Phytochemical evaluation of selected marine algae collected from Okha coast of Gujarat

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Abstract: *In present investigation on selected species of macro algae's viz., Grateloupia indica, Sargassum tenerimum, Scinaia fascicularis and Ulva lactuca which were collected from the Okha coast of Dwarka District of Gujarat State in India. Result for phytochemicals screening of crude methanolic extracts of all the tested species of macro algae's showed variation in term of presence and absence in the phytoconstituents. Maximum phenolic and flavonoid contents were reported in crude methanolic extracts of Sargassum tenerimum. However, in case of total antioxidant content by DPPH method maximum activity was reported in Grateloupia indica (69.90 %).*

Key Words: *Antioxidant, Species, Phytochemicals and Seaweeds,*

1. INTRODUCTION:

From marine ecosystem, seaweeds are now emerging as a potential source of new drugs. In many investigations it has been reported that seaweeds derived many important secondary metabolites which possessed broad range of biological activities. In India, seaweeds are very abundantly growing in coastal areas of different states where it is highly diversified and comprises mostly of tropical species, but boreal, temperate and subtropical elements have also been reported. Many of the rocky beaches, mudflats, estuaries, coral reefs and lagoons along the Indian coast provide ideal habitats for the growth of seaweeds. Distribution of economic seaweed resources along the Indian coast was first mapped by Thivy (Thivy, 1958). Edible seaweeds were widely consumed, especially in Asian countries as fresh, dried, or ingredients in prepared foods. Industrial and developmental sectors of natural product chemistry i.e., chemotaxonomy are also initiating the use of pigments as natural food dyes (Campo *et al*., 2000). In last few decades, researchers and scientists from various countries put their heads to gather to discover novel and effective drugs against resistant pathogenic strains to protect life-style related diseases from seaweeds sources. It has been now scientifically proved that these seaweeds have good source of potentially rich source of new drug candidates. The detection of new antimicrobial compounds from the natural resources is a promise to the rising emergency of antibiotic resistance and their side effects. It contains rich source of structurally novel and biologically active metabolites which finds various application in pharmaceutical industries.

In current investigation, four species of macro algae were used for phytochemical screening (Figure 1). *Grateloupia indica* (Borgesen) is a member of Grateloupiaceae family. It is a marine species which is distributed in the coastal areas of India at Okha (Gujarat), Maharashtra, Goa, Honawar, Bhatkal (Karnataka). Chattopadhyay *et al*., (2007) investigated the antiherpetic activity of galactan sulphate obtained from *Grateloupia indica*. *Sargassum tenerrimum* (J. Agardh) is one of important member of Phaeophyceae group which is belonging to the family of Sargassaceae. It is predominantly distributed in coasts of many Asian countries, and found along the coasts of Japan, China, Pakistan and India.

Scinaia fascicularis (Borgesen) Huisman is a marine red alga belonging to the Scinaiaceae family. Hayee-Memon *et al*., (1991) studied on phytochemicals constituents of *Scinaia fascicularis* in which methyl myristate was present in highest and laurate in smallest quantity; no unsaturated fatty acid could be detected. *Ulva lactuca* Linnaeus is a thin flat green algae growing on the rough and slimy surface. It is reported that this macro algae is distributed at world wide area. The margin is ruffled and often torn. It may reach 18 centimetres or more in length, though generally much less, and up to 30 centimetres across. The membrane is two cells thick, soft and translucent, and grows attached, without a branch.

2. MATERIALS AND METHODS:

2.1 Collection of macro algae's

In the present study, selected species of macro algae (viz., *Grateloupia indica*, *Sargassum tenerimum*, *Scinaia fascicularis* and *Ulva lactuca*) were collected from the Okha coast of Dwarka District of Gujarat State in India. These selected macro algae were used throughout the study for phytochemical test, total phenolic content, total flavonoid contents and total antioxidant activity.

2.2 Preparation of macro algae's extracts

For the phytochemical screening and analysis of total phenolic, total flavonoid and antioxidant activity the dried samples of selected macro algae's were extracted by soaking 10 g dry sample powdered material in 200 ml of methanol solvent at its respective boiling temperature for 24 hrs. in soxhlet apparatus, filtered through Whatman no.1 filter paper. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40°C.The dried filtrates (crude methanolic extracts of macro algae's) were reconstituted in known amount of methanol to obtain methanol extract of known concentration. The stock solution of crude methanolic extracts of macro algae's (1mg/ml) was prepared by dissolving a known amount of dry extract in 100 ml of methanol. The different working solutions of extracts was prepared from the stock solution using suitable dilution for further analysis of phytoconstituents.

2.3 Preliminary phytochemical analysis

The crude methanolic extracts of macro algae's were subjected to various phytochemical tests to determine the active constituents present in them. The details of the tests are as follows:

2.3.1 *Hager's test for alkaloids*:

To the crude methanolic extracts of macro algae's, saturated solution of picric acid was added. Yellow colors indicate the presence of alkaloids.

2.3.2 *Braemer's test for tannins*:

Take a 2–3 ml of crude methanolic extracts of macro algae's, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicate the presence of tannins in extracts.

2.3.3 *Shinoda test for flavonoids*:

To 2–3 ml of crude methanolic extracts of macro algae's, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. Pink red or red coloration of the solution indicate the presence of flavonoids in the extracts.

2.3.4 *Folin test for phenol*:

To 2–3 ml of crude methanolic extracts of macro algae's, 1 ml of Folin's reagent was added. Violet/brown coloration of the solution indicates the presence of phenol in the extracts.

2.3.5 *Sterols* (*Salkowski test*):

Ferric chloride and glacial acetic acid was added to the crude methanolic extracts of macro algae's and allowed to mixed properly. Aappearance of brown colors indicates the presence of sterols in tested extracts.

2.3.6 *Saponins* (*form test*):

Known amount of water was added in the crude methanolic extracts of macro algae's and properly shaken, appearance of froth indicates the presence of the saponins.

2.3.7 *Cardiac glycosides* (*Keller-kiliani test*):

Add the glacial acetic acid, $Fecl_3$, and H_2SO_4 in the crude methanolic extracts of macro algae's appearance of the green blue color indicates presence of cardiac glycoside.

2.3.8 *Triterpenes* (*Chloroform test*):

Add chloroform in crude methanolic extracts of macro algae's, warm it for 30 minutes and add concentrated sulfuric acid, after mixing it, appearance of red color will indicate the presence of triterpenes.

2.3.9 *Phlobatannins test*:

To the crude methanolic extracts of macro algae's, add 1% sulfuric acid and properly shake the mixture. Appearance of the red precipitates will indicate the presence of phlobatanins

2.4 Determination of total phenolic content

The amount of total phenol content in crude methanolic extracts of selected macro algae's were determined spectrophotometrically using Folin–Ciocalteu reagent (McDonald *et al*., 2001) with slight modifications. To 0.5 ml of each sample (0.1 mg/ml), 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of 20% Na_2CO_3 were added and incubated at 37 °C for 15 min. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Optizen 3220, Mecasys, Kore). For standard reading Gallic acid (standard phenolic compound 0.1 mg/ml) was used. Standard graph was compared with samples reading .Total phenolic content of extracts was noted in µg/g (on dry weight basis). All samples were analyzed in triplicates.

2.5 Determination of total flavonoid content

The aluminium chloride colorimetric method (Chang *et al*., 2002) was used to determine the flavonoid content in crude methanolic extracts of selected macro algae's. To 1.0 ml of crude methanolic extracts (1mg/ml) were mixed with 1.5 ml of methanol, 0.1ml of 10% aluminium chloride, 0.1 ml of 1M sodium acetate and 7.3 ml of distilled water was added then mixture was allowed to stand for 30.0 minutes at room temperature. The absorbance of reaction mixture was measured at 415 nm. Quercetin (Standard flavonoid compound 0.1 mg/ml) was used as standard for the calibration curve. Standard graph was compared with samples readings. Total flavonoid content of samples were expressed in µg/g (on dry weight basis). All samples were analysed in triplicates.

2.6 *In vitro* antioxidant assay by DPPH

DPPH radical is a widely used method to evaluate the free radical scavenging ability of natural compounds. The DPPH scavenging activity was determined using the method described by Shimada *et al*., (1992) with slight modification. Crude methanolic extracts of selected macro algae's (1 ml at 1mg/ml) was mixed with 1 ml of a 0.1 mM DPPH (Merck). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. L-Ascorbic acid (0.1mg/ml) was used as the standard.

$$\text{Radical scavenging activity } (\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where A_C = absorbance of the control and A_S = absorbance of sample.

All tests were run in triplicates (n = 3), and the average values were calculated.

3. RESULT AND DISCUSSION:

3.1 Preliminary phytochemical screening

Result of phytochemical screening of *Grateloupia indica, Sargassum tenerimum,* Scinaia *fascicularis* and *Ulva lactuca* were revealed that there were variation in term of presence and absence in the phytoconstituents of tested samples (Table No. 1). In case of *Grateloupia indica*, all the phytochemicals viz., alkaloids, flavonoids, phenols, tannins, steroids, saponins, cardiac glycosides, triterpenes and phlobatanin were present in crude methanolic extract. For crude methanolic extract of *Sargassum tenerimum* showed that alkaloids, tannin, flavonoids, phenols, steroids, saponins, triterpenes, cardiac glycosides types of phytoconstituents were present while remaining phytoconstituents were absents. In case of crude methanolic extract of *Scinaia fascicualaris*, except phlobatanins and saponins all other phytoconstituents were present. However, in crude methanolic extract of *Ulva lactuca* results showed that all the tested phytoconstituents were present except the phlobatanin. Similar line of research work also carried out by other scientists. In research of Kannan *et al*., (2014) reported the presence of a variety of chemical constituents, such as saponins, phenols, glycosides, flavonoids and alkaloids in these marine algae by TLC and HPLC method.

3.2 Determination of total phenolic content

For total phenolic content in all the tested crude methanolic extracts of selected macro algae's. Result revealed that the maximum total phenolic content was observed in case of *Sargassum tenerimum* extract in which 77.41± 2.11 µg/g (dry weight basis content) was reported. This extract was followed by *Scinaia fascicularis* extract in which 63.79 ± 1.33 µg/g (dry weight basis) of total phenolic content was noted. However, minimum total phenolic content was found in case of *Ulva lactuca* in which 28.94 ± 0.26 µg/g (dry weight basis) was observed (Table No. 2). Similar work was also done by Farasat *et al.,* (2013) in **antioxidant activity, total phenolic and flavonoid contents of some edible green seaweeds from Northern Coasts of the Persian Gulf, result showed that the** content of phenolic compounds varied from 5.08 ± 0.65 (*Ulva clathrata*) to 1.258 ± 0.126 (*U.intestinalis* (S5)) mg GAE g.

3.3 Determination of total flavonoids content

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants, algae etc. In the present finding, total flavonoids content in crude methanolic extracts of selected macro algae's (Table No. 2) showed that the maximum flavonoids content was observed in case of *Sargassum tenerimum* in which 36.67 ± 1.96 µg/g (dry weight basis). This was followed by *Grateloupia indica* in which 24.11 ± 0.98 µg/g (dry weight basis) of total flavonoids content was reported. However, minimum total flavonoids content was found in *Scinaia fascicularis* in which 8.24 ± 1.17 µg/g (dry weight basis). All these results indicate that flavonoids extracted from *Sargassum tenerimum* could be an important source of antioxidant molecules. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes; anti-inflammatory action (Frankel *et al*., 1995). Similar work was also done by Farasat *et al.,* (2013) in **antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from Northern Coasts of the Persian Gulf,** the flavonoid content of algal extracts varied from 33.094 ± 2.053 (*Ulva clathrata*) to 8.048 ± 1.119

(*U.intestinalis* (S5)) mg RE g-1. The flavonoid contents of two samples of *U.intestinalis* (S3 and S5) were significantly different and were higher in S3 (25.316 ± 2.198 mg RE g-1).

3.4 *In vitro* antioxidant assay by DPPH

Result of total antioxidant activity by DPPH method revealed that crude methanolic extracts of *Grateloupia indica, Sargassum tenerimum, Scinaia fascicularis* and *Ulva lactuca* showed variation in total antioxidant activity. Among all the tested samples maximum radical scavenging activity was noted in *Grateloupia indica* (69.90 %). It has been followed by *Sargassum tenerimum* with the radical scavenging activity of 51.13 %. However, minimum or lowest radical scavenging activity was observed in case of crude methanolic extract of *Scinaia fascicularis* with 26.05% (Table No. 3). Hence it could be concluded from present study that tested species of *Grateloupia indica, Sargassum tenerimum, Scinaia fascicularis and Ulva lactuca* leads to open a new source of natural secondary metabolites as well as antioxidants in herbal drug industries which would replace the synthetic ones and can reduce the level of toxicity.

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Figures:

(a) **(b)** **(c)** **(d)**

Figure 1: Close view of selected species macro algae's collected form Okha

(a) *Grateloupia indica* **(b)** *Sargassum tenerimum* **(c)** *Scinaia fascicularis* **(d)** *Ulva lactuca*

Table No. 1. Preliminary phytochemical analysis of *Grateloupia indica, Sargassum tenerimum, Scinaia fascicularis* and *Ulva lactuca*.

S.No.	Components	*Grateloupia indica*	*Sargassum tenerimum*	*Scinaia fascicularis*	*Ulva lactuca*
1	Alkaloids	+	+	+	+
2	Tannins	+	+	+	+
3	Flavonoids	+	+	+	+
4	Phenols	+	+	+	+
5	Steroids	+	+	+	+
6	Saponins	+	+	-	+
7	Cardiac glycosides	+	+	+	+
8	Triterpenes	+	+	+	+
9	Phlobatanin	+	-	-	-

Key: - = absent, + = Presence.

Table No. 2. Total phenol and flavonoids contents in the crude methanolic extracts of macro algae's.

Extract	Algal sample	Total phenolic content (µg/g dry weight basis)	Total flavonoid content (µg/g dry weight basis)
Crude methanolic extract	*Grateloupia indica*	46.01 ± 0.98	24.11 ± 0.98
	Sargassum tenerimum	77.41± 2.11	36.67 ± 1.96
	Scinaia fascicularis	63.79 ± 1.33	8.248 ± 1.17
	Ulva lactuca	28.94 ± 0.26	24.00 ± 2.16

Table No. 3. Antioxidant activity in crude methanolic extracts of macro algae's (DPPH).

Extract	Algal sample	Total Antioxidant content (%)
Crude methanolic extract	*Grateloupia indica*	69.90
	Sargassum tenerimum	51.13
	Scinaia fascicularis	26.05
	Ulva lactuca	38.67

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