

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(12)60275-5 ©2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Preliminary Phytochemical, UV–VIS, HPLC and Anti–bacterial Studies on *Gracilaria corticata* J. Ag

Krishnaveni Eahamban¹, Johnson Marimuthu @ Antonisamy^{1*}

¹Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

ARTICLE INFO

Article history:

Received 15 April 2012

Received in revised form 27 April 2012

Accepted 8 August 2012

Available online 28 August 2012

Keywords:

Phytochemistry

HPLC

UV–VIS

Bio–efficacy

Gracilaria corticata

ABSTRACT

Objective: The present study aimed to investigate the preliminary phytochemical analysis and UV–VIS, HPTC profiling and the antibacterial activity of *Gracilaria corticata* J. Ag extracts against the Gram positive and Gram negative bacteria. **Methods:** Preliminary phytochemical screening was carried out by Harborne method. The *G. corticata* extracts were tested against bacteria by the agar disc diffusion method. **Results:** The results of the presence study showed the presence of alkaloids, steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids, glycosides and sugars. Proteins, xantoproteins, coumarins and catechin did not show any positive result for their presence in any of the six extracts of *Gracilaria corticata* tested. The result of the present study revealed the various behavior character of *Gracilaria corticata* crude drug. The UV–VIS spectrum profile of *Gracilaria corticata* methanolic, petroleum ether, benzene and aqueous extracts profiles were recorded. The HPLC profile of *Gracilaria corticata* petroleum ether benzene and aqueous extracts were tabulated. The maximum (9/12 bacterial pathogens) degree of antibacterial activity was observed in isopropanol soxhlet extracts followed by isopropanol cold extracts (7/12 bacterial pathogens). **Conclusion:** The results of the present study showed that *G. corticata* may be rich sources of phytoconstituents which can be isolated and further screened for different kinds of biological activities, depending on their reported therapeutic uses.

1. Introduction

Seaweeds are one of the most important marine resources of the world and being used as human food, animal feed and raw material for many industries. For centuries, seaweed has been of botanical, industrial and pharmaceutical interest. In recent years research on the chemistry of seaweeds (or more generally marine organisms) has experienced a tremendous increase due to the need for compounds possessing bioactivities of possible pharmaceutical applications or other potential economic properties. Since marine organisms live in a significantly different environment from those of terrestrial organisms, it is reasonable to suppose that their secondary metabolites will differ considerably. After more than 25 years of fruitful research, marine natural product chemistry must now be considered to be approaching maturity. Seaweeds offer a wide range of therapeutic possibilities both internally and externally. Seaweeds are extensive profile source of secondary metabolites. Although

a majority of these (about 60%) are terpenes, but some fatty acids are also common (20%) with nitrogenous compounds. More recent reports indicate that seaweed is still employed in folk medicine in many parts of the world as treatments of a variety of diseases. The Japanese and Chinese have used seaweeds to treat goiter and other glandular problems since 300 BC. The Romans used them in the treatment of wounds, burns and rashes. To date, there are quite a lot of reports on antibacterial activity of solvent extracts from marine algae. However, there are very few reports pertaining to antifungal activity of crude solvent extracts from the seaweeds representing Phaeophyceae and Rhodophyceae [1]. Seaweeds have been considered as potential source of marine medicinal including antimicrobial, cancer therapies hypocholesterolemic and anthelmintic substances. Many scientists also reported antimicrobial activities in marine algae [2–5]. Several compounds from the ocean show pharmacological activities and bioactive compounds, primarily for treating deadly diseases like cancer, Acquired Immuno Deficiency Syndrome (AIDS) Arthritis etc., while some compounds have been used to treat inflammation etc. Marine algae are continuously exposed to many biotic and

*Corresponding author: Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

E-mail: ptcjohnson@gmail.com

Tel: +91 97869 24334; Fax: +91 462 2561765

abiotic pressures which influence the organism's physiology, which in turn leads to the production of multifunctional natural secondary metabolites. So far, more than 2400 SSM are described and many of the seaweeds secondary metabolites (SSM) are natural blueprints for the development of new drugs [6]. Several of these compounds are constitutive, existing in biologically active forms in healthy seaweeds. The major secondary metabolites produced by seaweeds are halogenated compounds displaying anti-bacterial, anti-fungal, anti-viral, anti-fouling and anti-feedent properties. Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of microorganisms. Although a number of phytochemical and bioefficacy studies were carried out at global level, only few reports are available on the bio-potential and biochemical studies on the seaweeds from Gulf of Mannar and Peninsular coast of India [7–9]. To fulfill the lacuna, the present study was aimed to explore phytochemical constituents present in *Gracilaria corticata* J. Ag and to screen antimicrobial activity of *G. corticata* extracts by using disc diffusion method.

2. Materials and Methods

2.1 Collection and Extraction

Gracilaria corticata J. Ag were collected by handpicking from the coast of Rasthacaud (Lat N 08008'308'' E77032'80'') Kanyakumari District, Tamil Nadu, India. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. Shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

2.1.1. Hot Extraction

To compare the hot and cold extraction, the dried and powdered materials (5 g) were extracted successively with 250 ml of petroleum ether, methanol, chloroform, acetone, benzene, isopropanol and water by using soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered by using Whatman filter paper (No.1) and then concentrated in vacuum at 40°C using Rotary evaporator. The residues obtained were stored in a freezer -20°C until further tests.

2.1.2. Cold Extraction

2 g of air dried powder of sample was extracted with 50 ml of solvents viz., ethanol, acetone, petroleum ether, chloroform, benzene and water for 72 h. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

2.2. Preliminary Phytochemical Analysis

The different extracts were tested for steroids, triterpenoids, reducing sugars, phenolic compounds, saponins, xanthoproteins, tannins, flavonoids, saponin, protein, glycosides and anthroquinones. Phytochemical screening of the extracts was carried out according to the standard methods [10–13].

2.3. Proximate Analysis

The extracts were examined under visible and UV light. These powdered materials were also treated with various reagents such as 50% nitric acid, acetone, ethanol, 50% sulphuric acid, 1N HCL and 1N NaOH and changes in colour were recorded.

2.4. UV-VIS spectrophotometer and HPLC analysis

The extract was centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The crude extracts containing the bioactive compound was analyzed spectroscopically for further confirmation. To detect the UV-VIS spectrum profile of the crude extracts of *Gracilaria corticata*, the extracts were scanned in the wavelength ranging from 200 – 1100 nm by using Shimadzu Spectrophotometer and the characteristic peaks were detected. HPLC method was performed on a Shimadzu LC-10 AT VP HPLC system, which was equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 µl loop and auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250 mm, 5 µm size) with a C-18 guard column was used. An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC-10 AT VP pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and reverse phase Luna 5°C 18 (2) Phenomenex column (250mm X 4.6mm) was used. The mobile phase components methanol: water (45:55) were filtered through 0.2 µm membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260–270 kgf/cm². The column temperature was maintained at 27°C. 20 µl of respective sample was injected by using Rheodyne syringe (Model 7202, Hamilton). The elution was carried out with gradient solvent systems with a flow rate of 1 ml min⁻¹ at ambient temperature (25–28°C). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µl while the wavelength of the UV-Vis detector was set at 254 nm [14, 15].

2.5. Anti-bacterial activity of the *G. corticata* extracts

The petroleum ether, methanol, chloroform, acetone, benzene, isopropanol and aqueous extracts of *Gracilaria*

corticata J. Ag. were tested against the selected pathogens viz., *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, *Serratia marcescens*, *Proteus mirabilis*, *Enterococcus faecalis* and *Streptococcus pyogenes*. Microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India. The antimicrobial activity was tested through disc diffusion method [16]. Nutrient agar was used as the standard test medium for the present study. Fresh cultures were prepared and used to inoculate 50 ml of Nutrient broth that was incubated at 35 °C for 18 h. Overnight broth cultures were prepared, adjusted in peptone–physiological salt solution (1 g peptone and 8.5 g/l NaCl) to yield approximately 106 bacteria/ml. The agar plates were prepared in 90 mm Petri dishes with 22 ml of agar medium giving a final depth of 3 mm. Different solvents extracts of *Gracilaria corticata* (125 µg/ml) loaded discs were placed on the inoculated agar surfaces. Solvents (75%) were used as negative control. Amikacin (30 µg) was used as positive controls. All plates were aseptically incubated at 37 °C for 18–24 h. The antimicrobial activity was estimated by measuring the radius of the inhibition zone (mm). Each test was performed in triplicate and the results were shown as means.

3. Results

By preliminary phytochemical screening, thirteen different chemical compounds (steroids, alkaloids, phenolic groups, saponins, tannin, flavonoids, anthraquinone, carbohydrates, carboxylic acid, coumarins, proteins, sugars and xantoproteins) were tested for their presence or absence in six different extracts of *Gracilaria corticata*. Thus out of (2 x 6 x 13 = 156) tests for the presence or the absence of the above compounds, 53 tests gave positive results and the remaining 103 gave negative results. The 53 positive results showed the presence of alkaloids, steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids, glycosides and

sugars. Proteins, xantoproteins, coumarins and catechin did not show any positive result for their presence in any of the six extracts of *Gracilaria corticata* tested. In the soxhlet extracts (6) of *Gracilaria corticata* steroids show the maximum presence in six different extracts followed by alkaloids and sugar in 4 extracts, phenol, saponin and tannin in 3 different extracts. Among the six different extracts, aqueous, benzene, methanol and petroleum ether extracts showed the presence of maximum number (5) of compounds. Next to that, chloroform and isopropanol extracts showed the presence 3 compounds (Table –1). Tannin showed the maximum presence in five different extracts of *Gracilaria corticata* followed by steroid, alkaloid, saponin and sugar in 4 extracts, phenol in 3 different extracts. Among the six different extracts, aqueous, benzene and petroleum ether extracts showed the presence of maximum number (5) of compounds. Next to that, isopropanol, chloroform and methanol extracts showed the presence 4 compounds (Table –1).

The behavior of the drug powder with different chemical reagent will also be helpful in characterization of the crude drug. In the present study, fluorescence analysis of *Gracilaria corticata* was carried out. The result of the present study revealed the various behavior character of *Gracilaria corticata* crude drug. The result of the present study depicted in the Table 2. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. In the present investigation the extractive values for *G. corticata* were produced. In addition the behavior of the drug powder with different chemical reagent also produced. It will be helpful in characterization of the crude drug and identify and classify the seaweeds.

The qualitative UV–VIS profile of *Gracilaria corticata* methanolic extract was taken at the wavelength of 300 nm to 1000 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 972, 866, 650, 600 and 336 nm with the absorption 0.068, 0.026, 0.199, 0.125 and 2.147 respectively (Table –3). The qualitative UV–VIS

Table 1
Preliminary Phytochemical Studies on *Gracilaria corticata* J. Ag

Compounds	<i>G. corticata</i> – Soxhlet (Hot)						<i>G. corticata</i> – Cold						Total
	C	M	I	B	Aq	P	C	M	I	B	Aq	P	
Alkaloids	+			+	+	+	+			+	+	+	8
Phenols		+	+	+				+	+	+			6
Flavonoids		+						+					2
Saponins				+	+	+	+			+	+	+	7
Proteins													0
Terpenoids		+						+					2
Steroids	+	+	+	+	+	+	+			+	+	+	10
Tannins				+	+	+		+	+	+	+	+	8
Xanthoprotiens													0
Catechin													0
Glycosides			+						+				2
Coumarins													0
Sugars	+	+			+	+	+		+		+	+	8
Total	3	5	3	5	5	5	4	4	4	5	5	5	53

C– Chloroform; B – Benzene; Aq– Aqueous; M– Methanol; I – Isopropanol; P– Petroleum ether

fingerprint profile of *Gracillaria corticata* petroleum ether extract (Table -3) was taken at wavelength from 400 nm to 1000 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 926, 668, 610 and 410 nm with the absorption 0.041, 0.55, 0.176 and 1.493 (Table -3). The qualitative UV-VIS fingerprint profile benzene extract of *Gracillaria corticata* (Fig. 1; Table -3) was picked a wavelength from 400 to 700 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at the nm of 670, 610 and 416 with the absorption 0.347, 0.118 and 1.841 respectively. The qualitative UV-VIS fingerprint profile of *Gracillaria corticata* aqueous extract was selected at the wavelength from 200 to 400 nm due to the sharpness of the peak and proper baseline. The profile showed the peaks at the nm of 302 with the absorption of 1.653 (Table -3).

The qualitative HPLC fingerprint profile of *Gracillaria corticata* petroleum ether extract was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at a retention time of 2.647 min and 4.080 min (Fig. 1). The qualitative HPLC fingerprint profile of *Gracillaria corticata* benzene extract was taken at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. The profile showed one prominent peak at a retention time 2.637 min and some moderate peaks also observed at a retention time 4.063 min, 5.9 min and 7.180 min (Fig. 2). Aqueous extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in the *Gracillaria corticata*. Four peaks with different retention time (1.950, 2.130, 2.417 and 2.637) were observed (Fig. 3).

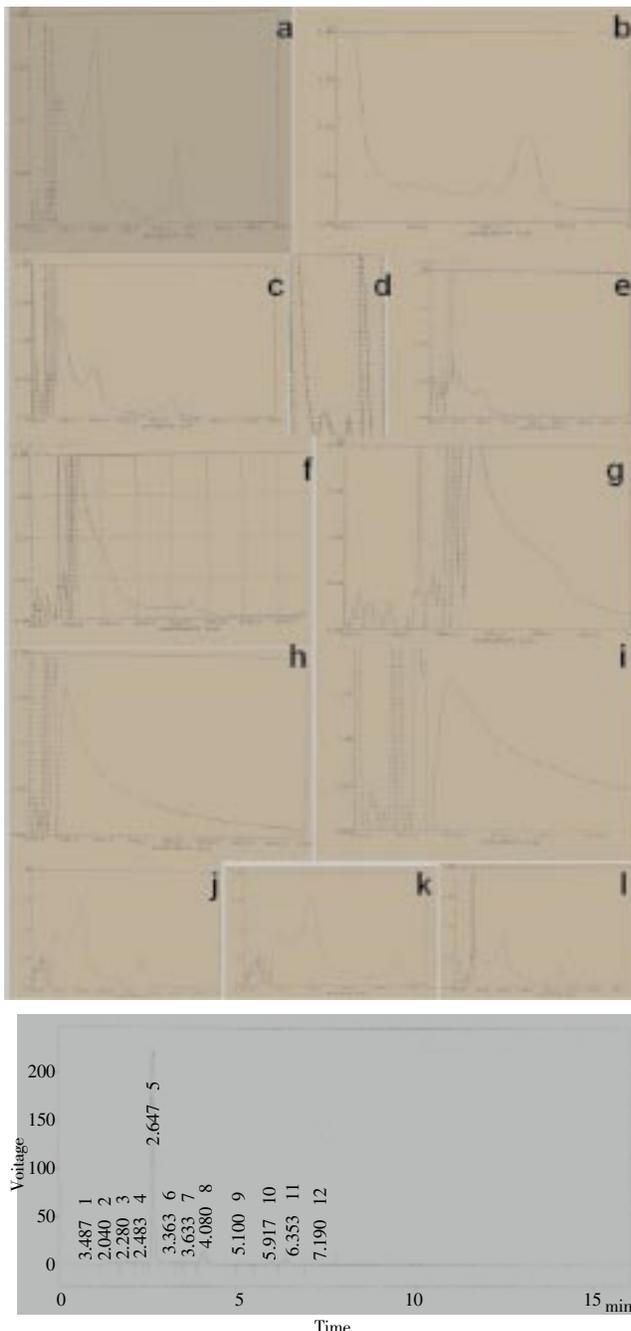


Fig. 1: HPLC profile of *Gracillaria corticata* J. Ag. - Petroleum ether Soxhlet Extract

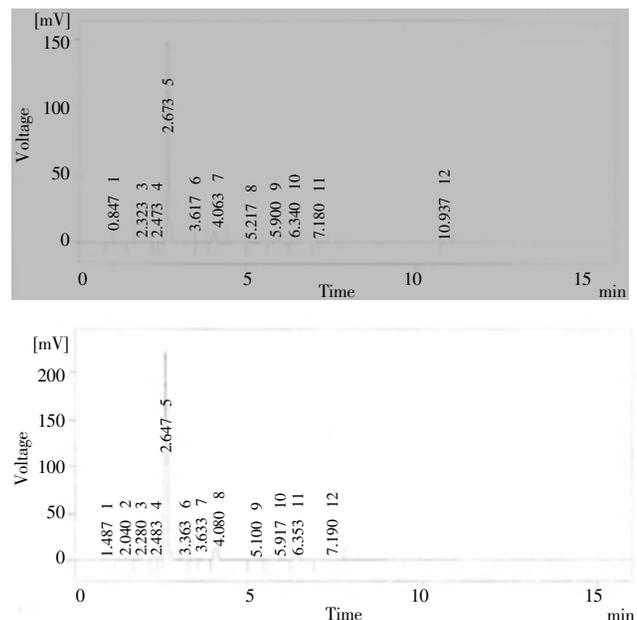


Fig. 2: HPLC profile of *Gracillaria corticata* J. Ag. - Benzene Cold Extract

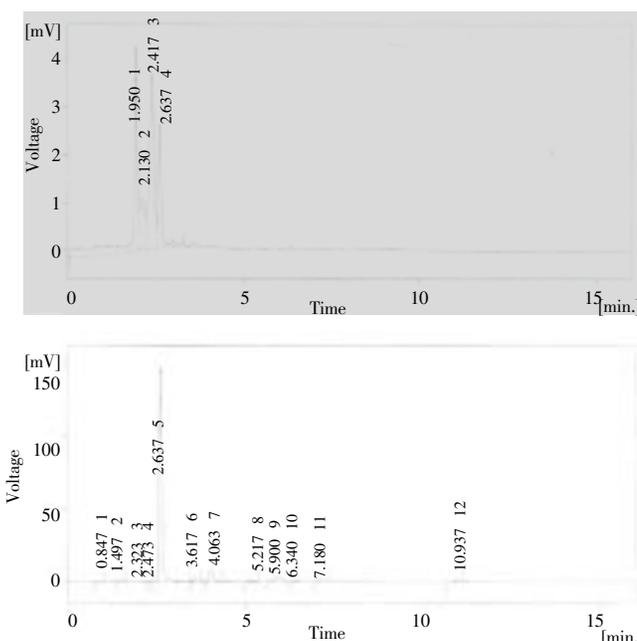


Fig. 3: HPLC profile of *Gracillaria corticata* J. Ag. - Aqueous Cold Extract

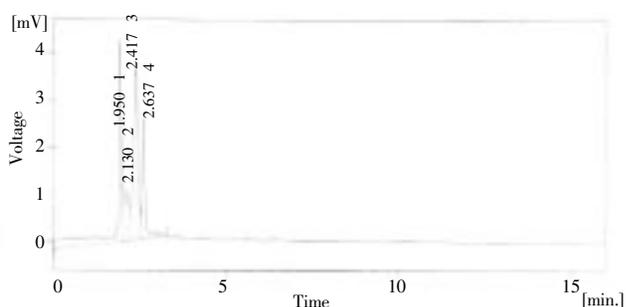


Fig. 4: HPLC profile of *Gracillaria corticata* J. Ag. - Aqueous Cold Extract

Antibacterial activities of crude extracts *Gracillaria corticata* were determined by paper disc diffusion method and the results are summarized in Table 4. The methanolic, chloroform, isopropanol, benzene and petroleum ether extracts were tested for antibacterial activity against twelve human bacterial pathogens. The maximum

(9/12 bacterial pathogens) degree of antibacterial activity was observed in isopropanol soxhlet extracts followed by isopropanol cold extracts (7/12 bacterial pathogens). Next to that methanolic extracts (cold and soxhlet) showed the antibacterial activity (5/12 bacterial pathogens). The isopropanol soxhlet extract of *G. corticata* showed the antibacterial activity against nine pathogens viz., *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *P. mirabilis*, *S. typhi*, *Acinetobacter* sps, *E. faecalis* and *B. cereus* with the inhibition zones 9 mm, 8 mm, 13 mm, 9 mm, 11 mm, 11 mm, 9 mm, 13 mm, 9 mm and 9 mm respectively (Table - 4).

4. Discussion

The m- etabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites, which are not found in terrestrial environments.

Table 2

Fluorescence Analysis of *G. corticata*

Solvents	<i>G. corticata</i> – Sox		<i>G. corticata</i> – Cold	
	Ordinary	UV	Ordinary	UV
Chloroform	Colour less	LG	YG	FG
Benzene	Y	LG	LY	FG
Methanol	YG	FG	YG	FG
Isopropanol	DG	DG	LG	colourless
Aqueous	Colour less	Colourless	Colourless	colourless

Table 3

UV–VIS Peak Values of Different Extracts of *Gracillaria corticata* J. Ag.

Solvents S. No.	Aqueous		Pet. Ether		Methanol		Benzene	
	λ	Abs	λ	Abs	λ	Abs	λ	Abs
1	302	1.653	926	0.041	972	0.068	670	0.347
2			668	0.55	866	0.026	610	0.118
3			610	0.176	650	0.199	416	0.841
4			410	1.493	600	0.125		
5					336	2.147		

Table 4

Antibacterial Activity of *Gracillaria corticata* J. Ag

Name of the Microorganism	Zone of Inhibition in mm								
	C Sox	M Sox	I Sox	C Cold	I Cold	B Cold	P Cold	M Cold	Amikaxin
<i>E. coli</i>	11	9	9	7	8		10	14	21
<i>K. pneumoniae</i>	16	7	8	11	7	9	8	8	25
<i>P. aeruginosa</i>		7	13			9			27
<i>S. aureus</i>	9		9	8	6		7		18
<i>P. mirabilis</i>			11			11	6		23
<i>Serratia</i> sps		7			10	7			29
<i>S. typhi</i>	8		11	8	8				20
<i>Acinetobacter</i> sps			9		13			9	25
<i>E. aerogenes</i>					10		5	8	20
<i>E. faecalis</i>			13		8				13
<i>S. pyogenes</i>	11	43		9		8			22
<i>B. cereus</i>	9		9	9		7	7	9	22
Total	6	5	9	6	7	6	6	5	

C - Chloroform; M - Methanol; I - Isopropanol; B - Benzene; P - Petroleum ether; Sox - Soxhlet extract; Cool - Cool Extract, Control - Amikaxin.

Thus, marine algae are among the richest sources of known and novel bioactive compounds [17]. The result of the phytochemical analysis of various solvent extracts revealed the presence of alkaloids, glycosides, saponins, steroids, phenols and Tannins in *G. corticata*. In the present study cold extraction and soxhlet extraction were implemented for the extraction. The result of the present study confirmed the previous observations and the soxhlet extracts showed more metabolites than the cold extraction. We observed that the sedimentation and turbidity rate of was high in the cold extracts compared to soxhlet extracts. The seaweeds known as medicinal, are rich in secondary metabolites which includes alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Tannins are polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. They are used in pharmaceutical preparations because of their astringent action. Tannins are known to possess general antimicrobial and antioxidant activities [18]. At low concentration tannins can inhibit the growth of microorganisms, and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism. Recent reports show that tannins may have potential value as cytotoxic and/or antineoplastic agents. Aside from the use of tannins as antimicrobial agents or prevention of dental caries, they are now being used in the manufacture of plastics, paints, ceramics and water softening agents. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications. In the present study we revealed the tannins presence in *G. corticata* extracts (Table - 1). The result of the present study suggest the *G. corticata* can be used as antiviral, antibacterial, anti-inflammatory, antiulcer and antioxidant and to treat ulcer, inflammations etc. Flavonoids, (a large group of naturally occurring plant phenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones), also known as nature's tender drugs, possess numerous biological/ pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective, and cytotoxic activities of flavonoids have generated interest in studies of flavonoid-containing plants. Of these biological activities, the anti-inflammatory capacity of flavonoids has long been utilized in Chinese medicine and the cosmetic industry as a form of crude plant extracts [19-21]. The presence of flavonoids in methanol extracts of *G. corticata* suggests that the seaweeds can be used as antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective, and cytotoxicity agent. The methanol extracts of *G. corticata* showed the presence of triterpenes. Triterpenoids have a range of unique and potentially usable biological effects and reference to the use of plants with high saponin / triterpenoid content can be found in the first written herbarium. Triterpenoids are studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. In the present study

the terpenoid presence was confirmed in the methanolic extracts of *G. corticata*. The results suggested that the methanolic extracts of the seaweed can be used as anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory agent. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce inhibitory effect on inflammation. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutraceuticals. Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, anti-inflammatory, anti-feedent, and hemolytic effects. The result of the present study revealed the presence of saponin in *G. corticata* benzene, aqueous, petroleum ether and chloroform extracts and hint that the seaweed can be used as an antimicrobial agent in the near future. Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties [22-23]. Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae.

The crude extract prepared by soxhlet apparatus and cold extraction was subjected to UV - VIS and HPLC for the identification of constituents present in crude extracts of *G. corticata*. HPLC identification test are required to confirm the presence of the active constituents and potential adulterant in ayurvedic drugs. In the present study, the UV - VIS and HPLC profile for the *G. corticata* was evolved. Thus the present studies on *G. corticata* exhibited novel markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs. Fluorescence, UV-VIS and HPLC analysis can be used as effective markers in identifying authentic from its adulterants. Therefore using newer analytical techniques as markers can be generated for the researches as a chain of markers for use of the common man to evaluate the quality of herbal drug and also incorporated in pharmacopoeias.

Rao et al [24] studied the antibacterial activity of the crude extracts of *Ulva compressa*, *Padina gymnospora*, *Sargassum wightii*, and *Gracillaria corticata*. They observed that the crude extracts were active against Gram-positive cultures of *Bacillus*. The present study result directly coincided with Rao *et al.*, [24] observations and supplemented that the crude extracts of *Gracillaria corticata* showed antibacterial activity against other bacterial pathogens also (Table - 4). The variation in the effectiveness of the extract against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. It has been suggested that the antimicrobial activity is mainly due to the presence of flavanoids, saponins, tannins, alkaloids, phenols, steroids and triterpenoids and other natural polyphenolic compounds or free hydroxyl groups. Present work revealed the presence of moderate antimicrobial activity in almost in all the seaweed extract with varied degree and was compared to the standard drugs used. Bacterial infection causes high rate of mortality in human population and aquaculture organisms. For an example, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like

mastitis, abortion and upper respiratory complications, while *Salmonella* sp. causes diarrhea and typhoid fever [25–28]. *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. Nowadays, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. It becomes a greater problem of giving treatment against resistant pathogenic bacteria. Moreover the cost of the drugs is high and also they cause adverse effect on the host, which include hypersensitivity and depletion of beneficial microbes in the gut. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives. Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from sea-weeds and used in medicine and pharmacy. The present study suggests that the *G. corticata* can be used to treat diseases like mastitis, abortion and upper respiratory complications, diarrhea and typhoid fever.

Conclusion

The above-mentioned results show that the *Gracilaria corticata* J. Ag examined may be rich sources of phytochemicals particularly flavonoids, triterpenes, steroids, tannins, alkaloids, phenol and glycosides, which can be isolated and further screened for different kinds of biological activities, depending on their reported therapeutic uses. Quantitative analyses of these phytochemicals may also be done to guide the researchers on which particular bioactive class of compounds may be subjected to subsequent target isolation.

References

- [1] Manilal A, Sujith S, Selvin J, Shakir C, Kiran GS. Antibacterial activity of *Falkenbergia hillebrandii* (Born) from the Indian coast against human pathogens. *FYTON* 2009; **78**: 161–166
- [2] Salvador N, Gomez-Garreta A, Lavelli L, Ribera L. Antimicrobial activity of Iberian macroalgae. *Sci. Mar.* 2007; **71**: 101–113.
- [3] Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran GS, Natarajaseenivasan K. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Annals of Microbiology* 2008; **58** (3): 535–541.
- [4] Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C, Gandhimathi R, Lipton AP. Antimicrobial potential and seasonality of red algae collected from the southwest coast of India tested against shrimp, human and phytopathogens. *Annals of Microbiology* 2009; **59** (2): 207–219.
- [5] Manivannan K, Karthikai devi G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. *Asian Pacific Journal of Tropical Biomedicine* 2011; 114–120.
- [6] El-Baroty GS, Moussa MY, Shallah MA, Ali MA, Sabh AZ, Shalaby EA. Contribution to the aroma, biological activities, minerals, protein, pigments and lipid contents of the red alga: *Asparagopsis taxiformis* (Delile) Trevisan. *J. Appl. Sci. Res.* 2007; **3**: 1825–1834.
- [7] Eluvakkal T, Sivakumr SR, Arunkumar K. Fucoidan in some Indian Brown Seaweeds found along the coast of Gulf of Mannar. *International Journal of Botany* 2010; **6**(2): 176–181.
- [8] Manivannan K, Thirumaran G, Karthikai Devi G, Anantharaman P, Balasubramanian T. Proximate Composition of Different Group of Seaweeds from Vedalai Coastal Waters (Gulf of Mannar): Southeast Coast of India. *Middle-East Journal of Scientific Research* 2009; **4** (2): 72–77.
- [9] Manivannan K, Thirumaran G, Karthikai Devi G. Biochemical Composition of Seaweeds from Mandapam Coastal Regions along Southeast Coast of India. *American-Eurasian Journal of Botany* 2008; **1** (2): 32–37.
- [10] Shyamala Gowri S, Vasantha K. Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts. *International Journal of PharmTech Research* 2010; **2**(2): 1569–1573.
- [11] Ngbede J, Yakubu RA, Njam DA. Phytochemical Screening for active compounds in *Cornarium schweinfurthii* leaves from Jos North, Plateau state. *Nigeria Res J Biol. Sci.* 2008; **3**(9): 1076–1078.
- [12] Onwukeame DN, Ikuegbvweha TB, Asonye CC. Evaluation of phytochemical constituents antibacterial activities and effects of exudates of *Pycanthus angolensis* weld warb on corneal ulcers in rabbit. *Trop. J. Pharm. Res.* 2007; **6** (2): 725–730.
- [13] Aparna Saraf. Phytochemical and Antimicrobial Studies of Medicinal Plant *Costus speciosus* (Koen.) *E-Journal of Chemistry* 2010; **7**(S1): S405–S413.
- [14] Sharanabasappa GK, Santosh MK, Shaila D, Seetharam YN, Sanjeevarao I. Phytochemical Studies on *Bauhinia racemosa* Lam. *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. *E-Journal of Chemistry* 2007; **4**(1):21–31.
- [15] Mallikharjuna PB, Rajanna LN, Seetharam YN, Sharanabasappa GK. Phytochemical Studies of *Strychnos potatorum* L. f. – A Medicinal Plant. *E-Journal of Chemistry* 2007; **4**(4): 510–518.
- [16] Paul Raj K, Irudayaraj V, Johnson M, Patric Raja D. Phytochemical and anti-bacterial activity of epidermal glands extract of *Christella parasitica* (L.) H. Lev. *Asian Pacific Journal of Tropical Biomedicine* 2011; **1**(1): 8–11.
- [17] Blunt JW, Copp BR, Hu WP, Munro MHC, Northcote PT, Prinsep MR. Marine natural products. *Nat. Prod. Rep.* 2007; **24**: 31–86.
- [18] Rievere C, Van Nguyen JH, Pieters L, Dejaegher B, Heyden YV, Minh CV, Quetin-Leclercq J. Polyphenols isolated from antiradical extracts of *Mallotus metcalfeanus*. *Phytochemistry* 2009; **70**: 86–94.
- [19] Veitch NC. Isoflavonoids of the Leguminosae. *Nat. Prod. Rep.* 2007; **24**: 417–464.
- [20] Jiang H, Zhan WQ, Liu X, Jiang SX. Antioxidant activities of extracts and flavonoid compounds from *Oxytropis falcate* Bunge. *Nat. Prod. Res.* 2008; **22**(18): 1650–1656.
- [21] Wu JH, Tung YT, Chien SC, Wang SY, Kuo YH, Shyur LF, Chang ST. Effect of Phytocompounds from the Heart-wood of *Acacia confusa* on Inflammatory Mediator Production. *J. Agric. Food Chem.* 2008; **56**: 1567–1573.
- [22] Kuda T, Kunii T, Goto H, Suzuki T, Yano T. Varieties of antioxidant and antibacterial properties of *Ecklonia stolonifera* and *Ecklonia kurome* products harvested and processed in the Noto peninsula. *Japan. Food Chemistry* 2007; **103**: 900–905.
- [23] Wang T, Jónsdóttir R, Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chemistry* 2009; **116**: 240–248.
- [24] Rao DS, Girijavallabhan KG, Muthusamy S, Chandrika V, Gopinathan CP, Kalimuthu S, Najamuddin M. Bio-activity of marine algae. In: Thompson M–F, Sarojini R, Nagabhusanam R, eds., *Bioactive Compounds from Marine Organisms with Emphasis on the Indian Ocean*. New Delhi, India, *Oxford & IBH Publishing*, 1999; 373–377.
- [25] http://en.wikipedia.org/wiki/Escherichia_coli
- [26] http://en.wikipedia.org/wiki/Staphylococcus_aureus
- [27] http://en.wikipedia.org/wiki/Pseudomonas_aeruginosa
- [28] <http://en.wikipedia.org/wiki/Salmonella>