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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *ULVA LACTUCA*

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ABSTRACT

Ulva lactuca, common seaweed abundantly found along the coasts of Raigad district of Konkan region of Maharashtra. Phytochemical screening of the extracts was carried out as per the standard procedure, using water, HCL (1%), ethanol, ethyl acetate, methanol, chloroform, benzene and petroleum ether. The different extracts of *Ulva lactuca* showed the presence of flavonoids, glycosides, phenolic compounds, saponins, steroids, tannin etc. The alga was extracted using Four different methods were used to evaluate the antioxidant activity of the extracts, including DPPH radical scavenging assay (1, 1-diphenyl-2-picryl hydrazyl), FRAP, Reducing power and Total phenolic contents. The results obtained suggest that *Ulva lactuca* is used as a best source of food and fodder, but has less important as a source of antioxidant.

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INTRODUCTION

The green algae are the most diverse group of algae, with more than 7000+ species growing in a variety of habitat. The green macro marine algae are found in both sandy and rocky beaches. Many green algal species can tolerate low salinity and colonize areas where rivers meet the sea. Some of the common seaweeds are *Ulva lactuca* causes "green tides" in the sea, *Caulerpa recemosa* (Sea grapes) and *Ulva intestinalis* (gut weed) etc, found abundantly along Indian coast line. (Dinabandhu sahoo, 2001). Algae are the source of amino acids, terpenoids, steroids, tannins, phenolic compounds. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in human to algicidal, nematicidal, and insecticidal (Jio Li Li, Jiang Zhang Wu and Zhang (2009). Algae are eukaryotic organisms inhabited in salty sea water and is recognized to synthesize several bioactive compounds, which show antimicrobial property. In addition, other substances identified as antimicrobial agents were chlorellin derivatives, acrylic microbial acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds and phenolic inhibitors (Bansemir *et al*., 2006). Seaweeds were considered for their medicinal value in the orient as long as 3000 B.C.

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The Chinese and Japanese used them in the treatment of goiter as well as in other glandular diseases. Although the Romans believed seaweeds to be useless, they also used them to heal wounds, burns, scurvy and rashes. The British used Porphyra species to prevent scurvy during long voyages (Uma Maheswara Rao, 1978). Marine algae contain active constituents which are used in traditional and complementary medicine. The active ingredients present in algae can cure diseases. Nowadays, higher percentage of populations prefers to use remedies of natural origin for curing illness as these claimed to produce less side effects Hoyt, (1970). The phytochemicals from marine algae are extensively used in various industries such as food, confectionary, textile, pharmaceuticals, dairy and paper mostly as gelling, stabilizing and thickening agents. The phytochemical screening of marine algae showed the presence of phenolic compounds. Phytoconstituents such as Phenolic compounds, Glycosides, flavanoids, alkaloids, Steroids, saponins, proteins, amino acids, terpenoids, sugar, fats etc (Thoudam Kirithika Kamala and Usha (2011). Seaweeds are effective in terms of antioxidant potentials as well as their phytochemical contents can serve as natural sources to obtain free radical scavengers and antioxidant agents. A large number of algae used for the treatment of diseases. The basic aim of the research was to find out the phytoconstituents and antioxidant potential in various extracts of the algal species *Ulva lactuca* using DPPH radical scavenging assay (1, 1-diphenyl-2-picryl hydrazyl), FRAP, Reducing power and Total phenolic contents.

MATERIALS AND METHODS

Collection of marine algae

In the present investigation, samples of macro marine algae were collected from the Raigad coast line during low tides. The macro marine algae were washed in sea water and fresh water thoroughly to remove the epiphytes and other contaminations. Then sample was transferred into a polythene bag with a small hole to leak out water drop wise and then shade dried. Collection of macro algae was done in labeled polythene bags and brought to laboratory, and marine algal samples were analyzed macroscopically for their morphological characters like colour, shape, size, texture etc. Then collected species of macro algae were preserved in 4% formalin solution. Herbarium specimens of each algal species were prepared for identification and confirmation of their taxonomic position. Identification of species was done by referring Taylor (1960), Deodhar (1987) and Dinabandhu sahoo (2001) and other previous publications.

Preparation of sample for qualitative phytochemicals analysis

For the phytochemical screening, fresh samples were used. Five grams of fresh sample weighed and homogenized with 50 ml of water, ethanol, and HCL (1%) solution separately. The extract was boiled for one hour, cooled and filtered. The filtrate was used for screening phytochemicals by using standard procedure (Harborne, 1973).

Preparation of organic extracts of sample for Antioxidant activities

The dried sample of seaweeds ground to coarse powder, weighed and wrapped in Whatman No.1 filter paper and successively extracted with 200 ml of different solvents such as benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether with their increasing order of polarity by soxhlation for 12-24 hours. The extract analyzed for the presence of antioxidant activities by referring standard procedure (Thoudam *et al*, 2011).

DPPH radical scavenging activity

DPPH is a stable inorganic radical. To determine the radical scavenging effect of marine algal extracts, DPPH (1, 1-diphenyl-2-picryl hydrazyl) method is used. This method is based on estimating the reduction of alcoholic DPPH solution in the presence of a hydrogen donor. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of marine algal extracts in methanol with different concentrations (0.5-2.5 mg/ml). Then the reaction mixture mixed thoroughly and kept in the dark at room temperature for 30 minutes and absorbance of the reaction mixture measured spectrophotometrically at 690 nm. BHT and BHA were used as references (Liyana-Pathiranan *et al*. 2005).

Total antioxidant activity (FRAP Method)

To evaluate total antioxidant activity of marine algal extracts FRAP (Ferric reducing antioxidant potential) method is used.

Results: Phytochemical screening

Table 1. Preliminary phytochemical study of *Ulva lactuca* Linn

Sr.No	Name of the Algal Species	Solvent used	a	b	c	d	e	f	g	h	i	j	k
1	*Ulva lactuca* Linn.	water	-	-	-	+	+	-	+	+	-	-	-
		HCL	-	-	-	+	-	-	-	-	-	-	-
		Ethanol	-	-	-	+	-	+	+	-	-	-	-
		Ethyl Acetate	-	-	-	+	-	-	+	+	-	-	-
		Methanol	-	+	+	+	-	-	+	+	+	-	+
		Chloroform	-	+	-	+	+	-	+	+	-	-	-
		Benzene	-	-	-	+	-	-	-	-	-	-	-
		Petroleum ether	-	+	+	-	-	-	+	-	-	-	-

Where, a: Alkaloids, b: Flavonoids, c: Glycosides, d: Phenolic compounds, e: Saponins, f: Steroids, g: Tannins, h: Carbohydrates, i: Proteins, j: Fats, k: Sugar and +: Present, -: Absent.

Antioxidant screening

DPPH-Radicals scavenging activity (%) of *Ulva lactuca* at 517 nm is given below,

Table 2. DPPH-radical scavenging activity of *Ulva lactuca* at 517 nm

Sr. No	Conc^{n} (mg/ml)	DPPH-Radical Scavenging Activity (%) of ***U.lactuca*** at 517 nm.					
		Benzene	Chloroform	Ethanol	Ethyl Acetate	Methanol	Petroleum Ether
1	0.5	4.854	7.671	2.977	3.853	11.267	2.213
2	1.0	9.431	14.285	6.203	6.225	28.169	4.262
3	1.5	12.343	23.015	9.305	9.090	47.887	7.049
4	2.0	15.256	28.042	12.158	11.857	63.380	9.344
5	2.5	18.307	35.185	15.756	14.426	79.577	11.147

FRAP-Total antioxidant activity of *Ulva lactuca* at 593 nm is as follows: (Concentration of extract used=10 mg/ml)

Table 3. FRAP-Total antioxidant activity of *Ulva lactuca* at 593 nm

Sr.No	Solvent used	Standard	Absorbance at 593 nm	FRAP-Total antioxidant activity at 593 nm
1	Benzene	S1 0.462±0.012	0.355±0.060	236.66
2	Chloroform	S2 0.786±0.025	1.152±0.041	768
3	Ethanol	S3 0.992±0.008	1.023±0.054	682
4	Ethyl Acetate	S4 1.294±0.016	0.598±0.033	398.66
5	Methanol	S5 1.608±0.035	0.915±0.036	610
6	Petroleum Ether	Blank 0.114±0.006	0.158±0.044	105.33

Reducing power of *Ulva lactuca* at 700 nm is as follows

Table 4. Reducing power of *Ulva lactuca* at 700 nm. (GRAPH: Conc (µg/ml) V/S Absorbance at 700 nm)

S.No	Conc^{n} µg/ml	Reducing power of *Ulva lactuca* at **700 nm**.						
		Standard	Benzene	Chloroform	Ethanol	Ethyl acetate	Methanol	Petroleum Ether
1	50	0.892	1.293	1.013	0.732	0.977	0.640	1.216
2	100	1.026	1.422	1.197	0.898	1.142	0.826	1.408
3	150	1.215	1.603	1.384	1.176	1.404	1.081	1.592
4	200	1.958	1.826	1.502	1.349	1.592	1.207	1.716
5	250	2.324	2.004	1.689	1.508	1.756	1.395	1.895

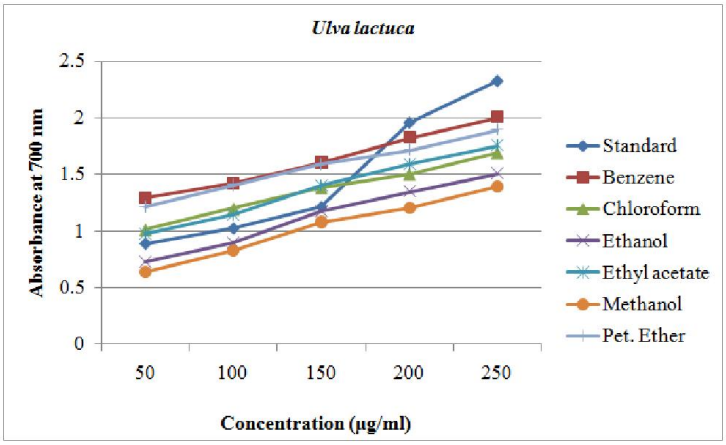

Figure 1. Standard &reducing power of solvent extracts of *Ulva lactuca* at 700 nm

Total phenolic content of *Ulva lactuca* at 765 nm in mg of gallic acid equivalent per gram is as follows, (Concentration of extract used=10 mg/ml)

Table 5. Total phenolic content of *Ulva lactuca* at 765 nm

S.No	Solvent used	Absorbance at 765nm	Total phenolic contentat 765 nm
1	Benzene	0.282±0.019	8.41±0.006
2	Chloroform	0.889±0.051	26.53±0.023
3	Ethanol	0.738±0.037	22.02±0.019
4	Ethyl acetate	0.712±0.020	21.25±0.015
5	Methanol	0.616±0.008	18.38±0.036
6	Petroleum ether	0.106±0.095	3.16±0.021

The stock solution included 300 ml acetate buffer (3.1g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 ml TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 ml HCL, and 20 ml $FeCl_3.6H_2O$ solution. The fresh working solution prepared by mixing 30 ml acetate buffer, 3 ml TPTZ and 3 ml $FeCl_3.6H_2O$ solution. The temperature of the solution was maintained to 37^0C before analysis. Then marine algal extracts (150µL) allowed to react with 2850 µL of the FRAP solution for 30 minutes in the dark condition. The coloured product (Ferrous tripyridyl triazine complex) read spectrophotometrically at 593 nm with single concentration i.e. 10 mg/ml and standard curve being linear between 100 and 1000 µL $FeSO_4$. Then results were expressed in µL Fe (II)/g dry mass and compared with that of BHT and BHA (Kasote *et al*, 2011).

Reducing power assay

The reducing power of marine algal extracts was assessed by using various concentrations i.e. 50-250 µL/ml of marine algal extracts in methanol (10 mg/ml), by using reference of standard BHT and BHA (1mg/ml), mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferric cyanate (2.5 ml). The mixture was incubated at 50^0C for 20 minutes and (10%, 2.5 ml) trichloroacetic acid was added. This solution is centrifuged at 3000 rpm for 10 minutes. Then upper layer of the reaction mixture (2.5 ml) is mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%), and absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicates the increased reducing power (Kasote *et al*. 2011).

Determination of total phenolic content

Total phenolic content in marine algal extracts were determined by using modified Folin-Ciocalteau reagent method. According to (Zahin *et al* 2009), gallic acid is a standard phenolic compound. The reaction mixture contained single concentration i.e. 10 mg/ml of the extract and Folin-Ciocalteau reagent. To 500 µL (10 mg/ml) of marine algal extracts in methanol, 2.5 ml of 1:10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na_2CO_3 (7.5% w/v) were added and mixed thoroughly and incubated at 45^oC for 15

minutes. Same procedure is followed for other marine algal extracts with benzene, chloroform, ethanol, ethyl acetate and petroleum ether respectively. The absorbance measured at 765 nm. The concentration of total phenolic content in the marine algal extracts was determined as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g dw) (Zahin *et al* 2009).

DISCUSSION

Ulva lactuca showed positive result for flavonoides, phenolic compounds, saponins, tannins, sugar and carbohydrates in the water extracts. In HCL extracts only phenolic compounds is present. In ethanolic extracts, Phenolic compounds, Steroids and tanins are present. In ethyl acetate extracts, phenolic compounds, tannins and carbohydrates are present. In methanolic extracts, flavonoids, glycosides, phenolic compounds, tannins, carbohydrates, proteins and sugar are present. In chloroform extracts, flavonoids, phenolic compounds, saponins, tannins and carbohydrates are present. In benzene extracts, only phenolic compound is present. Petroleum ether extracts, flavonoids, glycosides and tannins are present.

In antioxidant screening of Ulva lactuca, by using DPPH-Radical scavenging activity, showed highest value in methanolic extract (11.26%); followed by chloroform extracts (7.67%), benzene extracts (4.85%), ethyl acetate extracts (3.85%), ethanol extracts (2.97%) and petroleum ether extracts showed lowest radical scavenging activity i.e.(2.21 %). In FRAP-total antioxidant activity assay of Ulva lactuca showed highest value in the extracts of chloroform (768), followed by ethanolic extracts (682), methanolic extracts (610), ethyl acetate extracts (398.66), benzene extracts (236.66) and petroleum ether extracts showed less antioxidant activity (105.33).

Reducing power of Ulva lactuca, benzene extracts showed highest reducing power (1.293), followed by petroleum ether extracts (1.216), methanolic extracts (1.013), ethyl acetate extracts (0.977), ethanolic extracts (0.732) and methanolic extracts showed lowest reducing power (0.640). In determination of total phenolic content of Ulva lactuca, extracts of chloroform, showed highest value (26.53), followed by ethanolic extracts (22.02), ethyl acetate extracts (21.25), methanolic extracts (18.38), extracts of benzene (8.41) and petroleum ether extracts, showed lowest value (3.16). The results of present study indicate that the algal species *Ulva lactuca* is rich in secondary metabolites and effective in the terms of antioxidant potentials. It may be useful as food and feed and beneficial to improve strengths of human and animals.

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