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Evaluation of bioactive compounds in extracts obtained from three romanian marine algae species

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VICENTIU-BOGDAN HORINCAR[1], GEORGIANA PARFENE[1], GABRIELA BAHRIM[1]
[1] *Faculty of Food Science and Engineering, Dunarea de Jos University, Galati, Romania*
Str. Domneasca nr. 111, cod800201, Galati, Romania,Tel +40236-414871
e-mail: h_vicentiu@yahoo.com

Abstract

Algae are used for many purposes: in food industry, animal feeding, medicine, cosmetic industry and for soil enrichment. There are also many secondary ways for using algae: producing alginates and derivates of algae used in industry. Nowadays researchers use to analyze algae for medical purposes, because they have a strong potential against many diseases, in alimentation because they act like protective and functional additives.
In this present work it was studied the antioxidant activity and the total phenolics content of five different extracts of the three species of algae harvested from Romanian area of Black Sea shore (Ceramium rubrum, Cladophora vagabunda and Enteromorpha intestinalis) and it was performed a SPME-MS analisys for a screening of volatile compounds. From all five kinds of extractions the aim was to establish which solvent extracts better the phenolic compounds. After extractions it was studied the total phenolic content by Folin Ciocalteau method and antioxidant activity by DPPH assay was determined. The best results were obtained by extraction with water but also with others solvents good result were obtaineds. At the final of this study it was demonstrated that the wild marine algae from Black Sea have phenolic compounds with an antioxidative activity and also have some important volatile compounds that can be used in industry.

Keywords: *algae polyphenols, antioxidant activity, marine algae, DPPH assay, solvent extraction, volatile compounds, SPME, TPC*

1. Introduction

The beginnings of algae research was dated in 1768 when Samuel Gottlieb Gmelin developed the work Historia Fucorum. He was followed by W.H. Harvey (1811—1866) who was the first who divided the algae into four divisions, based on their pigmentation. This was the first use of a biochemical criterion in plant systematics. Harvey's four divisions are: Red Algae (Rhodophyta), Brown Algae (Heteromontophyta), Green Algae (Chlorophyta) and Diatomaceae (Dixon, 1973). Over the years, it was demonstrated the incredible effect of biocomponents of algae against terrible diseases like cancer. Recently phytochemicals in herbal plants have attracted a great deal of attention, mainly concentrated on their role in preventing diseases caused as a result of oxidative stress (Southon, 2000). Naturally growing seaweeds are an important source of food, especially in Asia. They provide many vitamins including: A, B1, B2, B6, niacin and C, and are rich in iodine, potassium, iron, magnesium and calcium (Simoons, Frederick J, 1991) In addition commercially cultivated microalgae, including both algae and cyanobacteria, are marketed as nutritional supplements, such as *Spirulina* (Morton, Steve L, 2008) *Chlorella* rich in vitaminC, *Dunaliella*, high in beta-carotene. The oils from some algae have high levels of unsaturated fatty acids. For example, *Parietochloris incisa* is very high in arachidonic acid, where it reaches up to 47% of the triglyceride pool (Bigogno, et al.,2002). Some varieties of algae contain the long-chain, essential omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), in addition to vitamin B12. The vitamin B12 in algae is not biologically active. Fish oil contains the omega-3 fatty acids, but the original source is algae (microalgae in particular), which are eaten by marine life such as copepods and are passed up the food chain(Allison Aubrey ,Morning Edition,

November 1, 2007) Algae has emerged in recent years as a popular source of omega-3 fatty acids for vegetarians who cannot get long-chain EPA and DHA from other vegetarian sources such as flaxseed oil, which only contains the short-chain alpha-linolenic acid (ALA).

Many studies reported best results of algae against the cancerous cells. Other studies revealed that many marine algae can be used like sources of natural antioxidants and like food additives (Jeremy Bechelli et al., 2010)

Black sea is a good environment for growth of many algae which grow here more abundantly as in other places. These algae are growing on the rocks under maximum 1 meter under water. *Ceramium rubrum* is a brown algae from *Rodophyta* family that are small algae growing to no more than 30 cm in length. They consist of an axis of cells surrounded by smaller cells forming a cortex. In most species there is a continuous cortex enclosing the axis, in others the cortical cells are arranged only in nodes at the junction of cells of the axes (Maggs and Hommersand, 1993). *Cladophora vagabunda* takes part in genus of reticulated filamentous *Ulvophyceae* (green algae). The genus *Cladophora* contains many species that are very hard to tell apart and classify, mainly because of the great variation in their appearances, which is affected by habitat, age and environmental conditions (Gestinariet al., 2010). It is growing in the neighbourhood of rocks and the population is looking like a green hair. *Enteromorpha intestinalis* is a part of *Ulva* family and is growing like the others on rocks under 0.5 to 2 meters in water. The thallus of *Enteromorpha* is tubular with the wall of the tube of a single cell layer thick. The thallus can be branched or unbranched, and there is a wide variety of forms within the genus. *Enteromorpha* is attached to the substrate by a disc-like holdfast (Eur. J, 2003) and can reach 2 meters in length.

This study is a part of the studies for food suppliers agents made from algae. Three species of algae, *Ceramium rubrum, Cladophora vagabunda* and *Enteromorpha intestinalis,* that are present on almost all Romanian sea shore were studied as sources of bioactive compounds with antioxidant activity.

2. Materials and methods

2.1 Chemicals

Methanol, ethanol, acetone, and hexane were at HPLC grade of purity and it was purchased from Merck KgaA Darmstadt, Germany. Folin–Ciocalteu's phenol reagent, DPPH (1,1-diphenyl-2-pikryl-hydrazin) and sodium carbonate was acquired from Sigma-Aldrich Chemie (Steinheim, Germany).

2.2 Plant Material

The algae biomass was harvested by hand from sea water and washed after with fresh water to remove the salt and sand. After that the biomass was left in freezer for 24 hours, at -70°C. It was performed the lyophilization and after that, the dry biomass was kept in freezer at -20°C for preservation until the next step.

2.3 Bioactive Compounds Extraction

Many solvents were use for extraction (water, methanol, ethanol, hexane, and acetone). 0.5 grams of algae biomass was weighted and mixed with 15 ml of every solvent. It was performed 20 minutes of ultrasonication at 40°C. The mixtures were left at 4°C and dark conditions for 12 hours. All the extracts were filtered with Ø 45µm filter paper and stored in dark conditions in freezer at -20°C until the analysis.

2.4 Dpph Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a well-known radical and a trap ("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized.
The free radical scavenging activity was measured by using the following protocol. In a glasstube was added 100 µl of seaweed extract and 1 ml solution of DPPH (3,5%) in methanol of 95% purity, after that was added methanol up to the volume of 4 ml. Also a blank probe was made with 1 ml of DPPH solution plus 3 ml of methanol of 95% purity. The absorbance was recorded at 515 nm against a methanol blank after 1 hour or resting in dark conditions.

2.5 Total Phenolics Determination (Tpc)

Firstly it was made solutions of gallic acid in different concentrations 50, 100, 150, 250, 500 mg/L what was served for the calibration curve(20 µl gallic acid solution at every concentration made, 1.58 ml water, 100 µl Folin-Ciocalteau reagent and 300 µl sodium carbonate, 2 ml in total). A blank sample was performed with 1.6 ml water, 100µl Folin-Ciocalteau reagent, and 300 µl sodium carbonate 20% (w/v), 2 ml in total. The samples were made with 20 µl seaweed extract, 1.6 ml H_2O, 100 µl Folin-Ciocalteau reagent and 300 µl sodium carbonate, 2 ml in total. The tubes were mixed and allowed to stand for 1 h in the darkness at room temperature. The absorbance was measured at 765 nm using a SHIMADZU 1700 UV–vis spectrophotometer. The estimation of phenolic compounds was carried out in triplicate, and the results were averaged.

2.6 Spme And Gc Analysis Of Volatile Compounds

For the first step in that analyze it had been performed a sample watering by adding in 5 ml of pure water 5 mg of sample. An SPME fiber (75 lm Carboxen-PDMS; Supelco, Inc., Bellefonte, PA, USA) was exposed to the sample headspace in a 40°C oven. The VFC were desorbed by inserting the SPME fiber into a GC injector (injector temperature 230 °C) in splitless mode connected with a fused-silica GC column (DB-1, 30 m, 0.53 mm ID, 1.5 lm film thickness) (J&W Scientific, Folsom, CA, USA) for 15 min. The initial temperature of the GC was set at 40 °C for 4 min, then the oven temperature was increased at a rate of 5 °C /min until reaching 230 °C which was maintained for another 3 min. The detector temperature was set at 250 °C.

2.7 Gc–Ms Analysis Or Volatile Compounds

For GC–MS analysis, a GC (HP 6890) coupled with a mass spectrometer (HP 5973, Hewlett-Packard, Palo Alto, CA, USA) was used, with the column (HP-1, 30 m, 0.32 mm ID and 0.25 lm film thickness). The GC operating conditions (temperature and time) were the same as described above. The mass spectrometer was operated in the electron-ionization (EI) mode at an ionization voltage of 70 eV. Runs of hydrocarbon mixture (ASTM D5307, 4-8182; Supelco, Bellefonte, PA, USA) were performed under the same GC conditions described previously, and the RI calculated were referred to those of the previously(X) published Kovat indices.

3. Results and discusions

3.1 Extraction Differences

It is well known that the yield of chemical extraction depends on the type of solvents with varying pH, polarities, extraction time and temperature, as well as on the chemical

compositions of the sample. Under the same conditions, the solvent and the chemical properties of the sample are the most important factors. Earlier, solvents such as methanol, ethanol, acetone and water have been commonly used for the extraction of phenolics from brown and green seaweeds (Yuan & Walsh, 2006; Duan, Zhang, Li, & Wang, 2006; Ganesan, Kumar, & Bhaskar, 2008; Lim et al., 2002). The differences of the extraction in this study is presented in *table 1* where is presented a classification of solvents that had a better extraction regarding at total phenolic amount.

3.2 Total Phenolic Compounds (Tpc)

The amount of total phenolics varied from 28.1 at 212.4 mg equivalent GA/100 g of dry alga powder (*Figure 1*).

The obtained results do not show a similarity at all the samples. In the case of *Cladophora V.* is another classification of how solvent affected the TPC as the other 2 seaweed extracts that had the same classification.(figure 1)

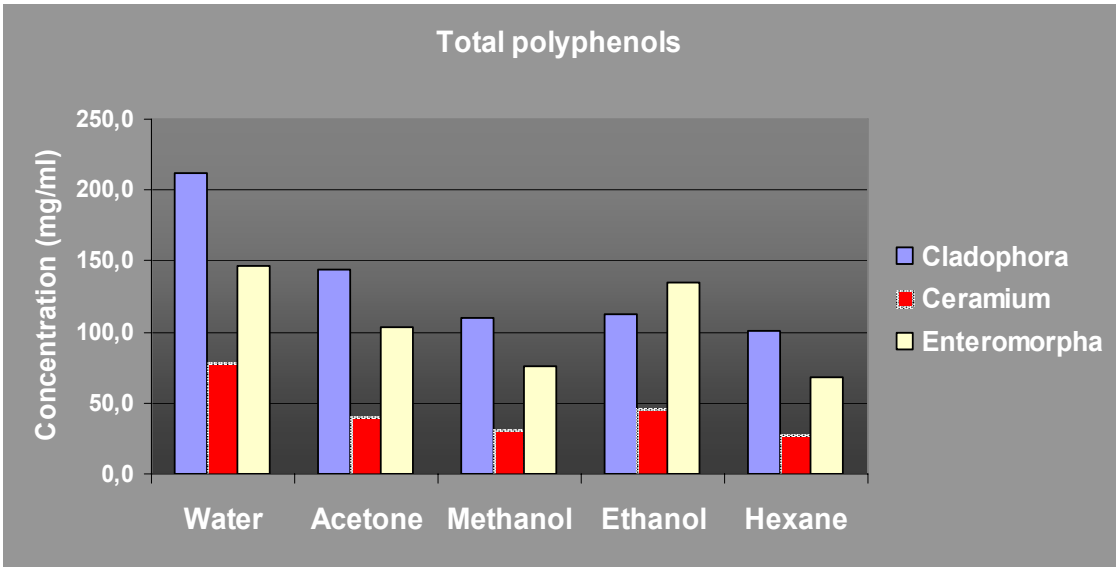

Figure1. Comparison of TPA regarding the different solvents

3.3 Dpph Radical Scavenging Activity

The results obtained in this analysis are not showing some connections with the TPC. As we can see comparing both the two Figure1 with Figure2, there are no similarities between the TPC and antioxidant activity of the seaweed extracts. The best antioxidant activity is obtained by *Enteromorpha* when the best TPC was obtained by *Cladophora vagabunda* , both values obtained in water extraction. In the table 2 is presented a comparison between the two analyses. The used solvents do not look to have a similarity of extraction of phenolic compounds, because in the graphic representation it cannot be highlighted an algorithm of extract nol the curves are almost in the same positions.

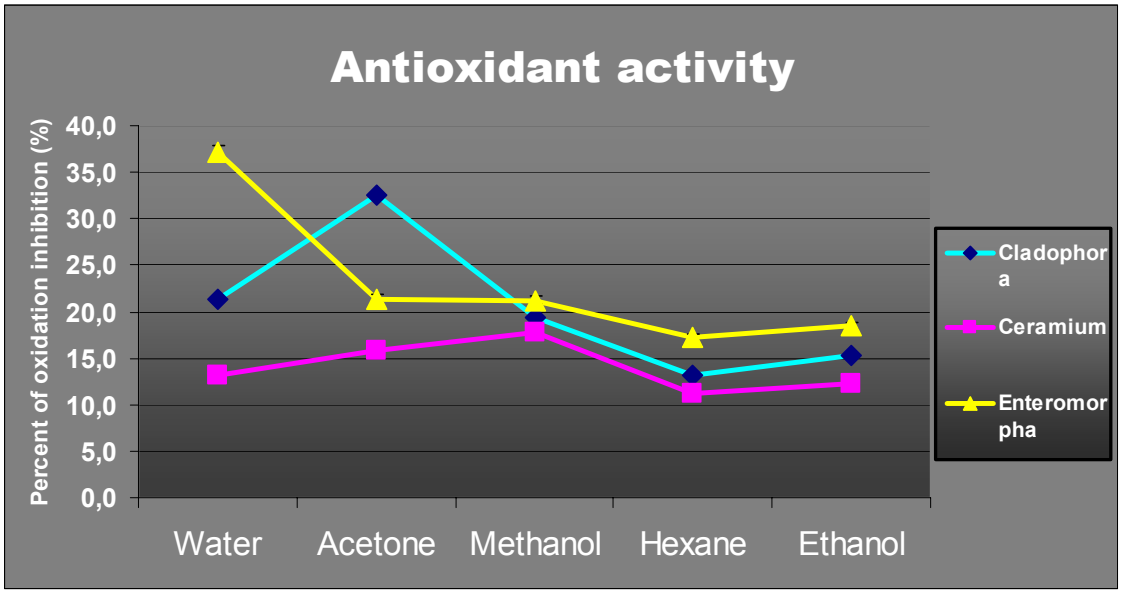

Figure 2. Comparison of differences for solvent extraction regarding percent of oxidation inhibition

	Solvent	TPC (mg/100 g)	Antioxidant activity (%)
Cladophora vagabunda	Water	212.4	21.4
	Acetone	144.2	32.5
	Methanol	109.9	19.3
	Ethanol	112.7	15.3
	Hexane	101.1	13.1
Ceramium rrubrum	Water	78.3	13.1
	Ethanol	46.2	12.2
	Acetone	41.1	15.9
	Methanol	31.6	17.8
	Hexane	28.1	11.2
Enteromorpha intestinalis	Water	146.4	37.2
	Ethanol	134.5	18.5
	Acetone	103.4	21.4
	Methanol	75.5	21.2
	Hexane	68.2	17.2

Table2. Comparison between results from TPC and DPPH radical scavenging activity

3.4 Volatile Compounds

After the GC analysis it was identified many volatile compounds in all the samples, but many of them were only traces. In the tables below will be presented the most representative volatile compounds of the samples. The first analyzed sample was *Ceramium rubrum.*. In this case the compound with the highest concentration was hexanal, with 11.15 %, followed by octane with 9.80 % and furanone a with 7.24 %. Also furanone b is present in *Ceramium rubrum*, but in a smaller amount (2.80%).

Table 3. Volatile compounds in *Enteromorpha intestinalis*

2,4,4 trimethyl 1 pentene	3.08
acetone	0.96
2 ethyl furan	2.07
pentanal	2.26
methyl isobutyl ketone	1.49
trichloromethane	7.27
hexanal	14.56
3 hexen 2 one	5.32
2,6 dimethyl 4 heptanone	1.81
hepatanl	2.31
2 hexanol	1.32
2 pentyl furan	1.37
o-Cymene	3.00
octanal	3.10
nonanal	5.99
furanone a	3.90
furanone b	1.33
b-Ionone	1.39

Table 4 Volatile compounds in *Ceramium rubrum.*

octane	9,80
pentanal	1,62
hexanal	11,15
3 hexen 2 one	4,01
2,6 dimethyl 4 heptanone	1,39
heptanal	2,87
2 hexanol	1,12
o-Cymene	3.58
octanal	3.49
nonanal	6.98
1 octen 3 ol	6.69
2,5,5 trimethyl 2 hexene	4.67
benzaldehyde	3.08
furanone a	7.24
furanone b	2.80
a-Ionone	1.97
b-Ionone	1.26

On *Cladophora vagabunda.* it was identified a high concentration of 3 hexen 2 one in a percentage of 27.87%. This compound was followed by the acetone (12.40%) and furanone b (3.20%). Here the concentration of furanone b is higher than in *Ceramium,* but furanone a is in a lower concentration, of 2.91 %. In *Enteromorpha intestinalis* it was recorded a maximum concentration of hexanal (14.56%), followed by trichloromethane (7.27%) and nonanal (5.99%). The compounds which were present in all analyzed samples in different concentrations was hexanal, furanone a, furanone b and b-lonone. This is the reason that the marine algae can be sources for these compounds, of use for industrial purposes.

Table 5 Volatile compounds in *Cladophora v.*

acetone	12.40
pentanal	1.70
methyl isobutyl ketone	1.45
hexanal	3.38
3 hexen 2 one	27.87
2,6 dimethyl 4 heptanone	1.60
2 hexanol	1.27
o-Cymene	2.74
2 octanone	1.13
nonanal	2.27
1 octen 3 ol	1.78
hexadecane	1.17
Menthol	1.71
furanone a	2.91
furanone b	3.20
b-Ionone	2.30

4. Conclusions

At the end of this study it was demonstrated that in all studied algae species it can be found interesting compounds that can be useful for our health. The phenolic compounds recorded on these three marine algae were in correlation with antioxidant activity, depending on the solvent that was used for the extraction and the algae species. The main conclusion is that we can easy extract phenolic compounds with water, and to have high antioxidant effects by using it.

Also there are many others compounds that can be used for many purposes. Also this study showed that in marine algae are present volatile compounds with a high importance in industry, such as furanone a, furanone b and hexanal.

It can be interesting to use algae in our lives because along with their antioxidant effect, they also contain great amounts of nutrients that are essential for a strong health.

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