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Research Paper

Bioactive Potentials of Red Seaweeds, Gracilaria Corticata J.Ag., and *Gracilaria Follifera*(Forssk.) Boergs

Botany

KEYWORDS : Gracilaria corticata, Gracilaria follifera

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ABSTRACT

The most common form of marine vegetation found in the sea is algae, which populate the pelagic zone to the lowest point of light penetration. The largest of the marine algae (green, brown, and red) or macrophytes, are found in the comparatively narrow coastal zone of the sea known as the sublittoral. Presence of metabolites such as fatty acids, steroids, carotenoids, lectin and mycosporine like amino acids, halogenated compounds polypeptides and toxins as well as other sulphated polysaccharides make these organisms an economically important product. Seaweeds or marine macro –algae are potential renewable resource in the marine environment. It has been used as anti-oxidant , anti-mutagen, anti-coagulant anti-microbial and anti-tumoragent. Majority of their antioxidant activity is due to flavones, isoflavones, flavonoids, anthocyanin, Coumarin, ligans, catechins and isocatechin. Seaweeds are rich in antioxidants such as carotenoids, pigments, polyphenols , enzymes and diverse functional polysaccharides. The main objective of the present study was to determine the biochemical composition, phytochemical composition and antioxidant activity of Gracilaria follifera and Gracilaria corticata collected from Thikkodi coast

INTRODUCTION

Sea vegetation is the most nutrient dense vegetation on planet Earth. They include algae, grasses, mangrove trees, shrubs, bacteria and to a lesser degree, fungi. The most common form of marine vegetation found in the sea is algae, which populate the pelagic zone to the lowest point of light penetration. The marine algae are represented by both unicellular and multicellular forms that externally resemble terrestrial higher plants. The classification into divisions is based on various properties such as pigmentation, chemical nature of photosynthetic storage products, the organization of photosynthetic membranes and other morphological features. Traditionally, they belong to four different groups, empirically distinguished since the mid-nineteenth century on the basis of color; blue-green algae,red algae,brown algae and green algae.

Today, various species of marine algae provide not only food but also produce extracts such as agar, carrageenans, and alginates. These extracts are used in numerous food, pharmaceutical, cosmetic and industrial applications. The industrial applications of macroalgae are also varied. Their polysaccharides are used in food, cosmetics, tooth paste, solid air fresheners, and paint, crop, textile, paper, rubber and building industries. According to their chemical structure, most isolated compounds belong to sulfated polysaccharides, phenolics, terpenoids, lactons, sterol and fatty acids (McDermid and Stuercke, 2003).

Phenolic compounds are commonly found in edible brown, green and red seaweeds, whose antioxidant properties have been correlated to their phenolic contents (Ganesan et al., 2008). There are several reports on the antioxidant activities of steroids terpenoids and saponins. The antioxidant activity of polysaccharide from *Bryopsis plumosa* was reported by Song et al., 2010. Vinayak et al., 2011 reported cytotoxic and antioxidant activities of crude methanol extract of the brown seaweed *Dictyopteris australis*. There exist several reports in the literature on the antioxidant activities of many *Gracilaria* species. Quality of protein and lipid in seaweeds are most acceptable for consumption compared to other vegetables mainly due to their high content in essential amino acids and relatively high level of unsaturated fatty acid (Peter et al., 2005).In contrast to terrestrial plant material less research has been conducted on the antioxidant potential of marine seaweeds. Reports on the antioxidant properties of seaweeds extracts from India are limited (Duan et al., 2006).

The main objective of the present study was to determine the biochemical composition, phytochemical composition and antioxidant activity of *Gracilaria follifera* and *Gracilaria corticata* collected from Thikkodi coast.

MATERIALS AND METHODS

Study area

The algal samples were collected from Thikkodi (11^0 29` N lat &$75^0$ 37` E long) (Plate-1) during February 2015.The station has an extensive rocky promontory with small bays of sand and posses rich algal vegetation. There is no fresh water influence.

Collection of seaweeds

The fresh plants of two species of seaweeds namely *Gracilaria corticata* J.Ag., and *Gracilaria follifera* (Forssk.)Boergs (Rhodophyceae) (Plate -2) were collected from Thikkodi.

The samples were immediately washed with seawater to remove the adhered sand and brought in plastic bags to the laboratory .The samples were washed thoroughly with tapwater to remove attached epiphytes and adhered dirt particles. Then they were again washed thoroughly three to four times with water for removing the debris and sand particles from the seaweeds.

Preparation of seaweed powder

The water was drained off and the seaweeds were spread on blotting paper to remove excess water. All the samples were dried at room temperature followed by 40^0 c in the hot air oven for two days. Then they were powdered and kept in airtight plastic bottles at room temperature.

Preparation of solvent extracts

50 gm dried crushed samples were extracted using each 250 ml solvent of petroleum ether, acetone, water and ethanol .The weighed quantity of plant parts were then homogenized in different solvents and then properly covered with aluminum foil and labeled. After twenty four hours of extraction, each extract was filtered through whatmans filter paper no.1 separately. The extract was evaporated to dryness at 40^0 c in a dryheat incubator, extract was then kept in the refrigerator until the time of use for following experiments. Afterwards a measured volume of solvent was used to dissolve the extract to required working concentration.

Qualitative phytochemical screening

Phytochemical screening was carried out by using standard procedure described by Harborne (1998).

Biochemical composition of seaweeds

The biochemical composition was carried out using dried powder of algae.

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Estimation of protein
The total protein was estimated by following procedure of Lowry *et al*., 1951.

Estimation of carbohydrate
The total carbohydrate was estimated by following the method of Dubois *et al*., 1956.

Total phenolic content
The amounts of total phenolics in the extract were determined with Folin-ciocalteu reagent according to the method of Singleton and Rossi (1965).

Total flavonoid content
Calorimetric technique was used for flavonoid estimation (Chang *et al*., 2002).

Antioxidant assay
DPPH radical scavenging assay
Scavenging effects of samples for DPPH radical were monitored according to the method of Yen & Chen (1955).

RESULTS
Phytochemical substance such as alkaloids, steroids, flavonoids, phenols, coumarins, cardiac glycosides, tannins, terpenoids, and saponins were determined in various extracts (Acetone, Ethanol, Water, Petroleum ether) of two species of algae (*Gracilaria corticata* and *Gracilaria follifera*) (Table-1,Fig-A&B).

Table 1.Qualitative phytochemical analysis of various extracts of *Gracilaria corticata* and *Gracilaria follifera*.

Solvents	Acetone		Ethanol		Water		Petroleum ether	
Seaweeds	GC_A	GF_A	GC_E	GF_E	GC_W	GF_W	GC_P	GF_P
PHYTO-CHEMICALS Alkaloids	+ +	+ +	++ +	+ + +	+ +	+	+	+ +
Steroids	+ +	+ +	+ +	+ +	_	+	_	+
Flavonoids	+ + +	+ + +	+ + +	+ +	+	_	+	+
Phenols	+ + +	+ +	+ + +	+ + +	+	+	+	+ +
Coumarins	+ +	+	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
Cardiac glycosides	–	+	+ + +	+ +	+ + +	+ + +	+ + +	+ +
Tannins	_	+	+ +	+ +	+ +	+ + +	_	+
Terpenoids	+	+	+ + +	+ +	+	_	+ +	+ +
Saponins	+	+	+ +	+ +	+ + +	+ +	_	_

GC - *Gracilaria corticata*
GF - *Gracilaria follifera*
(+) - Present (trace amount)**, (+ +) –** Abundant, **(+++)** – Very abundant,
(-) – Absent.

Alkaloids are very abundantly present in ethanol. Steroids are abundantly present in acetone and ethanol and absent in water and petroleum ether extract of *Gracilaria corticata*. Flavonoids are very abundantly present in acetone and ethanol extract of *Gracilaria corticata* and absent in water extract of *Gracilaria follifera*. Phenols are very abundantly present in acetone and ethanol extract of *Gracilaria corticata*. Coumarins are very abundantly present in ethanol, water and petroleum ether. Cardiac glycosides are abundantly present in water. Tannins are very abundantly present in water extract of *G.follifera*. Cardiac glycosides and tannins are absent as recorded in acetone extract of *Gracilaria corticata*. Terpenoids are abundantly present in ethanol extract of *G.corticata*. Trace amount of saponins are recorded in the acetone extract and absent in petroleum ether extract of two species. Biochemical composition such as protein and carbohydrate were estimated in two species of algae (*Gracilaria corticata* and *Gracilaria follifera*).

The maximum protein content was recorded in petroleum ether extract of *Gracilaria follifera* (36 .03 ± 0.03 %) and minimum in water extract of *Gracilaria corticata* (17.50 ± 0.01 %). In carbohydrate maximum value recorded in acetone extract of *Gracilaria follifera* (6.70 ± 0.01 %) and minimum in petroleum ether extract of *Gracilaria corticata* (2.38 ± 0.02 %).(Table-2, Fig-1&2)

Table 2.Biochemical composition of various extracts of *Gracilaria corticata* and *Gracilaria follifera*.

Parameters	Solvents	Gracilaria corticata	Gracilaria follifera
Protein (%DW)	Acetone	20.33 ± 0.46	32.13 ± 0.01
	Ethanol	19.03 ± 0.04	34.40 ± 0.14
	Water	17.50 ± 0.01	29.03 ± 0.04
	Petroleum ether	27.35 ± 0.21	36.03 ± 0.03
Carbohydrate (%DW)	Acetone	4.23 ± 0.01	6.70 ± 0.01
	Ethanol	3.39 ± 0.02	4.36 ± 0.01
	Water	6.67 ± 0.03	4.32 ± 0.02
	Petroleum ether	2.38 ± 0.02	3.42 ± 0.02

Values are mean ± SD ; Sample size (n) = 3.

Fig 1: Biochemical Composition of *Gracilaria corticata* Collected from Thikkodi.

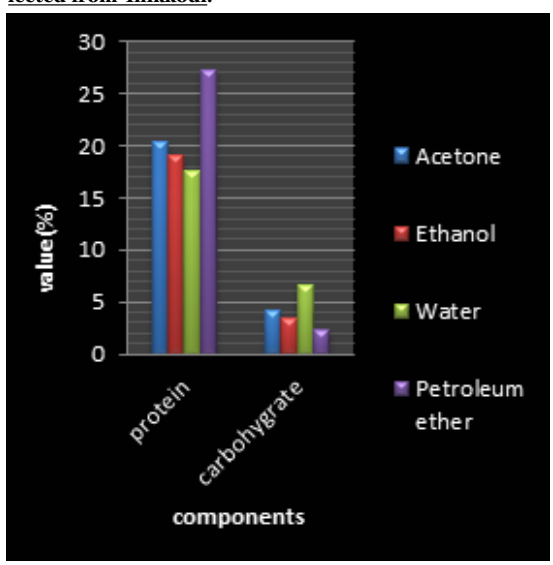
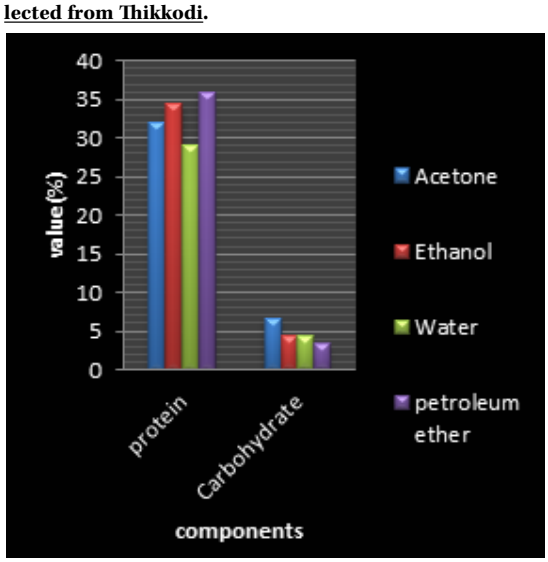


Fig 2:- Biochemical Composition of *Gracilaria follifera* Collected from Thikkodi.



The Table 3 showed the result of phenol and flavonoid content of various extract of *Gracilaria follifera* and *Gracilaria corticata*

Table 3. Total Phenol and Flavonoid content of various extracts of *Gracilaria corticata* and *Gracilaria follifera*.

Algae	Solvent	Phenol(mg/g)	Flavonoid (mg/g)
Gracilaria corticata	Acetone	4.42 ± 0.35	44.22 ± 0.31
	Ethanol	8.75 ±0.21	18.54 ± 0.06
	Water	2.16 ± 0.09	21.86 ± 0.01
	Petroleum ether	3.50 ± 0. 14	15.65 ± 0.05
Gracilaria follifera	Acetone	7.6 ± 0.14	28.66 ± 0.02
	Ethanol	8.79 ± 0.13	15.63 ± 0.01
	Water	7.44 ± 1.22	18.72 ± 0.004
	Petroleum ether	1.54 ± 0.44	8.83 ± 0.004

The phenol content of various extract of seaweeds varied from 8.75 ± 0.21mg/g) to 2.16 ± 0.09mg/g). In *Gracilaria corticata*, the maximum phenol content was recorded in ethanol extract (8.75 ± 0.21mg/g) and minimum in water extract (2.16 ± 0.09mg/g). In the case of flavonoid maximum recorded in acetone extract (44.22 ± 0.31 mg/g) and minimum in petroleum ether extract (15.65 ± 0.05mg/g).

Fig 3:- Total Phenolic Content and total flavonoid Content of *Gracilaria corticata*

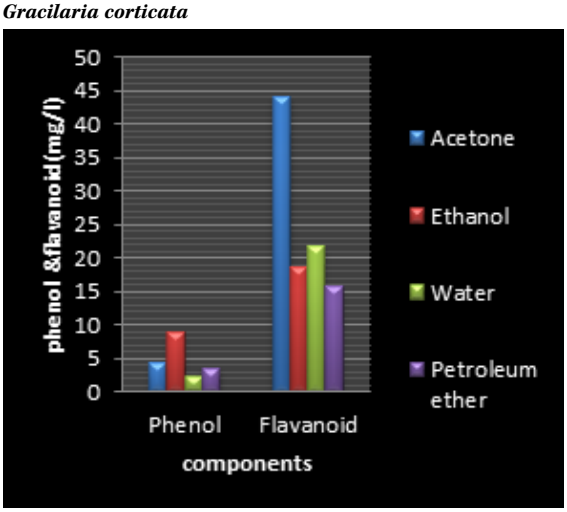
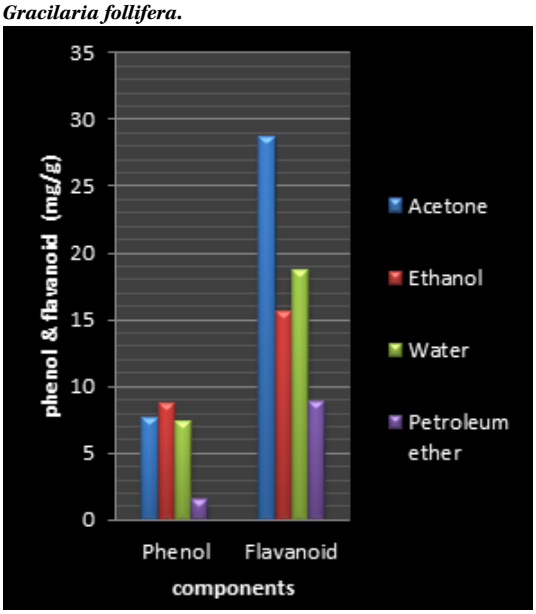


Fig 4- Total Phenolic Content and total flavonoid Content of *Gracilaria follifera*.



In *Gracileria follifera*,the maximum phenol content was recorded in ethanol extract (8.79 ± 0.13mg/g) and minimum in petroleum ether (1.54 ± 0.44 mg/g).The flavonoid content analysed and ranged from 28.66 ± 0.02mg/g to 8.83 ± 0.004mg/g.The maximum content recorded in acetone extract (28.66 ± 0.02mg/g) and minimum in petroleum ether extract (8.83 ± 0.004mg/g).

Table 4. DPPH-free radical scavenging activity of Acetone seaweed extract

Sl. No	Concentration (µg/ml)	% of activity (± SD)		
		Standard (Ascorbic acid)	Gracilaria corticata	Gracilaria follifera
1	100	90.5± 0.70	59.5 ± 0.70	52.21 ± 0.71
2	200	94.21 ± 0.70	62.62 ± 2.12	53.41 ± 1.41
3	300	96.42 ± 0.77	64.21 ± 0.71	56.20 ± 0.71
4	400	97.28 ± 0.64	65.00 ± 0.05	59.83 ± 2.82
5	500	98.20 ± 0.05	66.68 ± 0.03	60.03 ± 0.04

In *Gracilaria corticata*, the maximum value of DPPH-free radical scavenging activity of acetone and ethanol seaweed extract were recorded as 66.68 ± 0.03% and 51.58 ± 0.02% respectively at concentration 500µg/ml and the minimum value recorded as 59.5 ± 0.70% and 43.21 ± 0.70 % respectively at concentration 100µg/ml.(Table-4&5, Fig-5)

Table 5. DPPH-free radical scavenging activity of Ethanol seaweed extract

SL. NO	Concentration (µg/ml)	% of activity (± SD)		
		Standard (Ascorbic acid)	Gracilaria corticata	Gracilaria follifera
1	100	60 ± 1.41	43.21 ± 0.70	27.62 ± 2.12
2	200	62.5 ± 0.70	46.41 ± 1.41	28.41 ± 1.41
3	300	63.90 ± 0.14	49.41 ± 1.41	29.62 ± 2.12
4	400	64.5 ± 0.70	50.41 ± 1.41	32.41 ± 1.41
5	500	65.00 ± 0.05	51.58 ± 0.02	34.29 ± 0.30

In *Gracilaria follifera* the maximum value of DPPH-free radical scavenging activity of acetone and ethanol seaweed extract were recorded as 60.03 ± 0.04% and 34.29 ± 0.30%respectively at concentration 500µg/ml and the minimum value recorded as 52.21 ± 0.71 % and 27.62 ± 2.12% respectively at concentration 100µg/ml.(Table-4&5,Fig-6).

DPPH-free radical scavenging activity of water seaweed extract of *Gracilaria corticata* reported highest value was 60.07 ± 0.01% at 500µg/ml concentration and lowest value was 50.85 ± 0.79% at 100µg/ml concentration. In *Gracilaria follifera* reported highest value was 40.33 ± 0.46 % at concentration 500µg/ml and lowest value was 32.38 ± 1.97 % at concentration 100µg/ml. (Table-6, Fig-5&6).

Table 6. DPPH-free radical scavenging activity of Water seaweed extract

SL. NO	Concentration (µg/ml)	% of activity (± SD)		
		Standard (Ascorbic acid)	Gracilaria corticata	Gracilaria follifera
1	100	65.41 ± 1.41	50.85 ± 0.79	32.38 ± 1.97
2	200	65.83 ± 1.41	52.29 ± 1.46	34.38 ± 1.004
3	300	67.85 ± 1.20	55.43 ± 1.96	36.008 ± 0.013
4	400	69.30 ±2.05	57.59 ±0.94	38.10 ± 0.35
5	500	71.47 ± 3.32	60.07 ± 0.01	40.33 ± 0.46

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Table 7. DPPH-free radical scavenging activity of Petroleum ether seaweed extract

SL. NO	Concentration (µg/ml)	% of activity (± SD)		
		Standard (Ascorbic acid)	Gracilaria corticata	Gracilaria follifera
1	100	90.03 ± 1.48	42.07 ± 0.91	18.41 ± 1.41
2	200	92.30 ± 1.40	43.63 ± 1.83	20.41 ± 1.12
3	300	92.36 ± 1.91	44.07 ± 0.91	22.51 ± 1.43
4	400	95.23 ± 0.78	46.26 ± 0.37	23.67 ± 0.91
5	500	96.82 ± 2.12	47.28 ± 1.63	25.66 ± 0.02

DPPH-free radical scavenging activity of petroleum ether seaweed extract of *Gracilaria corticata* reported highest value was 47.28 ± 1.63 % at concentration 500µg/ml and lowest value was 42.07 ± 0.91% at concentration 100µg/ml. In *Gracilaria follifera* reported highest value was 25.66 ± 0.02 % at concentration 500µg/ml and lowest value was 18.41 ± 1.41% at concentration 100µg/ml. (Table-7, Fig-5&6).

Fig 5:- DPPH radical activity of Gracilaria corticata in different solvent extract.

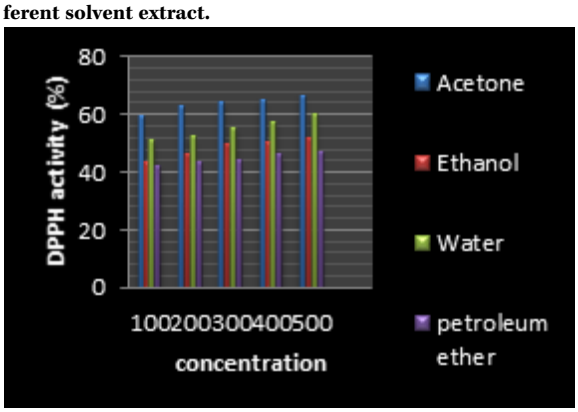
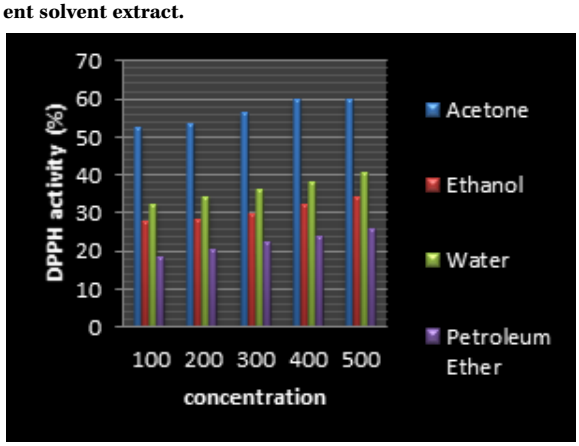


Fig 6:- DPPH radical activity of *Gracilaria follifera* in different solvent extract.



DISCUSSION

Marine algae are among the richest sources of known and novel bioactive compounds. In the case of Gracilaria corticata the result of the phytochemical analysis of various solvent extracts revealed the presence of alkaloids in ethanol, flavonoids and phenols in acetones, coumarins in ethanol and cardiacglycosides in water and petroleum ether, terpenoids in ethanol and saponins in water.

In Gracilaria follifera, the abundance of various solvent extracts revealed the presence of alkaloids, flavonoids and phenols in ethanol, coumarins and cardiacglycosides in petroleum ether and water respectively.In both species saponins are absent in petroleum ether extract.

Seaweed extracts are considered to be a rich source of phenolic compounds. The seaweeds known as medical are rich in secondary metabolites which include alkaloids, glycosides, flavanoids, saponins, tannins and steroids. In the present study maximum protein content was recorded in the petroleum ether extract of Gracilaria follifera and minimum in the water extract of Gracilaria corticata maximum carbohydrate was recorded in the acetone extract of Gracilaria follifera. In Gracilaria corticata phenol content is highest in ethanol and lower in water. In Gracilaria follifera maximum phenol content is noticed in ethanol extract and minimum in petroleum ether extract.

In the present study flavonoid content gives same result in both species (Gracilaria corticata and Gracilaria follifera)

Maximum value is recorded in acetone extract and minimum in petroleum ether extract. DPPH radical scavenging activity in acetone extract of Gracilaria follifera and petroleum ether extract of Gracilaria corticata gives higher free radical scavenging activity. The free radical scavenging activity increased as the concentration increased in all the extracts.

Total phenol content and radical scavenging activity of Gracilaria follifera did not show a clear correlation, however samples with a higher phenol showed a higher scavenging activity. An inverse relationship between phenolic content and DPPH free radical from the extract of Gracilaria corticata indicated that in this study the activity could be due to the presence of phenolics.

The DPPH radical scavenging activity of various extract demonstrated its oxygen radical absorbance capacity and indicated its potent antioxidant nature. A positive correlation has been documented between antioxidant capabilities and total flavonoid content for both algae, but not with the content of phenol. All the two species were found to have good antioxidant capacities and thus could be potential rich sources of natural antioxidants.

The bioactive potentials of *Gracilaria* species collected from Thikkodi region were evaluated. All the two species were found to have good antioxidant capacities and thus could be potential rich sources of natural antioxidants. Therefore it is suggested that further works may be performed on these least studied species for its industrial applications.

CONCLUSION

The present work deals with the study of phytochemical, biochemical and antioxidant parameters on macro algae. Phytochemical parameters considered include alkaloids, steroids, flavonoids, phenol, saponins, cardioglycosides, terpenoids, tannins, coumarins and biochemical constituents, proteins and carbohydrates. The macro algae were also analysed for the DPPH free radical scavenging activity.

The present study gives the result that *Gracilaria corticata* and *Gracilaria follifera* contain all the phytochemicals, but their rate of presence differ in different solvents.Most phytochemicals shows absence in petroleum ether solvent. This is because the algae are less soluble in petroleum ether. Compared to *Gracilaria corticata*, protein content and carbohydrate are rich in *Gracilaria follifera*. Both of the algae show DPPH free radical scavenging activity. Hence it can be used in medical field.

Fig 7:- Gracilaria corticata.

Fig 8:- Gracilaria follifera.

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