6	Miristic acid	Tetradecanoic acid	9.5	36.7
2	7.2		Pentadecanoic acid	–	2.1
3	8.0	Palmitic acid	Hexadecanoic acid	34.6	14.3
4	8.3	Palmitoleic acid	9-Hexadecanoic acid	2.5	43.0
5	10.1	Stearic acid	Octadecanoic acid	1.4	1.4
6	10.4	Oleic acid	9-Octadecanoic acid	17.9	0.9
7	10.5		11-Octadecenoic acid	–	1.6
8	11.3	Linoleic acid	9, 12-Octadecadienoic acid	9.9	–
9	12.5	α-Linolenic acid	9, 12, 15-Octadecatrienoic acid	9.2	–
10	17.2	Araquidonic acid	5,8,11,14-Eicotetraenoic acid	15.1	–

Table 5
Pigment composition in the *Himanthalia elongata* and *Synechocystis sp.* PLE extracts obtained with ethanol at 100 °C and 20 min.

ID	RT (min)	λ_{max} (nm)	Compound	*Himanthalia elongata*		*Synechocystis sp.*	
				Norm. area (%)	Conc. (mg/g)	Norm. area (%)	Conc. (mg/g)
1	4.40	278, 336, 444, 580, 632, 666	NI Chlorophyll	5.06			
2	5.35	268, 338, 392, 446, 582, 634	NI Chlorophyll	24.49			
3	7.46	448, 468	Fucoxanthin[a,b]	39.76	0.82		
4	8.59	372, 412, 506, 536, 608, 668	Violaxanthin[a,b]	2.82	0.05		
5	9.03	294, 446, 472, 500	Myxoxanthophyll[a,b]			5.74	0.58
6	9.24	442, 462	NI Carotenoid	4.29			
7	9.56	270, 320, 334, 438, 462	NI Carotenoid	6.41			
8	10.39	284, 424, 448, 476	Zeaxanthin[a,b]	9.97	0.13	19.02	1.64
9	11.27	416, 474, 640, 660	NI Chlorophyll			10.67	
10	11.51	336, 440, 618	NI Chlorophyll			11.65	
11	11.82	256, 342, 400, 456	NI Carotenoid			4.18	
12	12.24	388, 438, 654, 678	NI Chlorophyll			5.86	
13	12.62	364, 396, 436, 588, 634, 680	NI Chlorophyll			10.13	
14	13.23	340, 378, 416, 430, 574, 614, 656, 666	NI Chlorophyll			14.79	
15	13.79	420, 656	NI Chlorophyll			4.84	
16	14.06	296, 356, 416, 640, 660	NI Chlorophyll			2.05	
17	14.43	346, 418	NI Carotenoid			2.16	
18	15.12	478, 422, 450, 474	Echinenone[a,b]			3.08	0.24
19	17.67	338, 404, 432, 614, 666	NI Chlorophyll	3.59			
20	18.08	330, 378, 408, 430, 574, 610, 656	NI Chlorophyll			3.92	
21	22.43	278, 420, 452, 478	β-Carotene[a,b]			1.91	2.04
22	29.85	324, 370, 408, 502, 534, 606, 666	NI Chlorophyll	2.27			
23	31.69	324, 370, 408, 502, 534, 606, 666	NI Chlorophyll	1.34			

[a] Similar spectrum as standard.
[b] Retention time equal to standard.

extract. Quantitatively, the *Synechocystis* extracts obtained at 100 °C (the most active) possessed higher carotenoid content than the ones obtained at different temperatures and using other solvents. Therefore, a relatively strong influence of the presence of these compounds over the antioxidant activity can be suggested. However, it is possible to see in Table 5, how the total amount of carotenoids in the *H. elongata* extract is lower than in the *Synechocystis sp.* extract in spite of the higher antioxidant activity demonstrated by the first. In this respect, it should be considered that algae are complex matrices of compounds; therefore, in many extracts antioxidant activity would not be closely connected with a specific compound but with a mixture of compounds. Moreover, this mixture of compounds can act synergistically and, therefore, the antioxidant activity cannot be explained with the analysis of a specific algae component. In this respect, TEAC values of *Himanthalia elongata* are not, at least directly, related to carotenoids content. Therefore, a deeper analysis would be necessary to establish the presence of other antioxidant components (for instance, tocopherols) or the possible synergistic action of chlorophyll *a* and its derivatives with carotenoids and tocopherols as reported by other authors [28].

4. Conclusions

In this work, a general procedure to carry out the screening for new natural functional compounds is presented and applied to two different algae species (*Hymanthalia elongata* and *Synechocystis sp.*) for the first time. This procedure includes the use of novel natural sources together with environmentally friendly extraction techniques and advanced analytical techniques, and allows the characterization of bioactive extracts that could be potentially used for the food industry. The use of PLE allows the attainment of food-grade extracts obtained only with water or with other generally recognized as safe solvents, such as ethanol. Besides, this methodology allows to establish the correlation between the functional activities and the compounds found and it can be considered as a powerful tool in algae and microalgae metabolomics. The possibility to find functional interesting compounds from algae and microalgae using this methodology looks promising.

Acknowledgments

This work has been financed by Spanish Ministry of Education and Science (AGL2005-06726-C04-01/02/04 and CONSOLIDER INGENIO 2010 CSD2007-00063 FUN-C-FOOD) and Comunidad Autónoma de Madrid (S-0505/AGR/000153) projects. M.P. thanks CSIC for her I3P fellowship. M.H. would like to thank the Spanish Science and Innovation Ministry (MICINN) for the “Juan de la Cierva” contract.

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