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STUDY OF PHYTOCHEMICAL SCREENING AND REDUCING POWER OF GREEN ALGA-*Ulva fasciata* DELILE, COLLECTED FROM RAIGAD COASTLINE.

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Abstract:

Ulva fasciata is one of the most common green alga found abundantly along the coasts of Raigad district of Konkan region of Maharashtra. To study, Phytochemical screening, the algal 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 fasciata showed the presence of flavonoides, glycosides, phenolic compounds, saponins, steroids, tannin etc. The alga was extracted to evaluate the antioxidant activity, to find out reducing power. The results obtained suggest that Ulva fasciata is used as a best source of food and fodder for grazing or domestic animals & human beings.

Key words: *Ulva fasciata, Raigad coast, Phytoconstituents, Reducing Power.*

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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, *Ulva fasciata*, *Caulerpa racemosa* (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 L. Li X, Li T, Jiang P, Zhang L, Wu M, Zhang L. (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. 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 (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, J.W. (1970).

The phytochemicals from marine algae are extensively used in various industries. Phytoconstituents such as Phenolic compounds, Glycosides, flavonoides, alkaloids, Steroids, saponins, proteins, amino acids, terpenoids, sugar, fats etc (Thoudam B, Kirithika T, Kamala S, and Usha K. (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

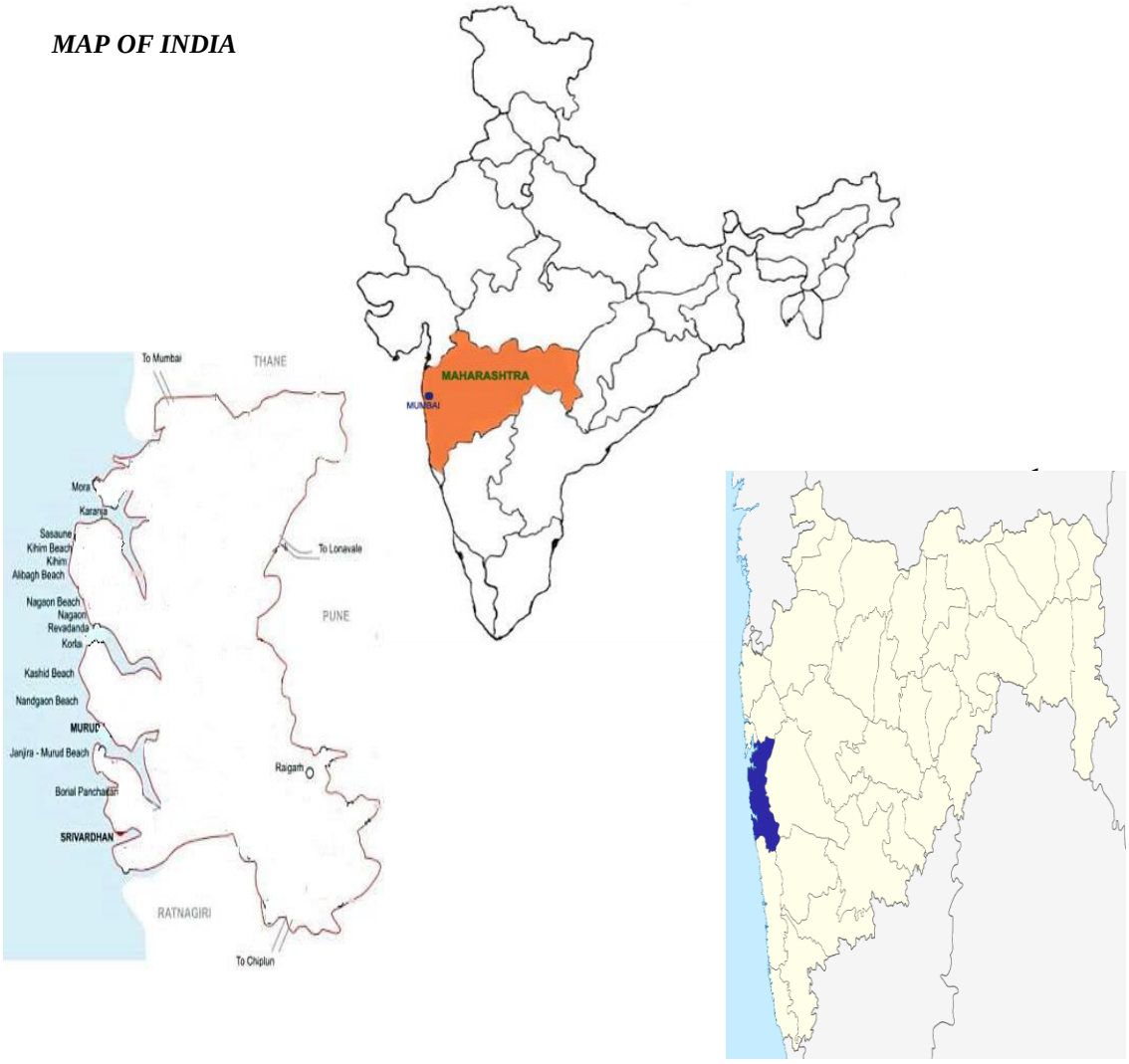
The basic aim of the research was to find out the phytoconstituents & reducing power of *Ulva fasciata*.

MATERIALS AND METHODS:

Collection of marine alga:

In the present investigation, samples of macro marine alga were collected from the Raigad coast line during low tides. 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.

Collection of macro alga 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 alga were preserved in 4% formalin solution. Herbarium specimens of 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.



Raigad coastline *Map of Maharashtra*

Fig.1: Location of study area (Raigad coastline).

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)

REDUCING POWER ASSAY:

Principle: The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activity had reported with the development of reducing power. The presence of antioxidants causes the reduction of Fe^{3+} to Fe^{2+}form.Therefore Fe^{2+} can be monitored by

measuring at 700 nm. It is an assay by electron transfer reaction.

Increase in the Fe^{3+} to Fe^{2+} transformation in the presence of test substance (sample) are electron donors and therefore can reduce the oxidized intermediates of lipid per oxidation processes. Thus they can act as primary and secondary antioxidants.

Chemicals:

1. **Phosphate buffer** (0.2 M, pH 6.6).
2. **10% Trichloroacetic acid:** 10 g TCA in 100 ml Distilled Water.
3. **1% Potassium ferricyanide:** 1 g of $K_3Fe(CN)_6$ in 100 ml Distilled Water.
4. **0.1% ferric chloride anhydrous:** 100 mg $FeCl_3$ in 100 ml DW (Prepared fresh sample).

Calculation:

OD indicates the reducing power.

Procedure:

The reducing power of algal extracts was assessed by using various concentrations i.e. 50-250 µL/ml of seaweeds extracts in methanol (10 mg/ml) and reference standard BHT and BHA (1 mg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide (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. Distilled water is blank. Increased absorbance of the reaction mixture indicates the increased reducing power (Kasote, *et al*., 2011).

RESULTS:

Phytochemical screening:

Table No.1: Preliminary phytochemical study of *Ulva fasciata*.

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

Where,

a= Alkaloid, **b**=Flavonoides, **c**= Glycosides, **d**= Phenolic compounds, **e**= Saponins, **f**= Steroids, **g**= Tannins, **h**= Carbohydrates, **i**= Proteins, **j**= Fats, **k**= Sugar, and

+= Present, **-**= Absent.

2. Reducing power of *Ulva fasciata* at 700 nm:

Tabe No.2: Reducing power of *Ulva fasciata* at 700 nm.

Sr. No	Conc^n µg/ml	Reducing power of ***Ulva fasciata*** at **700 nm**.						
		Standard	Benzene	Chloroform	Ethanol	Ethyl acetate	Methanol	Petroleum Ether
1	50	0.892	1.275	1.003	0.714	0.954	0.638	1.202
2	100	1.026	1.403	1.188	0.852	1.106	0.805	1.415
3	150	1.215	1.611	1.344	1.146	1.402	1.065	1.552
4	200	1.958	1.802	1.501	1.312	1.562	1.202	1.701
5	250	2.324	2.002	1.669	1.504	1.726	1.371	1.855

(GRAPH: Conc (μg/ml) V/S Absorbance at 700 nm)

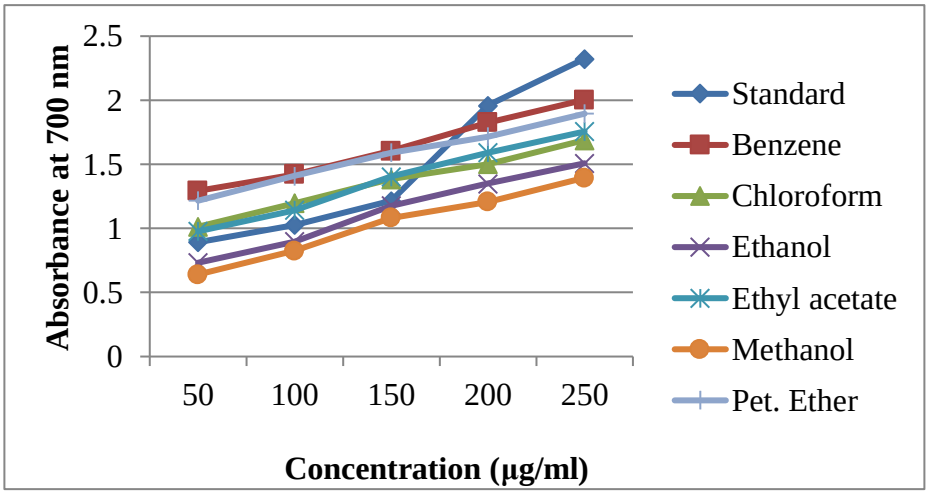


Fig. 2: Standard &Reducing Power of *Ulva fasciata* at 700 nm.

DISCUSSIONS:

1. Phytochemical Screening of *Ulva fasciata*:

Ulva fasciata 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 tannins are present. In ethyl acetate extracts, phenolic compounds, tannins and carbohydrates are present. In Methanolic extracts, flavonoides, glycosides, phenolic compounds, tannins, carbohydrates, proteins and sugar are present. In chloroform extracts, flavonoides, phenolic compounds, saponins, tannins and carbohydrates are present. In benzene extracts, only phenolic compound is present. Petroleum ether extracts, flavonoides, glycosides and tannins are present.

2. Reducing power of ***Ulva fasciata***:

In reducing power of *Ulva fasciata*, benzene extracts showed highest reducing power (1.275), followed by petroleum ether extracts (1.202), Chloroform extracts (1.003), ethyl acetate extracts (0.954), Ethanolic extracts (0.714) and Methanolic extracts showed lowest reducing power (0.638).

The results of present study indicate that the algal species *Ulva fasciata* is rich in secondary metabolites and effective in the terms of antioxidant potentials. It may be useful as food and feed for human and animals.

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