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STUDY OF PHYTOCHEMICAL SCREENING & ANTIOXIDANT ACTIVITIES OF *CLADOPHORA GLOMERATA* LINN COLLECTED FROM RAIGAD COAST OF KONKAN (M.S.) INDIA

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ABSTRACT

The marine macro algal species have great antioxidant potentials. They produce various numbers of bioactive compounds. The aim of present investigation was to evaluate the phytochemical screening and antioxidant activity of green alga *Cladophora glomerata* Linn belonging to family- Glomerataceae. This alga collected along the coasts of Raigad of Konkan region of Maharashtra. Qualitative phytochemical investigations indicate that the extract of *Cladophora glomerata* contained phytoconstituents like flavonoids, glycosides, phenolic compounds, saponins, tannin and proteins. The antioxidant potential investigated by using four methods like DPPH radical scavenging(%), FRAP Assay, Reducing power assay and determination of total phenolic contents. Obtained results from these methods clearly indicates that the *Cladophora glomerata* have a great antioxidant potential.

KEY WORDS: Raigad coast, *Cladophora glomerata* Linn, Phyto -constituents, Antioxidant potential.

INTRODUCTION

Marine macro algae constitute most important part of marine ecosystems. In present days, near about 90% of the marine plants are algae, which is known as primary source as a food for aquatic organisms (Untawale, A.G. and Dhargalkar, V.K. (1975). All over world, various species of marine algae were used extensively by humans as food, fodder, medicine etc, since several decades (Kuda, T., Tsunekawaa, M., Goto, H. and Araki, Y. (2005). Algae are rich in bioactive compounds with its medicinal potential against various diseases (Volka, R.B., Furkert, F.H. (2006). Algae are reservoirs of various important phytoconstituents like flavonoides, phenolic compounds, saponins, steroids, tannins, carotenoids, pigments, enzymes, proteins *etc*. Marine algae are the richest source of vitamin A, B_1, B_{12}, C, D and E. (Soobrattee, M.A, Neergheen, V.S, Luximon-Ramma, A, Aruoma L, Bahorun T. (2005).The various species of macro algae were reservoirs of antioxidant potential, due to that they have great importance in pharmaceutical industry (Stirk, W.A., Reinecke, D.L., Staden, J. (2007). The south coast of India is a richest source of macro algae, where luxuriant growth of several species of green, brown and red algae along the various coasts of Raigad and other coasts of konkan region (Venkataraman, K. and Wafar, M. (2005). The genus *Cladophora glomerata* has been studied for its antioxidant potential. The present study gives detailed information of *Cladophora glomerata* as antioxidant potential. This information is useful for preparing drugs in pharmaceutical industry. It is hoped that this information of *Cladophora glomerata* may be useful for integrated management of various diseases based on improved resistance and immunity power of human body. These natural origin substances have less chance of side effects on human health rather than the chemical based products.

MATERIAL & METHODS

Collection of marine algae:

In the present investigation, samples of macro marine alga *i.e. Cladophora glomerata* were collected by hand picking, during low tide along the coast of Raigad district, Maharashtra, India (17º53' and 19º08' N Latitude, 72º51' and 73º42' E Longitude). The macro marine alga was 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. Then collected macro algae was transferred in labeled polythene bags and brought to laboratory, and samples were analyzed macroscopically for their morphological characters like colour, shape, size, texture *etc*. Then collected species 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)

Total antioxidant activity of marine algal extracts was determined by FRAP (Ferric reducing antioxidant potential) method. 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 using single concentration of extract 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 of the extracts i.e. 10 mg/ml, 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).

RESULTS

TABLE 1: Preliminary phytochemical study of *Cladophora glomerata* Linn.

Sr. No	Name of the Algal Species	Solvent used	a	b	c	d	e	f	g	h	i	j	k
		water	-	+	+	+	+	-	+	+	+	-	+
		HCL	-	+	-	-	+	-	-	+	+	-	+
		Ethanol	-	+	+	+	+	-	+	+	+	-	+
1	*Cladophora glomerata* Linn.	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 activities of *Cladophora glomerata* Linn.

I. DPPH-Radical scavenging activity

TABLE 2: DPPH-Radical scavenging activity of *Cladophora glomerata* Linn, at 517 nm is given in table,

Sr. No	Conc^{n} (mg/ml)	DPPH-Radical Scavenging Activity (%) of *Cladophora glomerata* Linn at 517 nm.					
		Benzene	Chloroform	Ethanol	Ethyl acetate	Methanol	Petroleum Ether
1	0.5	3.744	6.349	4.342	2.371	27.464	14.836
2	1.0	8.183	13.227	7.196	5.039	40.845	18.442
3	1.5	13.453	24.338	10.669	7.509	52.112	21.065
4	2.0	19.278	34.126	15.012	10.671	71.830	23.196
5	2.5	27.045	42.857	18.858	13.438	86.619	26.672

II.FRAP-Total antioxidant activity

TABLE 3: Total antioxidant activity of *Cladophora glomerata* Linn at 593 nm is as follows: (Concentration of extract used=10mg/ml).

Sr.No	Solvent Used	Standard	Absorbance at 593 nm	FRAP-Total anti-oxidant activity at 593 nm
1	Benzene	S1 0.462±0.012	1.068±0.012	712
2	Chloroform	S2 0.786 ± 0.025	1.172±0.024	781.33
3	Ethenol	S3 0.992±0.008	1.032±0.018	688
4	Ethyl Acetate	S4 1.294±0.016	2.103±0.043	1402
5	Methanol	S5 1.608±0.035	1.749±0.027	116.6
6	Petrolium ether	Blank 0.114±0.006	0.352 ±0.034	234.66

III. Reducing power assay

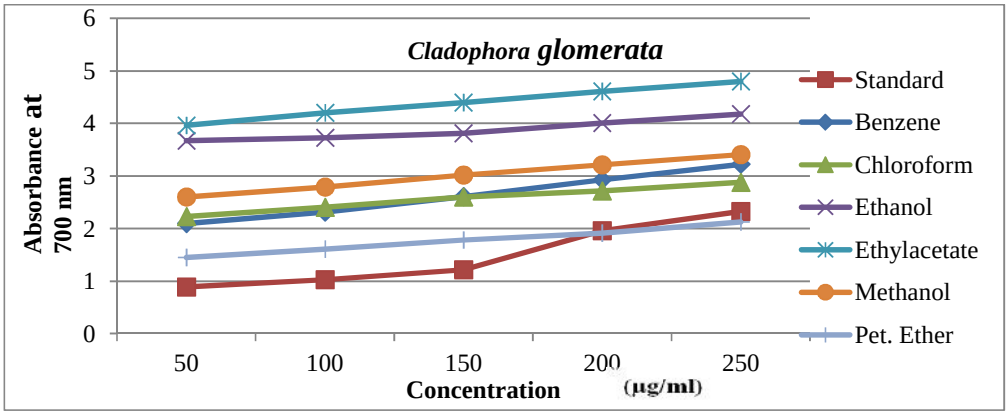
TABLE 4: Reducing Power of *Cladophora glomerata* Linn at 700 nm is given below,

Sr. No	Conc. µg/ml	Reducing power of *C. glomerata* linn at 700 nm.						
		Standard	Benzene	Chloroform	Ethanol	Ethyl acetate	Methanol	Petroleum Ether
1	50	0.892	2.094	2.227	3.670	3.960	2.603	1.453
2	100	1.026	2.312	2.403	3.725	4.202	2.788	1.612
3	150	1.215	2.604	2.598	3.812	4.394	3.016	1.784
4	200	1.958	2.928	2.716	4.006	4.608	3.212	1.915
5	250	2.324	3.225	2.882	4.182	4.796	3.407	2.124

IV.Total phenolic content

TABLE 5: Total phenolic content of *Cladophora glomerata* Linn, at 765 nm in mg of Gallic acid equivalent per gram is as follows, (Concentration of extract used=10mg/ml).

Sr.No	Solvent used	Absorbance at 765 nm	Total phenolic content at 765 nm
1	Benzene	0.481±0.034	14.35±0.030
2	Chloroform	0.457±0.062	13.64±0.026
3	Ethanol	1.059±0.025	31.61±0.046
4	Ethyl acetate	2.346±0.041	70.02±0.055
5	Methanol	2.462±0.048	73.49±0.023
6	Petroleum ether	0.215±0.032	6.41±0.007



DISSCUSSION

I. Phytochemical Screening of *Cladophora glomerata* Linn

Phytochemistry is the study of the natural products and the chemical constituents occurring in marine algae. The marine algae are known as medicinal rich in secondary metabolites like phenolic compounds, alkaloids, glycosides, flavonoides, saponins, tannins, steroids and related active metabolites. These constituents have a great medicinal value. They have been extensively used in the preparation of drugs and in medicinal industry. The qualitative phytochemical studies carried on water, HCL, ethanolic, ethyl acetate, methanolic, chloroform, benzene and petroleum ether extracts of *Cladophora glomerata* Linn were tested and eleven different constituents like alkaloids, glycosides, flavonoides, phenolic compounds, saponins, steroids, tannins, carbohydrates, proteins, fats and sugar etc, were tested.

Cladophora glomerata Linn gives positive result for flavonoides, glycosides, phenolic compounds, saponins, tannins, carbohydrates, proteins and sugar in the extracts of water. HCL extracts, showed presence of flavonoides, saponins, carbohydrates, protein and sugar. Ethanolic extracts, showed presence of flavonoides, glycosides, phenolic compounds, saponins, tannins, carbohydrates, protein and sugar. In ethyl acetate extracts, flavonoides, glycosides, phenolic compounds, saponin, tannin, carbohydrates, protein and sugar are present. Methanolic extracts, showed presence of flavonoides, glycosides, phenolic compounds, saponins, carbohydrates, proteins and sugar. The extracts of chloroform showed the presence of phenolic compounds, saponins, tannins and carbohydrates. Benzene extracts showed the presence of flavonoides, glycosides, saponins and carbohydrates, while in petroleum ether extracts, all constituents were absent. The extracts of water, ethanolic and ethyl acetate showed the presence of highest constituents. All constituents were absent in petroleum ether extracts.

II. Antioxidant activities of *Cladophora glomerata* Linn:

The antioxidant activity of marine algal extracts cannot be evaluated by only a single method, due to the complex nature of phytochemicals. In present work assay like DPPH, Reducing power, FRAP assay-Total antioxidant activity and determination of total phenolic content, were completed.

In the present study, the methanolic extracts of *Cladophora glomerata* showed maximum potent free radical scavenging activity on DPPH at 517 nm among the chloroform, benzene, ethanolic, ethyl acetate, methanolic and petroleum ether extracts. The methanolic extracts showed maximum (27.46%), followed by petroleum ether extracts (14.83%), chloroform extracts (6.36%), ethanolic extracts (4.34%), benzene extracts (3.74%) and ethyl acetate extracts, it is lowest (2.37%).

Cladophora glomerata, in FRAP assay, shows highest value in the extracts of ethyl acetate (1402), followed by chloroform extracts (781.33), benzene extracts (712), ethanolic extracts (688), petroleum ether extracts (236.66) and methanolic extracts showed the lowest value *i.e.* (116.6).In Reducing power, *Cladophora glomerata*, the extracts of ethyl acetate showed highest values (3.960), followed by ethanolic extracts (3.670), methanolic extracts (2.603), chloroform extracts (2.227), benzene extracts (2.094) and petroleum ether extracts gives less value (1.453).

In determination of total phenolic content of *Cladophora glomerata* from the results, it was observed that the methanolic extract has a higher phenolic content and it was found to be 73.49 µg GAE/g dry weight of extracts, followed by ethyl acetate extracts (70.02), ethanolic extracts (31.61), extracts of benzene (14.35), chloroform extracts (13.64), and petroleum ether extracts showed lowest value (6.41).

It is observed that *Cladophora glomerata* showed the presence of highest constituents in various extracts. It can also show maximum potent free radical scavenging activity. The methanolic extract had a higher phenolic content. These results of *Cladophora glomerata* indicates that it have a great antioxidant potential.

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