



Phytochemical screening and antioxidant activity of marine algae *Gracilaria corticata* and *Spirulina platensis*

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ABSTRACT

The aim of the present paper is to evaluate the phytochemicals, the antioxidant activity of marine algae *Gracilaria corticata* (*G. corticata*) and *Spirulina platensis* (*S. platensis*). The phytochemicals present in the selected marine algae *G. corticata* and *S. platensis* were screened and their antioxidant activities were tested. The marine algae was collected, shade dried, powdered and extracted with methanol. The presence of a variety of chemical constituents, such as saponins, phenols, glycosides, flavonoids and alkaloids were analyzed in these marine algae by TLC and HPLC method. Their antioxidant activities were studied by Fentons method and DPPH assay. Phytochemical screening showed the presence of active molecules. The selected algae is having antioxidant potential. From the study, it is clear that these *G. corticata* and *S. platensis* are the prospective sources of bioactive compounds.

Key words; *G. corticata*, *S. platensis*, saponins, phenols, glycosides, flavonoids and antioxidant.

INTRODUCTION

Algae is a mixed group of plants with an extensive fossil history. They are rich in many molecules like terpenoids, phlorotannins, alkanes, steroids, amino acids, phenolic compounds, halogenated ketones and cyclic polysulphides [1&2]. Marine algae is bright to make a prodigious variety of secondary metabolites with a wide-ranging of biological actions because they have bioactive compounds.

Antioxidants are molecules, capable of inhibiting the oxidation of molecules and protect the environment. Free radicals are produced mainly by oxidation reactions and, thereby these radicals can establish chain reaction. Because of these reactions in a cell, it gets damaged and finally leads to death. Antioxidants cause these sequences of reactions by eradicating free radical intermediates and reduce further oxidation reactions. Antioxidants do the above said by being oxidized themselves. So, antioxidants are acting often as dropping agents like ascorbic acid, thiols or polyphenols [3]. They are extensively used as ingredients in nutritional supplements and have been examined for the avoidance of diseases like coronary heart disease, cancer and even for altitude sickness.

Brown, red and green algae are rich in molecules with antiviral, antioxidant, antifungal and antimicrobial activities [4]. Because of its great nutritional value, *S. platensis* has been used since olden times as a resource of food [5]. It is prospered in nutrients like minerals, protein, carbohydrates, vitamins and (γ)-linolenic acid. In recent years, it is

getting more and more consideration, not only owing to its food value, but also for the progress of research of its potential pharmaceuticals [6]. *Gracilaria* is used as a foodstuff in Japan, Hawaii and Philippines. It is called ogonori or *ogo* in Japanese cuisine, as *gulaman* or *guraman* in Philippines [7]. The present research work was planned to examine the bioactive compounds and antioxidant activity of *G. corticata* and *S. platensis* marine algae extracts.

EXPERIMENTAL SECTION

A. Collection of *Gracilaria corticata* and *Spirulina platensis*

Gracilaria corticata and *Spirulina platensis* were collected from the coastal area of New Harbour, Tuticorin coast and Gulf of Mannar, Tamilnadu, India. They were used as the experimental algae to their biodiversity [8].

B. Collection of Marine Algae

Two dominant marine macroalgae such as *G. corticata* and *S. platensis* were collected at a depth of 50-100 cm from Tuticorin new harbor coast, Gulf of Mannar during season (October-January 2010). To remove epiphytes, debris and other marine organism, collected samples were washed with tap water systematically, then dehydrated with tissue paper and stored at -20°C till further analysis [9].

C. Extraction

The collected samples were shade dried and then milled into fine powder using pulverizer. The extraction was done by soxhlet apparatus (Methanol as solvent). The extracts were evaporated to total dryness by vacuum distillation and preserved in refrigerator at 4°C for further analysis [10]. The extracted Marine algae *Gracilaria corticata* and *Spirulina platensis* were used for the study of bioactive compounds and pharmacological studies.

D. Phytochemical studies of selected marine algae

Development of TLC Plate

The presence of active secondary metabolites of the *G. corticata* and *S. platensis* were qualitatively analyzed by TLC. The solid phase of silica gel was kept back at 100° C for 20 minutes in hot air oven. Silica powder was blended thoroughly by petroleum ether and made as slurry. 20 cm × 20 cm TLC glass plates were enclosed by slurry and dried air. Then, the plates were held in reserve back for 1 hour at 72°C in hot air oven. Using capillary tube, condensed filtrate was spotted on the plate. The diverse spots were divided using various solvent combinations as mobile phase based on the phytochemicals [11&12].

Separation of Alkaloids

The powdered marine algae *Gracilaria corticata* and *Spirulina platensis* were wetted by a partially diluted ammonium hydroxide and lixiviated with Ethyl acetate at room temperature for 24 hours. From the acidified filtrate, the organic part was separated and then with ammonium hydroxide (pH 11-12) basified. Then, it is removed with chloroform (3x) and reduced by evaporation. The evaporated material was used for chromatography. Using a solvent combination of chloroform and methanol (15:1), alkaloid spots were separated. After spraying with Dragendroff's reagent under Ultra Violet (254nm) and visible light [11], colour and hRf of alkaloids divided were analyzed.

Separation of Flavonoids

The powdered marine algae of the selected medicinal marine algae was treated with 10 ml methanol of water both for 5 min at 60°C. The filtrate was allowed to condense by desertion. Combination of water and ethyl acetate (10:1ml) was blended to filter the extracts. For chromatography, the ethyl acetate part retained and used. Using the chloroform and methanol (19:1) solvent combination the flavonoid spots were separated. Under Ultraviolet light (254m), hRf and colour of these spots were documented [11].

Separation of glycosides

The powdered marine algae of the selected marine algae was extracted with 70% Ethyl alcohol on a rotary shaker (180 thaws/min) for 10 hours, 70% lead acetate was included further to filtrate and centrifuged at 5000 rpm/10min. Further, supernatant was centrifuged by including 6.3% Sodium bi carbonate at 10,000 rmp/10 min. The remaining supernatants were dried up, re-dissolved in chloroform and recycled for chromatography. The glycosides were divided by the help of Ethyl acetate: Water: Methanol (80:10:10) solvent combination. By observing under Ultraviolet (UV 254nm) light, colour and hRf of the spots were documented [12].

Separation of phenols

The powdered marine algae of the selected marine algae were lixiviated using methanol on rotator shaker (180 thaws/min) for 24 hours. For chromatography, the reduced filtrate was cast off. Chloroform and methanol (27:0.3) solvent combination was used for split-up of phenols. After scattering the plates with Folin-Ciocalteus reagents which is heated for 10 minutes at 80°C, the hRf and colour of phenols were documented in visible light [12].

Separation of Saponins

The powdered marine algae was treated for 10 minutes with 10 ml 70% Ethyl alcohol by reflux in. The filtrate was thickened, added by saturating n-butanol for enrichment and mixed carefully. The butanol portion was reserved and applied for chromatography. Chloroform: Glacial acetic acid: Methanol: water (64:34:12:8) solvent mixture was used and the spoons were divided, then hRf and colour of these were documented by exposing Chromatogram to iodine vapors [11].

High performance liquid chromatography (HPLC)

It was analyzed for selected marine algae extracts, on Shimadzu spectrum HPLC-530 available in the research center, ANJAC, Sivakasi. The results were recorded.

E. Antioxidant properties of *G. corticata* and *S. platensis*

Antioxidant activity by Fentons reagent method

The antioxidant activity of methanolic extracts of *G. corticata* and *S. platensis* were determined by the Fentons method [13].

Free radical scavenging activity of DPPH method

The antioxidant activity for methanolic cuttings of *G. corticata* and *S. platensis* were separated by the TLC method for the selected marine algae were determined [13].

Add 50µl of methanolic extract of marine algae (100µg/ml) with every reaction and mixed with 1 ml of 0.1mm DPPH in methanol and 50mm Tris HCL buffer (450µl) of pH 7.4. Then, 50µl of methanol was used as the experimental control. The tubes were incubated at room temperature for 30 minutes. After 30 minutes, the reduction in the number of DPPH free radicals was measured at 517nm.

RESULTS AND DISCUSSION

To evaluate the antimicrobial and antioxidant properties of dried extracts, secondary metabolites were carried out using various solvents. The algae extracts of *Gracilaria corticata* and *Spirulina platensis* leaves were tested for the presence of alkaloids, flavonoids, phenols, glycosides, and saponins using thin layer chromatography technique (Table 1). In TLC, specific solvent systems were used to separate the specific compounds. Fig. 1 shows the TLC profile of the phytochemicals present in *Gracilaria corticata* and *Spirulina platensis*.

Table 1: TLC profile of *Gracilaria corticata* and *Spirulina platensis*

Name of the secondary metabolic compounds	Rf value of the <i>Gracilaria corticata</i>	Rf value of the <i>Spirulina platensis</i>
Alkaloids	0.50±0.03	0.35±0.02
Flavonoids	0.53±0.02	0.80±0.02
Glycosides	0.90±0.01	0.81±0.01
Phenols	0.70±0.01	0.55±0.03
Saponins	0.90±0.03	0.65±0.02

The alkaloids were separated as spots at the Rf value of 0.50±0.03 for *Gracilaria corticata* marine algae, flavonoids Rf value 0.53±0.02, 0.90±0.01 for glycosides, 0.70±0.01 for phenols and 0.90±0.03 for saponins. Similarly, the alkaloids were separated from *Spirulina platensis* and the Rf value 0.35±0.02, flavonoids Rf value was 0.80±0.02, 0.81±0.01 for glycosides, 0.55±0.03 for phenols and 0.65±0.02 for saponins and the results were tabulated. The TLC profile of secondary metabolites, alkaloids were found to be most abundant one in *Gracilaria corticata* extracts while flavonoids to be low in *Gracilaria corticata*. In *Spirulina platensis*, flavonoids were high and alkaloids were found to be low.

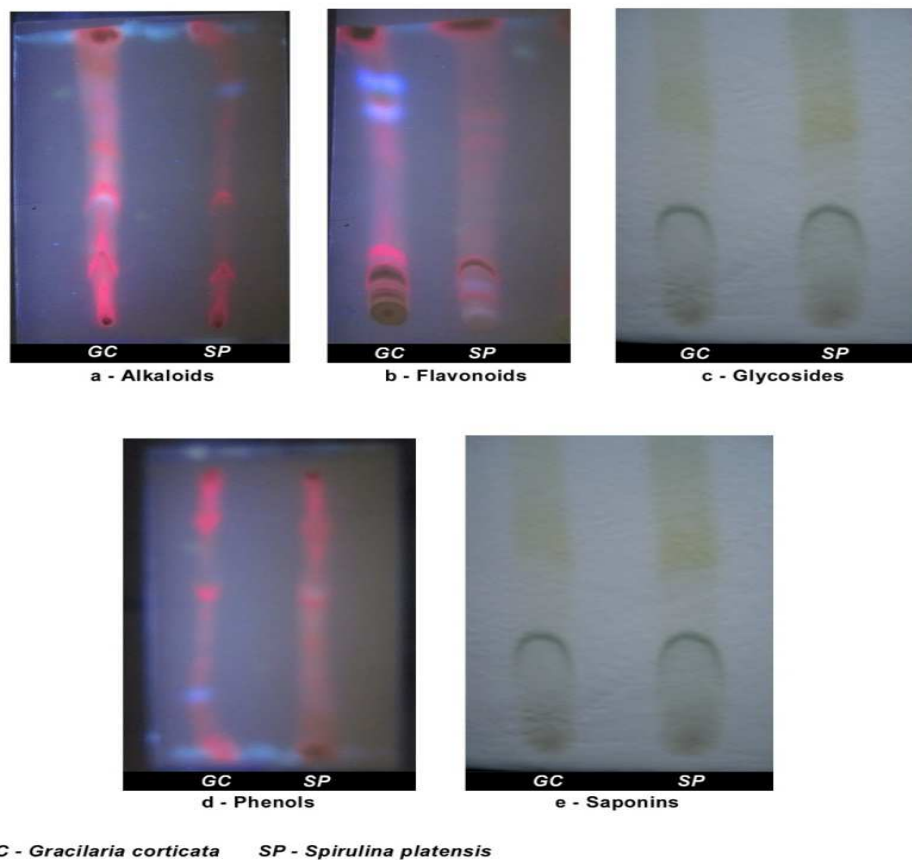


Fig. 1: TLC profile of the phytochemicals in Gracilaria corticata and Spirulina platensis

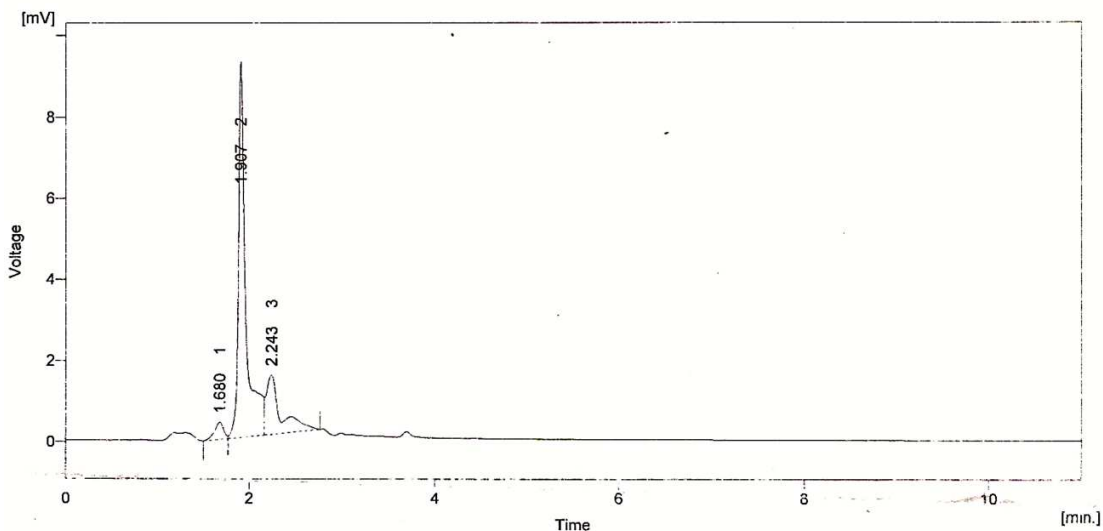


Fig. 2: HPLC analysis of Gracilaria corticata methanol extract

At 254 nm of wavelength, the qualitative HPLC marine algae extract report was studied. Moreover, the sharpness of its peaks, Rt min, percent area, suitable baseline and heights were documented. The marine algae *Gracilaria corticata* has a single main peak at 1.907 retention time in methanol extract and *Spirulina platensis* has two major

peaks at 1.907 and 2.160 retention time in the methanol extract (Fig. 2&3). The HPLC studies confirmed the bioactive compounds presenting in the sample.

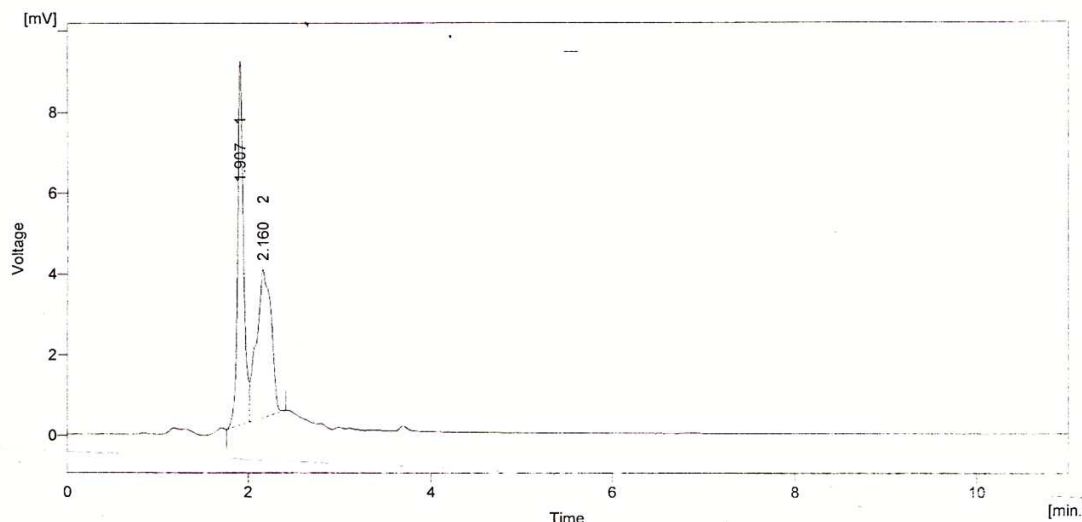


Fig. 3: HPLC analysis of *Spirulina platensis* methanol extract

It was found that the values such as 0.486 and 0.375 (%) for *Gracilaria corticata* and *Spirulina platensis*, respectively. This showed that they are of lower values than the standard. It was indicated that both the extracts of *Gracilaria corticata* and *Spirulina platensis* have better antioxidant activity and the observed results were tabulated (Table 2).

Table 2: Antioxidant activity from *Gracilaria corticata* and *Spirulina platensis* by Fenton reagent method

Sample	Concentration	<i>Gracilaria corticata</i>	<i>Spirulina platensis</i>
Standard	0.5ml	1.05ml	1.05ml
Test	0.5ml	0.250ml	0.250ml
Algae extracts control	-	0.663	0.790
Algae extract	-	0.375(50%)	0.486(94.4%)

Percentage values are given in parenthesis.

The DPPH assay is the furthestmost widely functional technique for viewing natural product's antioxidant activity since they can provide accommodation for a lot of models in a given period of time and spot ingredients (active) at low concentrations. The declining absorbance of DPPH radicals was mainly due to occurrence of an antioxidant and are because of hunting of radical by hydrogen contribution. The methanolic excerpts of *Gracilaria corticata* and *Spirulina platensis* showed DPPH radical scavenging activity by a strong positive correlation in a concentration reliant manner, with standard vitamin E, whereas the blank (dimethyl sulfoxide) short of marine algae extracts did not display any response (Table 3). Similar to Fentons, DPPH also showed a decrease in the absorption value with the increase in marine algae extract concentration from 150 to 190 μ l compared with the test control 1.93. This indicated that the marine algae extract showed better antioxidant activity.

Table 3: Antioxidant activity of marine algae extracts by free radical scavenging activity by DPPH method

Name of the marine algae	Test control	Marine algae extracts of various concentrations (μ l)				
		150	160	170	180	190
<i>Gracilaria corticata</i>		0.57 (70.4%)	0.65 (66.3%)	0.73 (62.1%)	0.86 (55.4%)	0.89 (53.8%)
<i>Spirulina platensis</i>	1.93	0.68 (64.7%)	0.72 (62.6%)	0.86 (53.8%)	0.90 (53.3%)	0.93 (51.8%)

Marine organisms are potentially fertile sources of extremely bioactive secondary metabolites that might symbolize functional leads in the development of novel pharmaceutical compounds [14].

Phytochemical screening of *Caulerpa racemosa* exposed the presence of alkaloids, phenolics, flavonoids and steroids [15]. Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from marine algae and used in medicine and pharmacy [16].

At $p < 0.05$, the contents of total phenolic of dehydrated methanolic extracts were considerably different and *H. elongata* showed maximum phenolic content at 151.3 ± 0.03 mg GAE/g of marine algae excerpt and as well had utmost DPPH scavenging activity by a 50% inhibition (EC50) level at 0.125 ± 0.001 $\mu\text{g/ml}$ of excerpt. *H. elongata* also reported to have the maximum flavonoid and tannin contents of 42.5 ± 0.02 mg QE/g and 38.34 ± 0.05 mg CE/g, respectively. The existence of any substances work as an antioxidant or free radical hunter may protect the body from the consequences of oxidative strain. Thus, antioxidants take part in the protection of cells against oxidative strain was caused by reactive species [13&17]. Quite a few molecules have been recognized in extracts equivalent to various carotenoids formerly known in *S.platensis* microalgae along with numerous degradation products. Further, few phenolic compound structures, possibly will be cautiously recognized. The antioxidant activity of excerpts could be credited to several of above stated plant materials [18, 19 & 20].

The preliminary phytochemical analysis of *Ulva fasciata* concluded that this alga is a beneficial one for its biological activity due to the presence of extra phytochemical. In the DPPH scavenging assay, it showed high antioxidant activity when it is compared to the *Chaetomorpha antennina* [21].

Marine algae is one of the chief marine living resources and they are an outstanding source of many vitamins as well as minerals like Ca, P, Na and K. Marine algae nutritional fibers execute a diverse array of functions such as antioxidant, anticoagulant, antimutagenic, antitumor etc., [22]. There are reports that marine algae were also a rich source of antioxidant compounds [23]. Antioxidant activity was determined by free radical scavenging (DPPH) and inhibition of lipid peroxidation in *Sargassum dentifolium*, *Jania corniculata* & *Laurencia papillosa*. Dichloromethane extract of each algal species established better antioxidant activities than the ethanol extract [24]. Quite a few studies have done for the antioxidant activity of natural products of freshwater and marine algae [25, 26, 27 & 28]. Several studies have focused on physiological qualities of some valuable antiviral or antioxidant compounds in blue green algae *Spirulina* [29]. *S. platensis* or its extracts show therapeutic ability, such as preventing the incidence of cancers, stimulate the immunological system, diminish the nephrotoxicity of pharmaceuticals, lessen blood cholesterol levels and noxious metals and offer defense against the damaging outcome of radiation [30].

After supplementation of *Spirulina* for 40 days (along with mercuric chloride, in olive oil, 800mg/kg body weight orally) lead to in reduced LPO level, serum glutamate oxaloacetate and serum glutamate pyruvate transaminase doings all along rise in liver reduced glutathione. The antioxidant enzymes actions such as superoxide dismutase, glutathione-S-transferase and catalase were concurrently reverted to close normalcy by *Spirulina* supply to mice (mercuric chloride intoxicated). The outcome revealed that *Spirulina* treatment augmented clearly the antioxidants protection mechanism, brought toxicity in mercuric chloride and delivers proof that it might require remedial part in free radical mediated diseases [31] and arthritis [32].

CONCLUSION

The present study revealed that the selected marine alga *Gracilaria corticata* and *Spirulina platensis* were the best source of bioactive compounds. They showed the presence of various phytochemicals. The investigation of antioxidant activities showed that they contain potent antioxidants. Thus, these marine algae and their bioactive compounds may be utilized for the development of natural antioxidants.

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