

# Antioxidant activity and lipid profile of three seaweeds of Faro, Portugal

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**Abstract** Three seaweeds, *Codium fragile* (Suringar) Hariot, *Ulva lactuca* (Linn.) members and *Eisenia arborea* (Areschoug) (Phaeophyta) were assessed for their antioxidant activities and lipid profile. Antioxidant activities of methanolic and aqueous extracts were analyzed for DPPH free radical scavenging, FRAP, total antioxidant, ABTS radical cation decolorizing, reducing power, total flavonoid, and total phenolic contents. Methanolic extract of *E. arborea* was found to contain high phenolic and flavonoid contents with higher antioxidant activities in all assays studied. Aqueous extract of *E. arborea* was the next active extract, whereas both the extracts of *C. fragile* were not highly anti-oxidative in nature. The methanolic extract of *U. lactuca* possessed higher activity and higher phenolic content compared to aqueous counterparts. This suggests the potential uses of seaweeds as a source for natural bioactive compounds with more antioxidant activities. Among these three seaweeds, *E. arborea* was found to have higher activities and their lipid and chemical constituents were identified by GCMS and LCMS.

**Keywords** Antioxidant · *Codium fragile* · *Eisenia arborea* · Seaweeds · *Ulva lactuca*

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## Introduction

Seaweeds have attracted the attention worldwide due to their commercial uses as fertilizers, nutraceuticals, alginate, agar, carrageenan, food, fine chemicals, and biofuels (Fornes et al. 2002; Van de Velde et al. 2002). The fact that some pigments, such as fucoxanthin, astaxanthin, carotenoid, and polyphenols such as phenolic acid, flavonoid, tannins were identified as the potential antioxidant compounds are widely distributed in seaweeds. They exhibit higher anti-oxidative activities as reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation (Heo et al. 2005). The role of antioxidants is to inhibit the development of oxidative rancidity in fat-based foods because oxidation is a naturally occurring process within the human body; a balance with antioxidants must exist to maintain health. Seaweeds are consumed by almost all the countries from Asia, Africa, America, Australia, and the European Union. These produce a great variety of secondary metabolites characterized by a broad spectrum of biological behavior such as antibacterial and antioxidant capacities.

Free radicals and other reactive oxygen species (ROS) are generated continuously not only via normal physiological processes but also by external stimulations. Normal physiological processes need oxygen in order to carry out their operations as a resultant by-product, like ROS are produced within the human body. If these harmful factors accumulate in cell, tissue, and other vital organs of the body, then our body will be exposed to dangerous circumstances. Of the external stimulations, ROS can be induced by tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Karawita et al. 2005). The ROS including superoxide ( $O_2^-$ ), hydroxyl radical ( $HO\cdot$ ), and hydrogen peroxide ( $H_2O_2$ ) have the

ability to react with a large variety of easily oxidisable cellular components such as proteins, lipids, nucleic acids, and carbohydrates. Their oxidative damages cause aging and many other diseases, including arthritis, strokes, heart diseases, atherosclerosis, diabetes, cancers, and neurodegenerative disorders (Lemberkovices et al. 2002).

Some studies (Lu and Foo 2000) have reported that there is an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of human diseases. Therefore, development and utilization of more effective antioxidant from natural resources is desired for use in foods or medicinal materials to replace the synthetic antioxidants. Many marine bio-resources have been attracted attention in the search for natural bioactive compounds to develop new drugs and healthy foods. Seaweeds have many phytochemicals with various bioactivities including antioxidant, anti-inflammatory, and anticancer. Among them, antioxidant activity is intensively focused due to the current growing demand from the pharmaceutical industry where they are interested in anti-aging and anti-carcinogenic compounds which possess health benefits. Therefore, many types of seaweeds have been examined to identify new and effective antioxidant compounds, as well as to elucidate the mechanisms of cell proliferation and apoptosis (Athukorala et al. 2003; Heo et al. 2005; Lee et al. 2004).

The aim of the present study is to investigate the in vitro antioxidant activities of aqueous and methanol extracts of seaweeds collected from the coast of Faro, Portugal and to evaluate their potential as natural anti-oxidative sources for food and medicinal industry.

## Materials and methods

### Seaweed collection

The seaweeds *Codium fragile* (Suringar) Hariot and *Ulva lactuca* Linn. (Chlorophyta) were collected during the period of July 2014 to September 2014, and *Eisenia arborea* f. *bicyclis* (Kjellman) (Phaeophyta) was purchased from a commercial shop, Faro, Portugal. After the collection of these seaweeds, salts, epiphytes, and sand particles were removed from them using tap water and rinsed with freshwater and stored in a refrigerator at  $-20\text{ }^{\circ}\text{C}$  until further use.

### Sample preparation and extraction

The seaweed samples were dried in shade and homogenized with a grinder before extraction. The powdered samples were then extracted for 24 h with Methyl alcohol (MeOH) using a Soxhlet apparatus at room temperature, and aqueous extracts were prepared by continuous shaking

at room temperature. Resultant extracts obtained were  $\text{Ea}^{\text{Aq}}$ ,  $\text{Ea}^{\text{Me}}$ ,  $\text{Cf}^{\text{Aq}}$ ,  $\text{Cf}^{\text{Me}}$ ,  $\text{Up}^{\text{Aq}}$ ,  $\text{Up}^{\text{Me}}$  (*C. fragile* (Cf), *U. lactuca* (Up), and *E. arborea* (Ea); superscripts Me and Aq indicate Methanol and Aqueous extracts, respectively). The extracts were concentrated under a vacuum in a rotary evaporator at  $40\text{ }^{\circ}\text{C}$ . Solid mass obtained was then dissolved in respective solvents and the concentration of all the extracts was adjusted to  $2\text{ mg/mL}^{-1}$ .

### DPPH free radical scavenging activity

Free radical scavenging activity of the seaweeds extracts was determined using a stable free radical DPPH according to a modified method (Yen et al. 2000). DPPH solution was prepared at the concentration of  $4 \times 10^{-4}\text{ M}$  in dimethyl sulfoxide (DMSO). During the assay, a  $100\text{ }\mu\text{L}$  seaweed extract and  $100\text{ }\mu\text{L}$  of freshly prepared DPPH solution were thoroughly mixed. The reaction mixture was incubated in the room temperature for 1 h. After which the absorbance was recorded at  $517\text{ nm}$  by ELISA reader (ELX tek Instrument Inc). The percentage of inhibition was calculated as  $[1 - (\text{A}_i - \text{A}_j)/\text{A}_c] \times 100$ , where  $\text{A}_i$  is the absorbance of extract mixed with DPPH solution,  $\text{A}_j$  is the absorbance of same extract mixed with  $100\text{ }\mu\text{L}$  DMSO, and  $\text{A}_c$  is the absorbance of control with particular solvent (without seaweed extract).

### Determination of total phenolic compound

Phenolic contents were determined using a similar protocol (Huang et al. 2006). Each  $1\text{ mL}$  of seaweed extracts,  $1\text{ mL}$  of  $95\%$  EtOH,  $5\text{ mL}$  of distilled water, and  $0.5\text{ mL}$  of  $50\%$  Folin–Ciocalteu reagent (Sigma Chemical, St. Louis, MO) were mixed. The mixtures were allowed to react for 5 min and then  $1\text{ mL}$  of  $5\%$   $\text{Na}_2\text{CO}_3$  was added and placed in the dark for 1 h. Absorbance was measured at  $725\text{ nm}$  and gallic acid standard curve was obtained for the calibration of phenolic content.

### Reducing power

Reducing power of the extracts was evaluated accordingly (Oyaizu 1986; Yen et al. 2000; Berker et al. 2009). Extract samples at different concentrations ( $0.25$ ,  $0.50$  and  $1\text{ mg mL}^{-1}$ ) were mixed with phosphate buffer ( $1.25\text{ mL}$ ,  $0.2\text{ M}$ ,  $\text{pH } 6.6$ ), and  $1.25\text{ mL}$  of potassium ferricyanide ( $1\%$ ) was incubated at  $50\text{ }^{\circ}\text{C}$  for 20 min, cooled, and mixed with  $1.25\text{ mL}$  of trichloroacetic acid ( $10\%$ );  $1.25\text{ mL}$  of this mixture was transferred to other test tubes to which distilled water ( $1.25\text{ mL}$ ) and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  ( $0.25\text{ mL}$ ,  $0.1\%$ ) were added. The mixture was centrifuged and kept at room temperature for 10 min before reading the absorbance at  $700\text{ nm}$ . Increased absorbance of the reaction mixture indicated increasing reducing power.

### Total antioxidant activity

Total antioxidant activity (TAC) was determined (Prieto et al. 1999). In brief, the methanolic extract at a concentration of  $1 \text{ mg mL}^{-1}$  was mixed with 3.0 mL reagent solution containing 0.6 M  $\text{H}_2\text{SO}_4$ , 28 mM sodium phosphate and 4 mM ammonium molybdate and incubated at 95 °C for 90 min in water bath. The absorbance was measured at 695 nm, using ascorbic acid as standard.

### ABTS radical cation decolorization assay

The experiments were performed using an improved ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) decolorization assay (Re et al. 1999; Zulueta et al. 2009). BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), and  $\alpha$ -tocopherol (Vitamin E) were used as the positive controls. All determinations were performed in triplicate. The inhibition percentage,  $I$ , of the absorbance was calculated as follows:

$$I = 100 (A_0 - A_1 / A_0), \quad (1)$$

where  $A_0$  is the ABTS+ absorbance value at the initial time and  $A_1$  is the ABTS+ absorbance value after 6 min of incubation.

### FRAP (Ferric reducing ability plasma) assay

The FRAP assay was performed according to the method described (Benzie and Strain 1999; Thaipong et al. 2006). It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH. Fe(II)-TPTZ has an intensive blue color which can be read at 593 nm. A 1.5 mL of freshly prepared FRAP reagent (25 mL of 300 mM  $\text{L}^{-1}$  of acetate buffer pH 3.6, 2.5 mL of 10 mM  $\text{L}^{-1}$  2,4,6 tripyridyl S triazine (TPTZ) in 40 mM  $\text{L}^{-1}$  of HCl, 20 mM  $\text{L}^{-1}$  of ferric chloride solution) was mixed with 50  $\mu\text{L}$  of seaweed extract (100  $\mu\text{g mL}^{-1}$ ) in 150  $\mu\text{L}$  of distilled water. The absorbance was monitored for 4 min (every 10 s) at 593 nm. The  $\Delta A$  is proportional to the combined ferric reducing or antioxidant power (FRAP value) of the antioxidants in the sample. The results are expressed as mMol of FRAP  $\text{L}^{-1}$  and were estimated using aqueous  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (200–1000 mM) as standard for calibration. The relative activity of the sample was compared with standard ascorbic acid (2–10  $\mu\text{g mL}^{-1}$ ).

### Determination of flavonoids content

The total flavonoid content (TFC) was estimated spectrophotometrically by the aluminum chloride method based on the formation of complex flavonoid aluminum

(Lamaison and Carnat 1990; Quettier-Deleu et al. 2000). One milliliter of sample was mixed with 1 mL of  $\text{AlCl}_3$  methanolic solution (2 % w/v). After incubation at room temperature for 15 min, the absorbance was read at 430 nm. The amount of TFC was estimated from the standard calibration curve of 10–100  $\text{mg mL}^{-1}$  quercetin.

### Statistical analysis

The data were statistically analyzed and the values are expressed as mean  $\pm$  SD. The Student's 't' test and 'P' values are also indicated (Fisher 1990).

## Results and discussion

### Total phenolic and flavonoid contents

The amount of total phenols was determined with Folin–Ciocalteu reagent. Gallic acid (GAE) was used as a standard compound and the total phenols were expressed as mg GAE  $\text{g}^{-1}$  dry wt. The results of the present study showed that the methanolic extract of *E. arborea* containing highest amount ( $80.11 \pm 5.8 \text{ mg GAE g}^{-1}$  dry wt.) of phenolic compounds exhibited the greatest antioxidant activity. The high scavenging property of *E. arborea* may be due to hydroxyl groups existing in the phenolic compounds. Both extracts (methanol and aqueous) of *C. fragile* and *U. lactuca* contain very meager amount of phenolic compounds.

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. Therefore, the total flavonoid content of the seaweed extracts was estimated by aluminum chloride method. The flavonoid content of *E. arborea*'s aqueous and methanolic extracts was  $12.05 \pm 0.71$  and  $16.49 \pm 3.78 \text{ mg quercetin g}^{-1}$  dry wt, respectively. Methanolic extract of *U. lactuca* contained higher content of flavonoids ( $55.04 \pm 4.97 \text{ mg Quercetin g}^{-1}$  dry wt.) compared to other extracts.

### Total antioxidant activity (TAA)

This is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and subsequent formation of green phosphate/Mo(V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents to ascorbic acid. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extract is due to these compounds. Methanolic extracts of all seaweeds showed potent antioxidant activity with the extracts of *E. arborea* and *U. lactuca* showing the highest activity ( $235.13 \pm 10.4$  and  $237.75 \pm 3 \text{ mg AAE g}^{-1}$  dry wt respectively) (Table 1). Pearson's correlation showed

significant relationship between TAA and flavonoid and phenol contents in the extracts ( $R^2 = 0.78$  and  $0.54$ , respectively) (Table 2). The relationship among TAA, phenol and flavonoid contents is significant; therefore, high antioxidant activity in seaweed extracts is dependent on the phenolic and flavonoid contents.

### Reducing power assay

The multifaceted aspects of antioxidants to inhibit oxidation can be analyzed by different assays. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. In the reducing power assay, the presence of reductant (i.e., antioxidants) in the sample (extract/antioxidant) would result in the reduction of  $Fe^{+++}$  to  $Fe^{++}$  by donating an electron. The methanolic and aqueous extracts of *E. arborea* showed highest reducing power ability ( $319.03 \pm 5.4$  and  $91.9 \pm 4.3$  mg TE  $g^{-1}$  dry wt, respectively) followed by the methanolic extracts of *U. lactuca* ( $35.06 \pm 2.29$  mg TE  $g^{-1}$  dry wt) (Table 1). A positive correlation existed between the reducing power ability of the extracts and their total phenolic content ( $R^2 = 0.89$ ) and negative relationship with the total flavonoid content (Table 2).

**Table 1** Total phenol (TPC) and flavonoid (TFC) contents and antioxidant activities of Methanol<sup>(Me)</sup> and aqueous<sup>(Aq)</sup> extracts of (Ea) *Eisenia arborea*, (Cf) *Codium fragile*, (Up) *Ulva prolifera*

	Ea <sup>Aq</sup>	Ea <sup>Me</sup>	Cf <sup>Aq</sup>	Cf <sup>Me</sup>	Up <sup>Aq</sup>	Up <sup>Me</sup>
TPC	65.28 ± 3.8	80.11 ± 5.8	5.92 ± 2.8	8.62 ± 0.6	5.04 ± 0.05	19.9 ± 1.2
TFC	12.05 ± 0.7	16.49 ± 3.8	2.44 ± 0.95	8.23 ± 5.5	2.31 ± 0.6	55.04 ± 4.9
TAA	83.88 ± 5.9	235.13 ± 10.4	7.28 ± 1.1	72.1 ± 4.7	49.94 ± 8.6	237.75 ± 3
RP	91.9 ± 4.3	319.03 ± 5.4	1.19 ± 0.17	9.10 ± 0.6	2.77 ± 1.1	35.06 ± 2.3
FRAP	21.05 ± 2.4	45.82 ± 3.4	12.19 ± 1.09	9.74 ± 2.8	4.84 ± 1.6	10.03 ± 3.8
DPPH	67.98 ± 0.7	29.54 ± 7.6	15.38 ± 3.4	8.09 ± 0.91	14.46 ± 2.4	5.47 ± 1.4
ABTS	89.9 ± 4.5	104.38 ± 3.8	69.48 ± 2.9	88.18 ± 1.5	49.03 ± 10.7	84.08 ± 5.8

The content is represented as follows TPC as mg GAE  $g^{-1}$  DW; TFC as mg Quercetin  $g^{-1}$  DW; TAA as mg AAE  $g^{-1}$  DW; RP and FRAP as mg TE  $g^{-1}$  DW; DPPH as % inhibition

**Table 2** Correlation between antioxidant activities, phenolic and flavonoid contents of the seaweed extracts

ABTS	DPPH	FRAP	RP	TAA	TFA	TPC	
1	0.336492	0.752441	0.709828	0.628918	0.333025	0.74608	ABTS
	1	0.423928	0.361295	-0.06527	-0.22562	0.723547	DPPH
		1	0.982559	0.557811	0.008547	0.897538	FRAP
			1	0.649046	0.081024	0.887115	RP
				1	0.78618	0.541354	TAA
					1	0.098469	TFA
						1	TPC

### FRAP assay

The literature shows that FRAP method is sensitive in the measurement of total antioxidant power of plant homogenates and pharmacological plant products. Ferric to ferrous ion reduction at low pH causes the formation of a colored ferrous tripyridyltriazine complex. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Methanolic extracts of *E. arborea* showed higher FRAP value of 45.82 mg TE  $g^{-1}$  dry wt, whereas other extracts had a moderate range of activity. There was again a positive correlation between FRAP and the total phenolic content of the extracts (Table 2).

### DPPH assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) analysis is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity. It is a stable free radical because of its spare electron delocalization over the whole molecule. The donation of H+ to the DPPH radicals made a corresponding change from violet color to pale yellow in the solution. The order of DPPH scavenging against the seaweed extracts was found to be in the order of *E. arborea* (aqueous) > *E. arborea* (methanol) with the extracts of *C. fragile* and *U. lactuca* showing moderate

inhibition activities. It was further found that radical scavenging effects of extracts were positively correlated (Table 2) to the phenolic content present in extracts ( $R^2 = 0.72$ ).

### ABTS assay

The effect of seaweed extracts assayed to be scavenging the ABTS<sup>+</sup> radical is shown in Table 1. The highest values were obtained in the methanolic extracts of *E. arborea* ( $104.38 \pm 3.8 \mu\text{M TE g}^{-1}$  dry wt.) followed by the aqueous

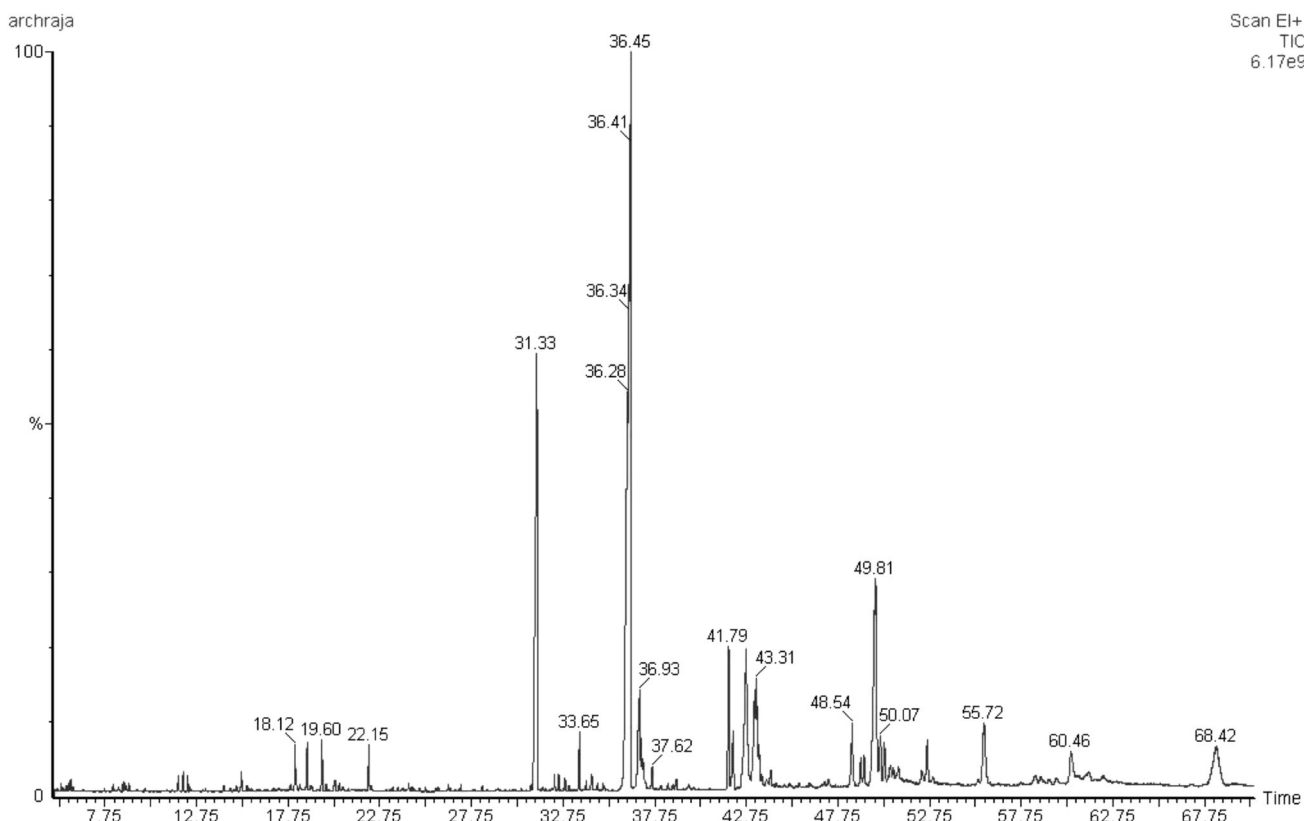
extract of *E. arborea* ( $89.9 \pm 4.5 \mu\text{M TE g}^{-1}$  dry wt), methanolic extracts of *C. fragile* ( $88.18 \pm 1.5 \mu\text{M TE g}^{-1}$  dry wt), and *U. lactuca* ( $84.08 \pm 5.8 \mu\text{M TE g}^{-1}$  dry wt). The results strongly correlated with FRAP, TAA, and TPC (Table 2).

### Fatty acid profile of methanolic extract of *E. arborea*

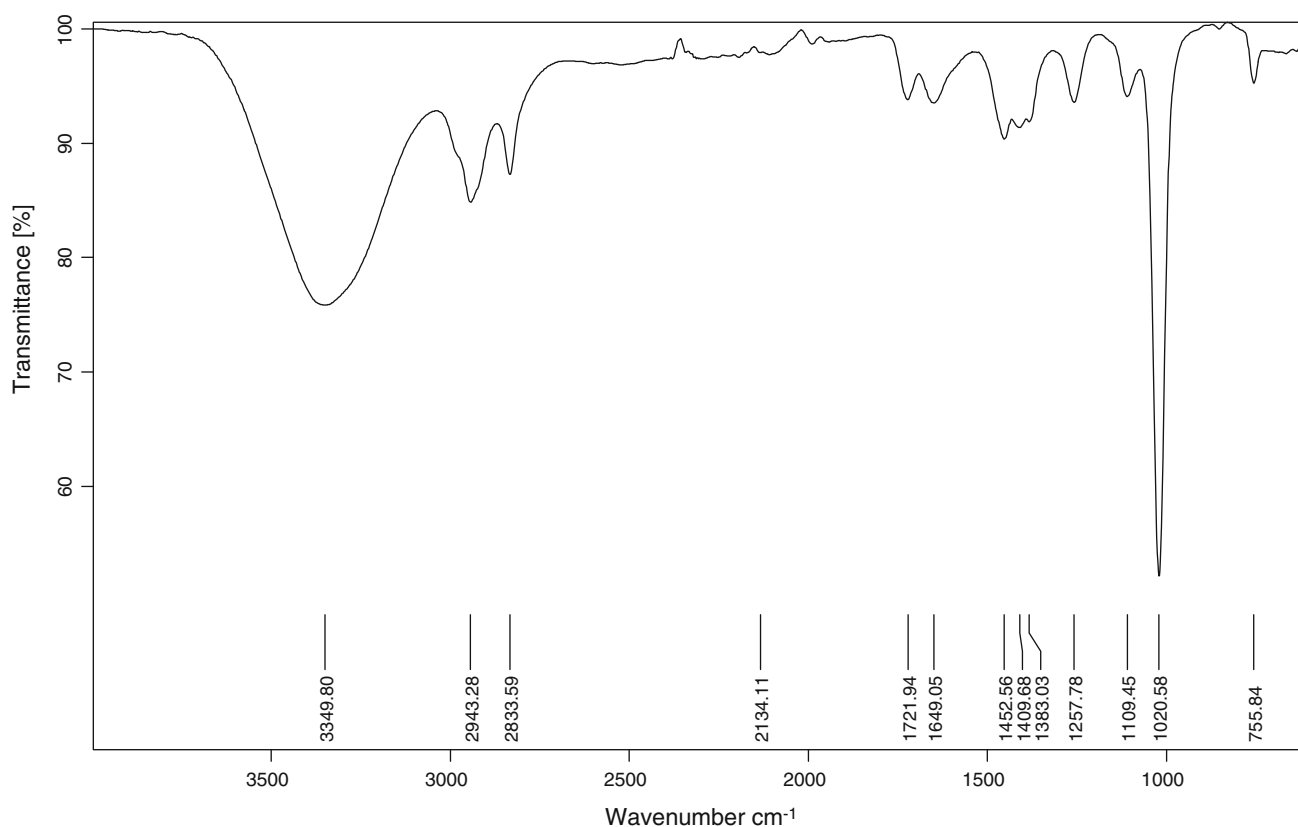
Since from the above studies, methanolic extract of *E. arborea* was known to possess higher antioxidant activity as it was screened for its chemical composition. The fatty acid composition of the most active extract, i.e., methanolic extract of *E. arborea*, is summarized in Table 3. Palmitic acid was the most abundant saturated fatty acid followed by myristic acid (Fig. 1). Furthermore, the methanolic extract of *Eisenia* contained minor levels of Pentadecanoic acid (C15:0), palmitoleic acid (C16:1), and stearic acid (C18:0). Among unsaturated fatty acids, there was a high content of elaidic acid followed by palmitoleic acid. In a study of Hernández-Carmona (Hernández-Carmona et al. 2009) described that a high concentration for arachidonic acid, medium concentration for linoleic acid, alpha linolenic acid, and eicosapentaenoic acid and significant lower concentration in docosahexaenoic acid were found in *E.*

**Table 3** Fatty acid groups present in *Eisenia arborea* given in mean  $\pm$  SD

	Fatty acid groups	Relative %
C14:0	Myristic acid	$12.99 \pm 0.72$
C15:0	Pentadecanoic acid	$4.15 \pm 0.84$
C16:0	Palmitic acid	$24.26 \pm 3.2$
C16:1	Palmitoleic acid	$17.76 \pm 3.7$
C16:1 d11	11-Hexadecanoic acid	$1.99 \pm 0.17$
C18:0	Stearic acid	$5.71 \pm 2.89$
C18:1n9t	Elaidic acid	$25.73 \pm 3.56$
C20:0	Arachidic acid	$7.39 \pm 1.52$



**Fig. 1** GCMS of *Eisenia arborea* extract



**Fig. 2** FTIR Spectra of methanolic extract of *Eisenia arborea*

*arborea*. Seasonal variation of lipid content was also noted throughout the year.

#### **Chemical composition of methanolic extract of *E. arborea***

Qualitative analysis of the methanolic extract of *E. arborea* was made on the basis of LC–MS results. The mass spectra were compared with the data from FTIR spectra of the extract. The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectra of the extract are shown in Fig. 2, where the focus is on the peaks at 1721, 1257, 1649, 2833, 2943  $\text{cm}^{-1}$  that correspond to acid carbonyl C–O Stretching, C=C (Alkene stretching), aliphatic CH Stretching, aromatic CH Stretching, carboxylic acid hydroxyl group, respectively. The results pertaining to LC–MS analysis leads to the identification of number of compounds (Table 4) from the LC fractions of the methanolic extract of *E. arborea*. The extract contains essential aminoacids such as methionine and conditionally nonessential aminoacids such as serine, proline, and asparagine. The nutritional value of the macroalgae depends on the presence of essential aminoacids, and *E. arborea* has been shown to possess seventeen aminoacids with high

concentrations of glutamic acid, aspartic acid, and leucine (Hernández-Carmona et al. 2009). It also contains the D-aminoacid and D-alanine. Various seaweeds such as *Hizikia fusiformis* (Harvey) Okamura, *Heterochordaria abietina* (f. simplex) Tokida, and *Sargassum nigrifolium* (Yendo) have been shown to contain D-alanine (Nagahisa et al. 1995). The biochemical and physiological role of D-alanine in higher organisms and their role in metabolic system of marine macroalgae are still to be interpreted.

Apart from the aminoacids, varied number of small molecules have been proposed to be present in the extract (Table 4) that ranged from molecular weight 76 to 453 with few important ones such as caffeic acid, piperine etc., having better biological activity. But, *E. arborea* and *E. Bicyclis* are long known for the presence of phlorotannins, phloroglucinol, and its polymers like eckol, phlorofuroeckol A, and dieckol, 8,8'-bieckol (Nagamura et al. 1996). Bieckols isolated from *E. bicyclis* has been shown to possess DPPH and ABTS radical scavenging activity as well as reducing power. They were also found to effectively suppress the detrimental effects of singlet oxygen  $^1\text{O}_2$  on type II photosensitization (Kwon et al. 2013). The antioxidant activity of the phlorotannins revealed their activity which depends on the degree of polymerization of phloroglucinol, with low molecular weight phlorotannins

**Table 4** Possible groups of products identified from LC–MS of the methanolic extract of *Eisenia arborea*

Molecular weight	Compound name	Molecular formula
74	Propionic Acid	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>
89	D-Alanine	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>
	Oxamic acid	C <sub>2</sub> H <sub>3</sub> NO <sub>3</sub>
	Sarcosine	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>
90	Lactic acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>
	Methoxy acetic acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>
102	2-Methylbutyric acid	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>
	Pivalic acid	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>
	Valeric acid	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>
	2-Oxobutyric acid	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>
	Acetoacetic acid	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>
105	L-Serine	C <sub>3</sub> H <sub>7</sub> NO <sub>3</sub>
115	L-Proline	C <sub>5</sub> H <sub>9</sub> NO <sub>2</sub>
	Maleamic acid	C <sub>4</sub> H <sub>5</sub> NO <sub>3</sub>
124	2-Pyrazine carboxylic acid	C <sub>5</sub> H <sub>4</sub> N <sub>2</sub> O <sub>2</sub>
130	2-Methylhexanoic acid	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>
	Citraconic acid	C <sub>5</sub> H <sub>6</sub> O <sub>4</sub>
	Mesaconic acid	C <sub>5</sub> H <sub>6</sub> O <sub>4</sub>
	Heptanoic acid	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>
	Ketoleucine	C <sub>6</sub> H <sub>10</sub> O <sub>3</sub>
132	Alpha-Methylene-beta gamma-Dihydroxybutyric acid	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>
	Asparagine	C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub>
	D-Ethylmalonic acid	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>
	Glutaric acid	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>
	Methylsuccinic acid	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>
146	2-Oxoglutaric acid	C <sub>5</sub> H <sub>6</sub> O <sub>5</sub>
149	D-Penicillamine	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub> S
	DL-Threo-beta-hydroxy Aspartic acid	C <sub>4</sub> H <sub>7</sub> NO <sub>5</sub>
	L-Methionine	C <sub>4</sub> H <sub>7</sub> NO <sub>5</sub>
165	3-Dimethoxy amino benzoic acid	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>
177	Allin	C <sub>6</sub> H <sub>11</sub> NO <sub>3</sub> S
180	2(4-Methoxyphenyl) Propionic acid	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>
	3,4-Methylenedioxy phenylacetic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>
	4-Hydroxy phenyl pyruvic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>
	Acetyl salicylic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>
	Caffeic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>
	Phenyl malonic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>
183	4-Pyridoxic acid	C <sub>8</sub> H <sub>9</sub> NO <sub>4</sub>
	DL-Homocysteic acid	C <sub>4</sub> H <sub>9</sub> NO <sub>5</sub> S
196	(3,4-Dimethoxy phenyl) acetic acid	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>
	(2,5- Dimethoxy phenyl) acetic acid	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>
	3-(4-Fluoro benzyl)Propionic acid	C <sub>10</sub> H <sub>9</sub> FO <sub>3</sub>
	4-Ethoxy carbonyl benzoic acid	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>
205	5-Methoxy-3-indole acetic acid	C <sub>11</sub> H <sub>11</sub> NO <sub>3</sub>
216	12-Hydroxy-dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>3</sub>
	2,6-Dichlorocinnamic acid	C <sub>9</sub> H <sub>6</sub> Cl <sub>2</sub> O <sub>2</sub>
	2-(Trifluoro methyl) cinnamic acid	C <sub>10</sub> H <sub>7</sub> F <sub>3</sub> O <sub>2</sub>
223	9-Nitroanthracene	C <sub>14</sub> H <sub>9</sub> NO <sub>2</sub>

**Table 4** continued

Molecular weight	Compound name	Molecular formula
226	2,2-Diphenyl Propionic acid	C <sub>15</sub> H <sub>14</sub> O <sub>2</sub>
	2,4-Dinitrophenyl acetic acid	C <sub>8</sub> H <sub>6</sub> N <sub>2</sub> O <sub>6</sub>
	3,4,5-Trimethoxy phenyl acetic acid	C <sub>11</sub> H <sub>14</sub> O <sub>5</sub>
	Prephenic acid	C <sub>10</sub> H <sub>10</sub> O <sub>6</sub>
246	Octopine	C <sub>9</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub>
	Gamma-glutamylvaline	C <sub>10</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub>
248	4-Iodobenzoic acid	C <sub>7</sub> H <sub>5</sub> IO <sub>2</sub>
270	Heptadecanoic acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
285	Morphine	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>
	Piperine	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>
293	<i>N</i> -acetyl muramic acid	C <sub>11</sub> H <sub>19</sub> NO <sub>8</sub>
294	Thioglycolic acid trimethylsilyl ester	C <sub>10</sub> H <sub>22</sub> O <sub>4</sub> SSi <sub>2</sub>
312	Ethisterone	C <sub>21</sub> H <sub>28</sub> O <sub>2</sub>
316	Cis-1,2-cyclohexanedicarboxylic acid bis(trimethylsilyl ester)	C <sub>14</sub> H <sub>28</sub> O <sub>4</sub> Si <sub>2</sub>
326	2-(2-Benzothiazolyl)azo-4-( <i>N,N</i> -Dimethyl) amino benzoic acid	C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> S
329	8-Hydroxyamoxapine	C <sub>17</sub> H <sub>16</sub> ClN <sub>3</sub> O <sub>2</sub>
367	Bicuculline	C <sub>20</sub> H <sub>17</sub> NO <sub>6</sub>
377	5'-Iodo 5'-deoxyadenosine	C <sub>10</sub> H <sub>12</sub> IN <sub>5</sub> O <sub>3</sub>
381	Trans-zeatin-9-glucoside	C <sub>16</sub> H <sub>23</sub> N <sub>5</sub> O <sub>6</sub>
384	<i>S</i> -adenosyl-L-homocysteine	C <sub>14</sub> H <sub>20</sub> N <sub>6</sub> O <sub>5</sub> S
453	4,7-Di(4-ethoxy phenyl)-6-phenyl-1,2,5-thiadiazol (3,4-c)pyridine	C <sub>27</sub> H <sub>23</sub> N <sub>3</sub> O <sub>2</sub> S

being more effective (Nagamura et al. 1996). The *E. arborea* was found to contain phlorotannins with dibenzodioxin structural elements (Glombitza and Gerstberger 1985). Six phlorotannins were shown to possess anti-degranulation activity on cell lines and inhibitory effects on enzymes involved in eicosanoid synthesis (Sugiura et al. 2009). They inhibited the  $\beta$ -hexosaminidase release from the rat basophilic leukemia cells and possessed antiallergic effects on rats (Sugiura et al. 2008). Many of the investigations have shown that phlorotannins from brown algae have antioxidant, anti-inflammatory, antidiabetic, antitumor, antihypertensive, antiallergic, hyaluronidase enzyme inhibition and in matrix metalloproteinases inhibition activity (Sugiura et al 2007; Thomas and Kim 2011).

The above results strongly suggest that antioxidant potential of the seaweeds with *E. arborea* is the most effective one followed by *U. lactuca* and *C. fragile*. The methanolic extracts possessed higher activity and higher phenolic content compared to their aqueous counterparts. This reveals the potential use of seaweeds as a source of natural bioactive compounds with antioxidant activities.

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