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Phytochemical screening, RP-HPLC and FTIR Analysis of *Kappaphycus alvarezii* (Doty) Doty EX P.C Silva: Macro red algae

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Abstract
Sea weeds are immense source of bioactive molecules for the exploration of novel drugs. The study was aimed to identify the phytochemicals present in the red algae *Kappaphycus alvarezii.* The sample was collected from the sea coast of Thoothukudi, Tamil Nadu. The different solvent extracts from *K. alvarezii* was found to be rich in phenols and carbohydrate. The carbohydrate content of the methanolic extracts of algal extract was 18.26 mg/g.f.wt, phenol 23.01mg GAE standard, gallic acid equivalent/100 g dry wt. The phytochemical analysis revealed the presence of carbohydrate, protein, alkaloids, glycosides, flavonoids, steroid, phenolic compounds and absence of tannin in the extracts. Also *K. alvarezii* extracts revealed macromolecules contents such as carbohydrate contents of 18.26 g, protein was 11.49 g and fat content of 1.01 g /100 g of dry wt. RP-HPLC analysis showed the presence of a pool of phenolic acids with higher level of sinapic acid followed by phloroglucinol. Subsequently, the different solvent extracts of *K. alvarezii* were subjected to fourier transform infrared spectroscopy for the analysis of functional groups. The results based on spectral data of FTIR revealed the presence of aliphatic constituents containing carbon, ketones, alkyl halides and hydroxyl groups. Thus, the phytochemical analysis of the algae showed immense potential values in terms of primary and secondary metabolites and therefore it can be used as functional and pharmaceutical food.

Keywords: Phytochemicals, phenolic acids, RP-HPLC, FTIR, sea weeds

Introduction
Seaweeds are considered as important marine living resources and are utilized by the humans in different ways. These seaweeds were the main source of phycocolloids, namely agar-agar, carrageenan and algin, which were extensively used in various food, confectionary, textiles, pharmaceuticals, dairy and paper industries. Most commonly these were used as gelling, stabilising and thickening agents [1]. In India seaweeds are found abundantly along the South Eastern and North Eastern parts of the coast. There were approximately 60 known species of seaweeds were commercially important in India out of reported 680 known species. *Kappaphycus alvarezii*, was extensively used by Japanese as edible seaweed for more than centuries. It was also reported that peoples in different part of the world such as New Zealand, Canada, Ireland, Scotland have been consuming seaweeds from ancient times onwards. Some Governmental and commercial organizations exploit seaweeds for animal and human food, soil manure, salt extractions (soda, iodine, etc.), production of colloids, in pharmaceuticals and in cosmetics. This represents an important economy mostly in the south east Asian countries where they are not only largely harvested but also intensively and largely employed for the nutrition of human being [2]. Since these macro algae were nutritionally valuable thy were used as fresh or dried vegetables, salads, or as ingredients in a wide variety of food preparations such as in soups. Sea weeds can play a vital role in various aspects compared to other aquatic resources. These seaweeds can be used to compensate the food crisis for some extent inoder to erect the economy of several countries and to fulfill the malnutrient conditions. There were reports of several biological activities from macro algae derived compounds. It was also reported that the secondary metabolites from many of the seaweeds have been used in traditional medicines for many centuries, due to their therapeutic potentials [3]. Due to various biotic and abiotic pressures faced by these marine algae which influence physiological nature of the cell, that leads to the production of several secondary metabolites for their defense. Several of these metabolites are constitutive, which were existing in biologically active forms. Further, there are several reports on antimicrobial and pharmacological activities of different solvent extracts from marine algae [4]. Many phytochemicals such as alkaloids, flavonoids steroids, glycosides, terpenes, tannins and phenolic, compounds were present in the marine algal extracts. The extracts from various algae seems to be a rich source of phenolic compounds.

The extracts also contain terpenes and fatty acids. They serve as source of medicine, ornamental purposes, flavouring, food additives and preservatives. Now a days FT-IR can be used as a potential tool to unravel the chemical and molecular composition of unknown compounds.

2. Materials and Methods

2.1. Sample preparation

The marine alga *Kappaphycus alvarezii* was collected during April 2015, from the Thoothukudi coast (8° 48' N, 78° 11' E), Gulf of Mannar. The thallus of *K. alvarezii* were first shade dried and cut into pieces, and then powdered in a grinder. The sample powder was then kept in air-tight container for further analysis. The algal extracts were prepared as per the methodology of Indian Pharmacopoeia [5]. 20 g each of dried algal powder were taken in a soxhlet apparatus and extracted successively with 250 ml each of petroleum ether, ethyl acetate and ethanol. The extraction was repeated several times and was then filtered through Whatman filter paper No.1. It was then evaporated at room temperature. The extract so obtained was analyzed for phytochemical screening of compounds, to evaluate the total phenolic content and to determine the functional groups present.

Initially, the different solvent extracts were subjected to qualitative analysis for the identification of phytochemicals present. Subsequently, the total phenol content, protein, sugar were quantified using standard protocols. Various chemical tests were done in different solvent extracts by using the standard procedures as described by [6] for the preliminary analysis of bioactive constituents. Total carbohydrate content of algae was calculated by using DNS reagent and the absorbance was measured at 540 nm with spectrophotometer. The soluble protein was estimated following the protocol of [7]. The amount of protein present in the algal extract was calculated spectrophotometrically at 670 nm after 30 minute using appropriate blank.

2.2. Estimation of total fat

Take 20g of sample. Then the sample was dissolved in 100 ml of water and transferred to a separating funnel subsequently acidified with 1M sulphuric acid. Petroleum ether was then added in different volume. Mixed ether solutions were taken in a separating funnel and washed with distilled water until the washings were free from acid. The ether solution was transferred to a tared flask, removed the ether and dried the residue of fatty acids to constant weight at 80 °C.

2.3. Determination of Total Phenolic Content

The total phenolics content in the selected seaweed was determined with the Folin- Ciocalteu reagent according to the method of [8]. Here used gallic as a standard. The total phenolics so obtained were expressed as mg/g gallic acid equivalents (GAE). The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour which was measured spectrophotometrically at 760 nm.

Also, the Vitamin C was determined by using the procedure as outlined by [9] was done in triplicates and the results were expressed as mg GAE/g of the sample.

2.4. RP - HPLC Analysis

RP-HPLC method was performed on a Shimadzu LC-10 AT VP HPLC system, equipped with a model LC-10AT pump, UV–VIS detector an auto injector SIL-10AT. A Hypersil - BDS C-18 column (4.6 · 250 mm, 5 lm 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 Luna5-C18 (2) Phenomenex column (250 mm 4.6 mm) was used. The mobile phase components were potassium hydrogen phosphate and acetonitrile in a ratio of 75:25were filtered through 0.2 l membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1 ml/min which yielded a column backup pressure of 260–270 kgf/cm^2.The column temperature was maintained at 27 ºC. 50 µl of the sample was then injected by using Rheodyne syringe. The elution was carried out with gradient solvent systems with a flow rate of 1 ml min-1 at an ambient temperature (25–28 ºC). The mobile phase was prepared daily, filtered through a 0.45 lm and sonicated before use. Running time taken was 15 min. Volume of sample for injection was 20 whilst the wavelength of the UV–VIS detector was set at a wavelength of 254 nm [10].

2.5 FT-IR analysis

The FT-IR studies have been followed by the method described by [11]. Dried powders of different solvent extracts of algae were used for FTIR analysis. 10 mg of the dried algal powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs and subjected to a pressure of about 5x106 Pa in an evacuated die to produce a clear transparent disc of diameter 13 mm and thickness 1mm. IR spectra region 4000-400 cm-1 were recorded at room temperature on a perkin Elmer fourier transform spectrometer equipped an air cooled DTGs (deuterated tri glycine sulfate) detector. For each spectrum, 100 scans were CO added at a spectral resolution of 4cm-1. The frequencies for all sharp bands were accurate to 0.01 cm-1.

3. Result and Discussion

Some phytochemical substances present in the plants have medicinal properties that have a finite biological action on the human body metabolisms. Different phytochemicals in these, were reported to have a broad range of biological activities which may help in protection against various diseases. For example, chronic diseases can be prevented by the alkaloids present in the plants. It was also reported that the saponins protect against hypercholesterolemia and also have antibiotic properties. Most of the algal steroids and triterpenoids have the analgesic properties. Central nervous system activities can be induced by steroids and saponins. Phytochemical screenings of the various solvent extracts were used to study the presence of alkaloids, flavonoids, steroids, saponins, glycosides and triterpenoid present in it.

Petroleum ether and ethanolic extract showed strong positivity (++/ +++) for major phytochemicals like reducing sugar, glycosides, alkaloids and terpenoids. However, ethyl actate performed poorly and showed negativity (-) in almost all phytochemicals (Table 1). The phytochemicals seen in the red algae was medicinal such as anti-inflammatory, anti-diabetic and analgesic activities and for central nervous system activity. It has been recently reported that the alkaloids, phenols and glycosides are nowadays used in various antibiotics are used in treating against various pathogenic strains.

Table 1: Qualitative analysis of phytochemicals in different solvent extracts from *Kappaphycus alvarezii*

	Petroleum Ether	Ethyl Acetate	Ethanol
Reducing Sugar	++	+	+
Glycosides	++	-	+
Flavanoids	+	+	++
Alkaloids	++	+	++
Tannins	-	-	-
Terpenoids	+	-	++
Steroids	+	+	+

+++= abundant, ++ = moderate, + = low, - = absent

Carbohydrate content was a major component in dried seaweed (dry weight basis) i.e., 18.26 g/100g DW. The protein content was 11.49 g / 100g DW. These results were also similar to previous studies by other researchers where generally higher carbohydrate and protein contents were found in green and red seaweeds (10% to 47% DW) [12]. The total fat content was found to be 1.01 g /100 g of dry wt which was comparatively low.

The content of total polyphenols was determined and found to be 23.01 mg/g tissue. Previous studies also found that the total phenolic contents varies with species and generally the green seaweeds have higher free-radical scavenging properties, followed by the brown seaweed, then the red seaweeds [13]. Vitamin C was also found to be 7.89 mg/g.f.wt

Results of the FTIR Spectra of the petroleum ether, ethyl acetate and ethanol crude extracts of *K. alvarezii* revealed the presence of different functional groups (Table 2). The FTIR analysis gives peaks at 3535.52, 3512.37, 3454.51, 3402.4 and 3321.42, 3186.40, 3165.19, 3145.90, 3105.39, 3224.98, 3205.69, 3186.40, 3165.19, 3145.90, 3130.447 cm-1, which indicated the presence of OH stretching in ethyl acetate and ethanol fractions depicting the presence of alcohols and phenols. While Peaks obtained at3371.57, 3315.63, 3248.13, 3321.42, 3381.21, 33444.57, 3323.35, 3302.13, 3282.84, 3263.56, 3242.34, 1610.56, 1637.56 cm-1 are N-H bending for primary amines with 952.84, 802.39, 680.87, 673.16, 823.60, 885.33cm-1 N-H wagging that indicated the presence of secondary amines in all of the three extracts. The FT-IR peaks obtained in all the three extracts at 2678.41, 1710.86, 1683.68, 3300.20, 3284.77,3 261.63, 3242.34, 3230.77, 3201.83 showed C=O stretching for carboxylic acids. The double bond =C-H medium stretching of petroleum ether extract and ethanolic extract at 3088.03, 3089.96, 3072.60, 3047.53, 3026.31 indicates the presence of alkenes where as in ethyl acetate extract shows a –C=C- stretch at 1676.14 indicates alkenes presence. Also in these three extracts present were the medium band for Alkanes: C-H stretching at 2920.23, 2850.79, 2918.30 cm-1; medium C-H bending for alkanes at peaks 1454.33, 1462.5, 1452.3 cm-1and C-H medium rocking at 1365.60, 1354.03, 721.38 cm-1 in petroleum ether extract. The C=O stretching at 1654.92, 1637.56, 1708.93, 1697.36cm-1 pointed at α and β-unsaturated aldehydes or ketones in all the three extracts. Other strong peaks at 1332.81, 1292.31, 1261.45,1294.24, 1259.52,1261.45 cm-1 C-N stretching indicates the presence of Aromatic amines where as C-N stretch at 1107.14,1087.85,1039.63,1022.27 and 1037.70 in extracts indicated Aliphatic amines. Moreover, the medium peaks generated at 2850.79, 2735.06, 2850.79 cm-1 represent H-C=O: C-H stretching for aldehydes, with strong absorption peaks at 1710.86, 1683.68, 1676.14, 1708.93, 1697.36 cm-1 are assigned to C=O stretching vibration in carbonyl compounds; which may be characterized by the presence of high content for unsaturated aldehydes, esters and ethers. Alkyl halides were represented by –C-H wag- weak stretching at 1228.66, 1207.44, 1193.94, 1294.24, 1259.52cm-1; and –C-Cl stretchbending at 866.04, 839.03, 800.46, 802.39, 823.60, 673.16, 650.01, 601.79, 586.36, 559.36 cm-1 ; and C- Br stretch at 680.87, 536.21 cm-1 in ethyl acetate and ethanolic extract. The observed sharp peaks in these extract at 1365.60,1354.03, 1597.06, 1554.63 cm-1 for N-O asymmetric stretching and N-O symmetric bend stretching in ethyl acetate extract at 1597.06, 1462.04, 1294.24 cm-1, revealed the presence of nitro compounds. A strong haoalkene, C-Cl appeared at 839.03 cm-1 only in petroleum ether extract. Also the extract indicates the terpenoids and flavanoids as indicated by peaks for C=O at 2850.79 in all the three extracts and 2735.06cm-1 in petroleum ether extract, represented as H-C=O: C-H stretching for aldehydes. The unsaturated aromatic lactones with C=O at the 1710.86, 1683.68, 1654.92,1637.56,1708.93, 1676.14 cm-1 in petroleum ether, ethyl acetate extract and a single peak at 1697.36 in ethanolic extract indicated the presence of coumarin, glycosides. Peak values 3321.42, 3300.20, 3284.77, 3261.63, 3242.34, 3230.77, 3201.83, 3535.52, 3512.37, 3454.51, 3402.4 cm-1 indicated the presence of phenolic compounds were depicted by O-H stretch. Anthraquinones were present as aromatic ethers with C-O stretch at 1261.45, 1259.52, 1037.70, 1261.45, 1043.49, 1371.39cm-1 in all the three extracts. The medium band for C-H stretch at 2920.23, 2850.79, 2735.06, 2918.30cm-1 in all the three extracts; the -C=C- medium stretch at 1654.92 cm-1 in petroleum ether extract and peak 1676 cm-1 in ethyl acetate extract revealed large quantities of terpenes.

Table 2: FTIR spectra analysis of *Kapppaphycus alvarezii*

	Absorption spectrum, Frequency (cm -1)			
Functional groups	**Component (Peaks)**	**Petroleum ether extract**	**Ethyl acetate Extract**	**Ethanol extract**
Amines 1^0& 2^0 amines	N-H stretch	3371.57,3315.63, 3248.13	3321.42,3300.20, 3284.77,3261.63, 3242.34,3230.77, 3201.83,3186.40	3381.21,3344.57, 3323.35,3302.13, 3282.84,3263.56, 3242.34
	N-H bend	1637.56,1610.56	1462.04	1637.56
	N-H wag	952.84,935.55, 866.04,839.03	931.62,802.39, 680.87	885.33
Alkenes	C=C-H Stretch	3088.03	–	3089.96,3072.60, 3047.53,3026.31
	C=C-C symmetric stretch	1610.56,1637.56, 1654.92	1676.14	

Alkanes	C-H Stretch C-H rock H-C-H bend	2920.23,2850.79 1365.60,1354.03, 721.38 1462.04	2850.79 – –	2850.79 –
Aldehydes	C-H Stretch of C=O C=O Stretch	2850.79,2735.06, 1710.86,1683.68, 1654.92,1637.56	1708.93,2850.79	1637.56,1697.36
Ketones	C=O Stretch	1654.92,1683.68, 1710.86	1708.93,1676.14	1637.56,1697.36
Carboxylic acids	H bonded OH stretch C=O stretch OH bend	2735.06,2671.41 1710.86,1683.68, 1654.92 935.55	3321.42,3300.20, 3284.77,3261.63, 3242.34,3230.77, 3201.83 1708.93,1676.14 931.62	3224.98,3205.69, 3186.40,3165.19, 3145.90,3130.47, 3107.32,3089.96, 3072.60,3047.53
Aromatic rings	C=C-C asymmetric Stretch C=C-C symmetric Stretch	1413.82,1454.33 –	3086.11,3066.82, 3043.67,1462.04 1597.06,1591.27	3089.96,3072.60, 3047.53,3026.31 –
Aromatic amines	C-N stretch	1332.81,1292.31, 1261.45	1259.52,1294.24	1261.45
Alkyl halides	C-H wag C-Cl stretch C-Br stretch	1228.66,1207.44, 1193.94 866.04,839.03, 800.46,721.38	1259.52,1294.24 680.87	1261.45 823.60,673.16, 650.01,601.79, 586.36,559.36 536.21
Aliphatic amines	C-N stretch	1107.14,1087.85, 1039.63,1022.27	1037.70	1043.49
Esters and ethers	C-O stretch	1292.31,1261.45, 1228.66,1207.44, 1193.94,1107.14, 1087.85	1037.70,1259.52, 1294	1261.45,1043.49
Phenols & alcohols	O-H stretch bonded	– 	3186.40,3165.19, 3145.90,3105.39	3535.52,3512.37, 3454.51,3402.43, 3224.98,3205.69
Nitro groups	N=O Stretch N=O bend	– –	1597.06,1591.27, 1462.04 –	1554.63,1543.05 1371.39
Aromatics	C-C stretch C-H “oop”	– –	– –	1406.11 885.33,823.60, 673.16
Proteins	C=O stretch	–	1597.06,1591.27, 1676.14	1543.05,1554.63, 1637.56,1697.36, 1697.36
Carbohydrates	O-H stretch	1022.27,1039.63, 1087.85,1107.14	1037.70	1043.49
Lipids	C-H stretch	2735.06,2850.79, 2920.23	2918.30,2850.79	2850.79
Terpenoids	C=O stretch	2850.79	2850.79	2850.79
Anthraquinones	C-O stretch	1261.45	1259.52, 1037.70	1261.45,1043.49,1371.39
Coumarin, Glycosides	C=O stretch	1710.86, 1683.68, 1654.92, 1637.56,	1708.93, 1676.14	1697.36
Terpenes	C-H stretch -C=C-stretch	2850.79, 2920.23, 2735.06, 1654.92	2850.79, 2918.30 1676.14	2850.79 –

The FT-IR studies conducted on algae and seaweeds, extracts revealed the surfaces of algae had the toxic interaction sites of carboxyl, amino acid and hydroxyl groups of algae [14, 15]. also reported the several functional groups such as amides, alcohols, phenols and phosphorous compounds so present in the FTIR analysis of crude powder of *Sargassum wightii*, which can be corroborated by the present findings. Algae like biological molecules show complex vibrational spectra that include combination and overtones in all bands. But metal-legend stretching frequencies and properties of functional groups coordinated to toxic centers offer useful information. C–O stretching, NH2 rocking C-O and CH2 stretching bands are metal sensitive and are shifted as the metal is changed, but NH2 vibrations are very sensitive to the intermolecular interactions and its effect for example, hydrogen bonding. Such interactions make it difficult to discuss the rigidity of the metal-nitrogen bond from the frequency shift [16]. In some spectra of *K. alvarezii*, bands were also seen at 1022.27, 1039.63, 1087.85, 1107.14 cm-1 petroleum ether extract, 1037.70 cm-1 in ethyl acetate extract and 1043.49 cm-1 in ethanol extract. These band positions match those attributed to the *ν*(C-O-C) stretching of polysaccharides [17]. According to a recent report by [18], these bands were noticed in different materials at slightly different frequencies at peaks 1147, 1086 and 1025 cm-1). It was noticed that the strongest absorbers between 1200 and 1000 cm-1 were carbohydrates. Nucleic acids like several classes of other compounds, also shows same absorption bands with same functional groups in the same spectral regions. [19] attempted a phytochemical study, FT-IR, GC-MS analysis of *Solanum torvum* methanolic leaves

extract. [20] analyzed different techniques for plant phenolic compounds. [21] screened compounds in *Eclipta* species by using FTIR and HPLC.

Since the thallus contain rich amount of total phenol, it was subjected to fractionation by RP- HPLC revealed the presence of a pool of phenolic acids when compared with the respective standards (Table: 3, Fig:1). The major molecules are Chlorogenic acid (66 µg/g), Sinapicacid (µg/g), Hydroxylbenzoic acid (µg/g), Gallic acid (61.8 µg/g), Phloroglucinol (270.9 µg/g), Vanillic acid (µg/g), Cinnamic acid (65.8 µg/g), Catechol (79.4 µg/g) and Ferulic acid (206.6 µg/g). Most of these are proven molecules with high therapeutic values. Phenolic acids show beneficial effects in terms of antioxidant, anti-ageing and protect cardiovascular issues. Further, their attributes reduce oxidative stress, lipid peroxidation, free radical generation and low density lipoprotein (LDL) cholesterol-oxidation.

Table 3: RP-HPLC profile of phenolic acids in *Kappaphycus alvarezii*

Compound	Concentration (µg/g)
Chlorogenic acid	66.01
Sinapicacid	3385.84
Hydroxylbenzoic acid	0.542
Gallic acid	61.837
Phloroglucinol	270.907
Vanillic acid	66.87
Cinnamic acid	65.780
Catechol	79.412
Ferulic acid	206.611

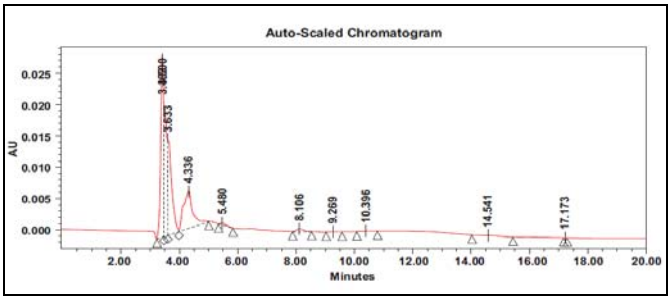

Fig 1: RP-HPLC analysis of *Kappaphycus alvarezii*

Moreover, other phenolic acids noticed were gallate, hydroxyl benzoate, chlorogenate, phloroglucinol, vanillate, cinnamte, and ferulate. These also possess positive biological effects on human health and ameliorate oxidative stresses. Phenolic compounds are found remarkably in medicinal plant species, including seaweeds, and have been reported to have wide range of biological activities including antioxidant. The HPLC analysis of the algae *Amphiroa anceps* revealed that the polyphenolic compounds present in them were an effective source of antioxidants. Therefore, the seaweed extracts could have potential applications in food industries. [22] also reported that the phenolic compounds are one of the most effective antioxidant.

4. Conclusions

The phytochemical screening shows that the thallus of the red algae is rich in phytochemical constituents. Inoder to investigate the fingerprint and to predict the composition of chemical groups present in plants FTIR analysis can be used as an ideal tool. The present results clearly indicate that *Kappaphycus alvarezii* extract possess significant phenolics and therefore capable of antioxidant activities like ascorbate. This study indirectly showed that the phenolic compounds were a pivotal for its free radical scavenging activity. Thus, the study has provided biochemical basis for alternative uses of the macro algae in the treatment and prevention of various diseases and disorders. Future perspectives include isolation, purification of lead molecule and to justify its biological potentialities.

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6. References

1. Kaliaperumal N, Chennubhotla VSK, Kalimuthu S. Seaweed Resources of India. CMFRI Bull, 1987; 14:51-99.
2. Ruperez P, Ahrazem O, Leal JA. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. J Agric Food Chem, 2002; 50(4):840-845.
3. Fitton JH. Antiviral properties of marine algae. In: Critchley AT, Ohno M, Largo DB. (Eds.), World Seaweed Resources. Windows and Macintosh, ETI Information Service, Workingham, UK, 2006, 7.
4. Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C, Gandhimathi *et al*. Antimicrobial potential and seasonality of red algae collected from the southwest coast of India tested against shrimp, human and phytopathogens. Ann. Microbiol. 1998; 2:207-219.
5. Anonymous, Pharmacopoeia of India,II edn. Govt. of India Press, Nasik, India.1966.
6. Harborne JB. Phytochemical methods, Chapman and Hall, London, 1973.
7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J. Biol. Chem. 1951; 193:265-295.
8. Singleton VL, Rossi JA. Colorumetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Viticult. 1965; 16:144-158.
9. Ranganna S, In Manual of analysis of fruit and vegetable products.9th edition, Tata Mc Graw Hill, New Delhi. 1997.
10. Sharanabasappa GK, Santosh MK, Shaila D, Seetharam YN, Sanjeevarao I, Phytochemical studies on *Bauhinia racemosa* Lam. *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. EJ. Chem. 2007; 4:21-31.
11. Jagmohan. Organic spectroscopy principles and applications, 2nd Edn, Narosa publishing House, Daryagani, Delhi, 2005.
12. Polat S, Ozogul Y. Fatty acid, mineral and proximate composition of some seaweeds from the northeastern Mediterranean coast Ital. J Food Sci. 2009; 21:317-324.
13. Matanjun P, Mohamed S, Mustapha NM, Muhammad K, Ming CH. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J Applied Phycol. 2008; 20:367-373.
14. Mishra A, Jha B. Isolation and characterization of extracellular polymeric substances from micro-algae *Dunaliella salina* under salt stress. Bioresource Technology, 2009; 100:113.
15. Marimuthu J, Essakimutju P, Narayanan J, Antham B, Tharamaraj RJJM, Arumugam S. Phytochemical characterization of brown seaweed *Sargassum wightii*. Asia Pacific J of Trop disease, 2012, 109-113.

16. Doshi H, Ray A, Kothari IL. Bioremediation potential of live and dead *Spirulina*: spectroscopic, kinetics and SEM studies. Biotechnology and Bioengineering, 2007; 96(6):1051-1063.
17. Brandenburg K, Seydel U. Fourier transform infrared spectroscopy of cell surface polysaccharides. In: Mantsch HH and Chapman D (ed.) Infrared Spectroscopy of Biomolecules, Wiley: Chichester, 1996, 203-278.
18. Diem M, Chiriboga L, Yee H. Infrared spectroscopy of human cells and tissue. VIII. Strategies for analysis of infrared tissue mapping data and applications to liver tissue. Bioplymers, 2000; 57(5):282-290.
19. Nithyadevi R Sivakumar. Phytochemical Screening and GC-MS, FT-IR Analysis of Methanolic Extract Leaves of *Solanum torvum* Sw. J International Journal of Research Studies in Biosciences, 2015; 3(9):61-66.
20. Ali Khoddami, Meredith A, Wilkes, Thomas H. Roberts. Techniques for Analysis of Plant Phenolic Compounds, Molecules. 2013; 18:2328-2375.
21. Thenmozhi M, Bhavya PK, Rajeshwari Sivaraj. Compounds identification using HPLC and FTIR in *Eclipta alba* and *Emilia sonchifolia*. International Journal of Engineering Science and Technology, 2011; 3(1):292-298.