IJPSR (2013), Vol. 4, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 April, 2013; received in revised form, 28 May, 2013; accepted, 15 August, 2013; published 01 September, 2013

EVALUATION OF MARINE ALGAE *KAPPAPHYCUS ALVAREZIL* AS A SOURCE OF NATURAL PRESERVATIVE INGREDIENT

G. Renuga*, Ali Osman and A. Babu Thandapani

Ultra College of Pharmacy, Department of Pharmaceutical Biotechnology, Madurai, Tamil Nadu, India

Keywords:

Antioxidant, *Kappaphycus alvarezii*, Macromolecules, Nutrient, Spectral Analysis

Correspondence to Author:

Dr. G. Renuga

Professor, Department of Pharmaceutical Biotechnology, Ultra College of Pharmacy, Madurai, Tamil Nadu, India

E-mail: renugabiog@gmail.com

ABSTRACT: Marine organisms possess an inexhaustible source of useful chemical substances for the development of new drugs. The present project focused to identify the bioactive compounds from the seaweed *Kappaphycus alvarezii.* The experimental samples were collected from the sea coast of Rameshwaram, Tamil Nadu, in the form of fresh living sample. The fresh solvent extracts of *K. alvarezii* were found to be rich in total carotenoid content and vitamin C. The concentration of vitamin C was found to be 9.2 mg/g.f.wt, Polyphenol 27.2mg GAE standard, gallic acid equivalent/100 g.dry.wt, β-carotene 6.52 mg/g dry.wt and total carotenoid 0.769 mg/g.f.wt. The phytochemical analysis demonstrated the presence of high carbohydrate, protein, alkaloids, glycosides, flavonoids, steroid, and phenolic compound and absence of tannin in the extracts also *k. alvarezii* extracts revealed macromolecules contents such as carbohydrate contents of 53 g, Protein 16.2g and fat content 1.01 g /100 g of dry wt. Micronutrients were estimated that calcium content was 5.3g/100g and iron content was found to be more in 1.14 mg, phosphorus content of 18.7 mg, magnesium 20.6mg /100 mg of dry wt. Furthermore, the extracted material was submitted to structural analysis, by Infrared spectroscopy and nuclear magnetic resonance spectroscope (H-NMR) and chemical composition analysis. The results based on spectral data IR and H-NMR revealed the presence of aliphatic constituents containing Carbon, and hydroxyl groups and both spectral studies were inferred the presence of polysaccharide compound in the crude extract when compared with standard carrageenan used in the spectral studies and the chemical structure of the isolated compound from *K. alvarezii* were established by spectral techniques (IR and H-NMR). Bioactive compound prepared from experimental species showed economic value, quality as polysaccharide, it can be concluded that the *K. alvarezii* may serve as functional food with vital pharmaceutical and biological value.

INTRODUCTION: Marine organisms are rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produces by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(9).3548-55

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(9).3548-55

Seaweeds are considered a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Marine macroalgae, commonly known as seaweeds, are one of the living renewable resources of the oceans with potential food applications consumption of seaweeds as sea +vegetables in human diets has been the common practice in several Asian countries [1].

The prevention and treatment of these infectious diseases by applying products from marine organisms appears as a possible alternative.

Hence, the interest in marine organisms as a potential and a promising source of pharmaceutical agents has increased during the last years [2, 3].

Research in natural products of marine algae has made significant advance in the recent years. Marine algae are known to produce a variety of compounds and some of them have been shown to possess biological activity of potential medicinal value in the last three decades the discovery of metabolites with biological activities from seaweeds has increased significantly. Marine macro algae or seaweeds are one of nature's most biologically active resources as they possess a wealth of bioactive compounds. It is exploited for both human and animal health applications

Seaweeds are known to be a healthy food with low calorie content and high fiber and mineral content, with significant amounts of protein, Vitamins and trace elements and a wide range of secondary metabolites not found in other organisms. Because of this additional uses as a natural source of functional ingredients this resource is particularly attractive [4].

A most characteristic feature of plant is their capacity to synthesized an enormous variety of low molecular weight compounds, the so called secondary metabolites although only 20-30% of higher plants have been investigated so far, several ten thousands of secondary metabolites have mass spectrometry, nuclear magnetic resonance or x ray diffraction [5].

Red alga is economically important tropical seaweed which is highly demanded for its nutraceutical and pharmaceutical applications 6. In this content, the present study was planned to evaluate the macromolecules and micronutrients potentials, the phytochemical components, antibacterial activity of solvent extract and a source of the selected red algae biomass namely *Kappaphycus alvarezii.*

MATERIALS AND METHODS:

Collection of Seaweed samples: *Kappaphycus alvarezii* collected from mandapam region in Ramanathapuram District Tamil Nadu. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of living sample. The marine red algae *Kappaphycus alvarezii* was collected in the month of December. Red algae samples were cleaned are rinsed with sterile water to remove any associated debris. Sample was kept under sunshade for 7 days. After drying the sample, it was ground thoroughly to powder form. The powder was then used for the solvent extraction methods.

This powder was stored in cold conditions in an airtight container and analysis was carried out within three months of processing. The collected sample identified by Dr. K. Eswaran, Principal scientist at Central Salt and Marine Chemicals Research Institute (CSMCRI), Mandapam and voucher specimen (KA/UCP/02/ 2013) was maintained in the department and used for all research experiments.

Methanolic extracts of Samples: The Solvent extraction of methanol and Acetone was adapted by the method described as in (Jeyanthi Rebecca) [7]. The algae after drying were weighed (5g) and then chopped. The chopped samples were finely powdered using mixer grinder. The finely powdered samples were dissolved in organic solvents and it was kept for 48 hours at room temperature and mixed at regular intervals. After 48 hours the sample homogenized in solvent was filtered by using Whatman filter paper No. 3 for the preparation of crude filtrate for further experiments.

Analysis of Pharmaceutical Potentials: Phytochemical screening of solvent extracted samples were carried out according to the standard methods as described by Trease and Evans [8] and Harper [9] for carbohydrates, proteins, alkaloids, tannins, flavonoids, steroids, glycoside, phenol saponin, terpenoids, quinons and mucilage. Estimation of Antioxidant potential like total carotenoid of solvent extracts of *K. alvarezii* was analysed by spectrophotometric method [Kulandaivelu and Gnanam) [10].

Vitamin C was analysed by dichlorophenol indophenols method by Chinoy and Singh [11] and polyphenol was analysed by spectrophotometric method [12] and quantitative analysis on the amount β-carotene was performed using high performance liquid chromatography reverse phase column [13].

Analysis of Macro & Micronutrients: *Kappaphycus alvarezil* were collected then selected seaweeds were washed thoroughly in seawater and then in tap water. The seaweeds were again washed in distilled water, the remaining water was drained and the fresh seaweeds were used for macromolecules and micronutrients analysis. Macromolecules such as carbohydrate were analyzed by Anthrone method, protein was measured by Lowrey's method and fat content was estimated by Soxhlet method. The seaweeds contain a wealth of mineral elements. The micro nutrients such as iron, phosphorus, calcium, magnesium and niacin were analyzed by standared methods of analysis of AOAC 14 association of analytical chemicals.

Heavy metal analysis: Heavy metals like cadmium, lead, arsenic, and mercury were analyzed by AAS. Methanolic extracts of samples were digested with triple mixture of nitric acid: Sulphuric acid: Perchloric acid [11:6:3] until a clear solution was obtained when dissolved in HCl. This solution was made upto 25ml with triple distilled and de ionized water and the digested samples were analyzed by atomic absorption Spectrophotometry [15].

Spectral analysis: Fourier transformed infrared spectroscopy (FTIR) of sample crude extract was performed using a Perkin Elmer spectrometer (16PC) with a resolution of 4cm -1 in the range of 400-4000 cm^{-1}. The intensity of maxima of the infrared absorption bands were determined by the baseline method. The H -NMR Spectra were recorded at 80^{o}C on a varian mercury plus spectrometer (400MHz) using chloroform as solvent.

RESULT AND DISCUSSION: The seaweed *Kappaphycus alvarezii* was collected during Dec 2012, from the Mandapam coast (Latitude 9 ^{o}17'N, Longitude 79 ^{o} 22' E), Gulf of Mannar. The collected samples were found to be red in colour namely K*appaphycus alvarezii* specimen identified by Scientist's in charge at the Centre for Marine and Fisheries Research Institution, Mandapam (**Fig. 1**). K*appaphycus alvarezii* is one of the larger tropical red algaes in the world and also has one of the fastest growing rates, able to double in size every 15 to 30 days.

The alga is quite tough, freshly and firm, growing up to 2 meters tall. Branches are very heavy and highly irregular, forming dense tangles of seaweed.

FIG. 1: FRESH SAMPLE OF SEA WEED (*KAPPAPHYCUS ALVAREZII*)

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary metabolites produced by *Kappaphycus alvarezii* may be potential bioactive compounds of interest in pharmaceutical industry. Phytochemical screening study of *K. alvarezii* extract revealed that the extract had significant quantity alkaloids, flavonoids, steroid, Terpenoids and absence of tannins. Preliminary phytochemical analysis of the sea weed reported in **Table 1** showed that contained carbohydrate, protein, gum, mucilage, fat, and phenols. Poly phenol found to be plentiful as reported by [5].

TABLE 1: PHYTOCHEMICAL SCREENING OF SELECTED *KAPPAPHYCUS ALVAREZII* EXTRACTS

Phytochemicals parameters	*Kappaphycus alvarezii*	
	Methanol Extract	Acetone Extract
Carbohydrates	+	+
Proteins	+	+
Alkaloids	+	+
Glycosides	_	_
Flavonoids	+	+
Steroids	+	+
Tannins	-	_
Phenols	+	+
Terpenoids	+	+
Quinons	+	+
Mucilage	+	+
Saponins	+	+

+ Positive symbol indicates the presence of the compound; - Negative symbol indicates the absence of the compound;

Total polyphenol content was 27.2mg GAE standard, gallic acid equivalent/100 g.d.wt. Total content of carotenoid estimated by spectrophotometric method was found to be 0.769 mg/g f.wt and the content of vitamin C were analyzed by dichlorophenol indophenols method was found to be 9.2 mg/g f.wt, β- carotene 6.52 mg/g of dry.wt. Antioxidant potential of the red algae *Kappaphycus alvarezii* was determined by measuring vitamin C content, total carotenoid was found to have the highest value and ascorbic acid equivalent antioxidant capacity 9.2 mg/ g f.wt.

Hence, the presence of vitamin C and carotenoid showed its ability to act as an antioxidant and also polyphenol compound from *K. alvarezii* could serve as an effective antioxidant.

HPLC data of β-carotene in *K. alvarezii* extracts showed the β-carotene peak (RT = 10.11/minute). The concentration of β- carotene 6.52 mg/gsample. HPLC results revealed that one compound is present in large besides other impurities which is confirmed as β- carotene.Quantitative analysis on the amount of β- carotene was performed using HPLC reverse phase column.

The data obtained in this study was similar to the observation of [2]. Reported the vitamin C quantity of Ulva species was also one of the sea lettuce belongs to a group of edible green algae widely distributed along the coaste of the world's Oceans.

Recently, sea weeds have received significant attention for their potential as natural antioxidants. Antioxidants activity of marine algae may arise from carotenoid and ascorbic acids. These compounds directly or indirectly contribute to inhibition or suppression of free radical generation.

Experimental data on nutrient content of the *k. alvarezii* extracts revealed that the carbohydrate contents of 53 g, Protein 16.2g and fat content 1.01 g /100 g of dry wt. Calcium content was 5.3g/100g and iron content was found to be more in 1.14 mg, phosphorus content of 18.7 mg, magnesium 20.6mg /100 mg of dry wt. **Table 2** revealed that *K. alvarezii* sea weeds were found to be rich in macro and micro nutrients which was comparable to the findings of [1].

TABLE 2: DETERMINATION OF MACRO & MICRO NUTRIENTS OF *KAPPAPHYCUS ALVAREZII*

S. No.	Parameters	Content
1	Carbohydrate	53g
2	Protein	16.2g
3	Fat	1.01g
4	Calcium	5.3g
5	Iron	1.14mg
6	Sulphate	20.2mg
7	Magnesium	160mg
8	Phosphorus	869mg
9	Sodium	22.4mg
10	Potassium	13.4mg

AAS analysis revealed the presence of arsenic, cadmium and lead were detected in *K. alvarezii* whereas **fig. 2** showed the contents of heavy metals in extracts of sea weeds. According to [2], contents of metals had meet safety regulations in terms of toxicological criteria. The estimated level of metal in term of ppm must be considered less than the upper limit of the standard value. In the present data, on heavy metal concentration in the *K. alvarezii* was within the tolerable value reported as quality criteria for the potential nutritional properties of seaweeds as raw or semi processed material in the formulation of sea food products.

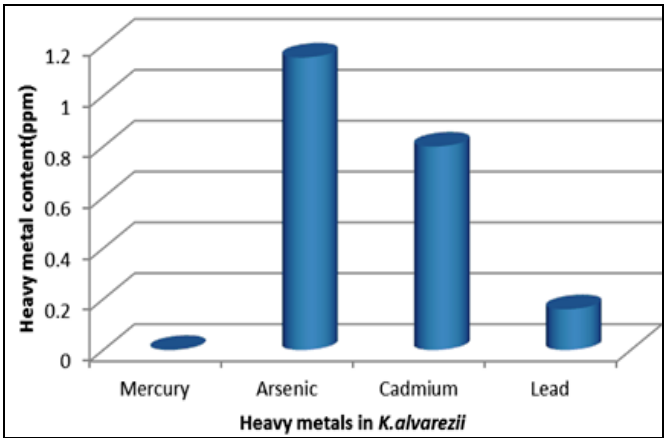

FIG 2: ESTIMATION OF HEAVY METALS IN *K. ALVAREZII*

The bioactive fraction residues were characterized in H-NMR studies (**Fig. 4**). The residue of *K. alvarezii* has a bunch of signals in the aliphatic region of the H-NMR. As there was a responsible signal in the C, H region, the presence of polysaccharides in the extract was confirmed. Here, the signals in the region 1.57 at standard and 1.59 at sample suggest the presence of carbohydrate identity in the extracts. Base on literature regarding the phytochemical screening and other application.

Since, sea weeds are the main source of carrageen in the world. Further, the carrageenan could be used as standard in H-NMR in order to confirm the presence of carrageenan compound in *K. alvarezii* residue for exploring its potential activity. From the spectral data it was concluded that the compounds exhibiting activity are probably carbohydrates. The results based on spectral data IR (**Fig. 3**) revealed the presence of aliphatic constituents containing Carbon, and hydroxyl groups, both spectral studies FT-IR and H-NMR inferred the presence of polysaccharide compound in the crude extracts of *K. alvarezii*, when compared with standard carrageenan used in the studies. Based on the observation the compound may be one of the macro molecules having the characteristic of CH, OH, and it can be considered the presence of polysaccharide. Once it has purified it will be useful for various application. By the both spectral studies FT-IR and H-NMR inferred the presence of polysaccharide compound in the crude extract when compared with standard carrageenan.

IR spectrum:

Wavelength cm^{-1}	Description
1654.62 cm^{-1}	C-O stretching
2927.73 cm^{-1}	C-H stretching
3426.90 cm^{-1}	O-H stretching
1405.55 cm^{-1}	C-C stretching
927.71 cm^{-1}	C-H deformation
1071.81 cm^{-1}	C-O-C stretching
1230.04 cm-1	S = O sulphate esters

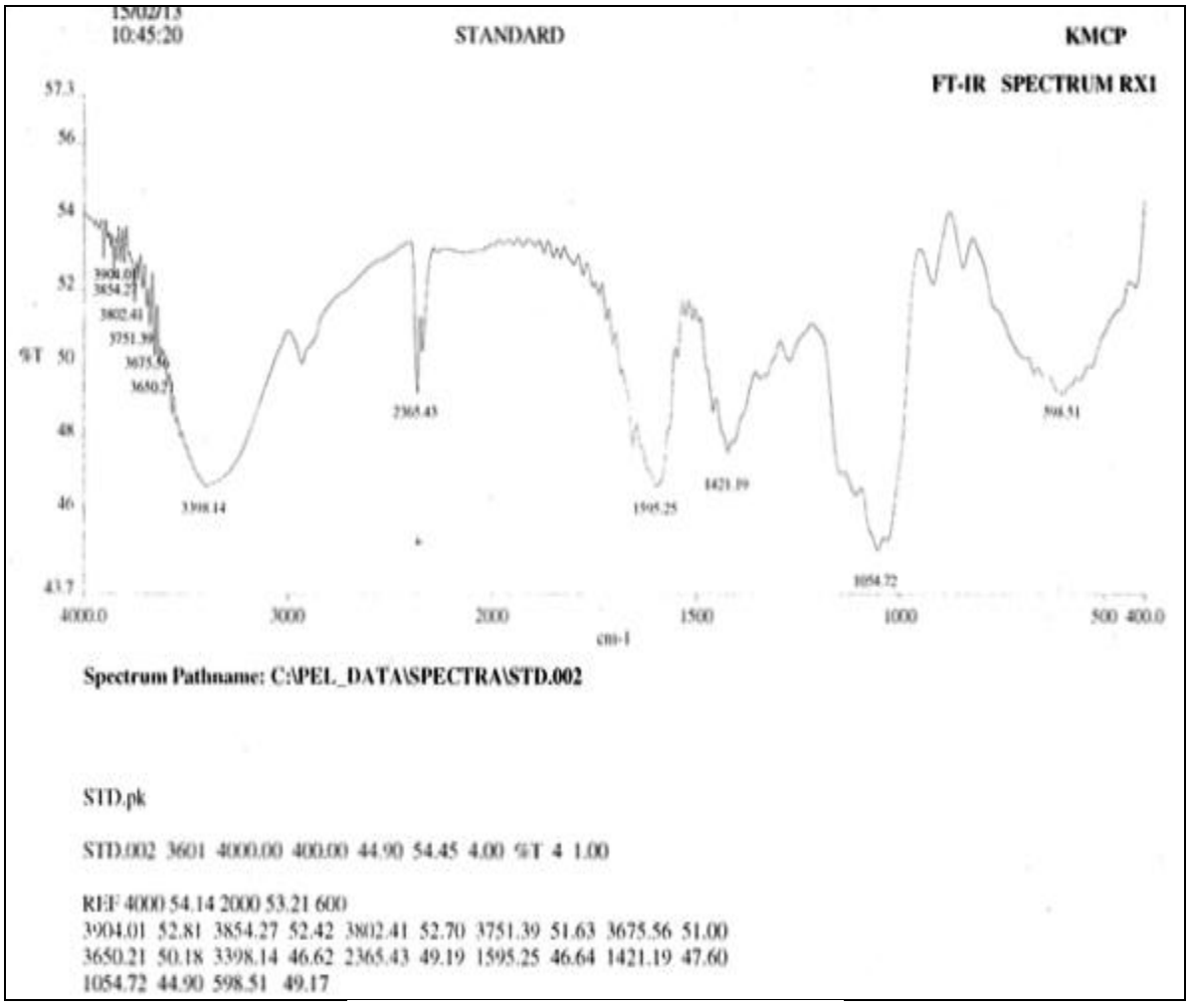

FIG. 3A: FT-IR SPECTRAL ANALYSIS

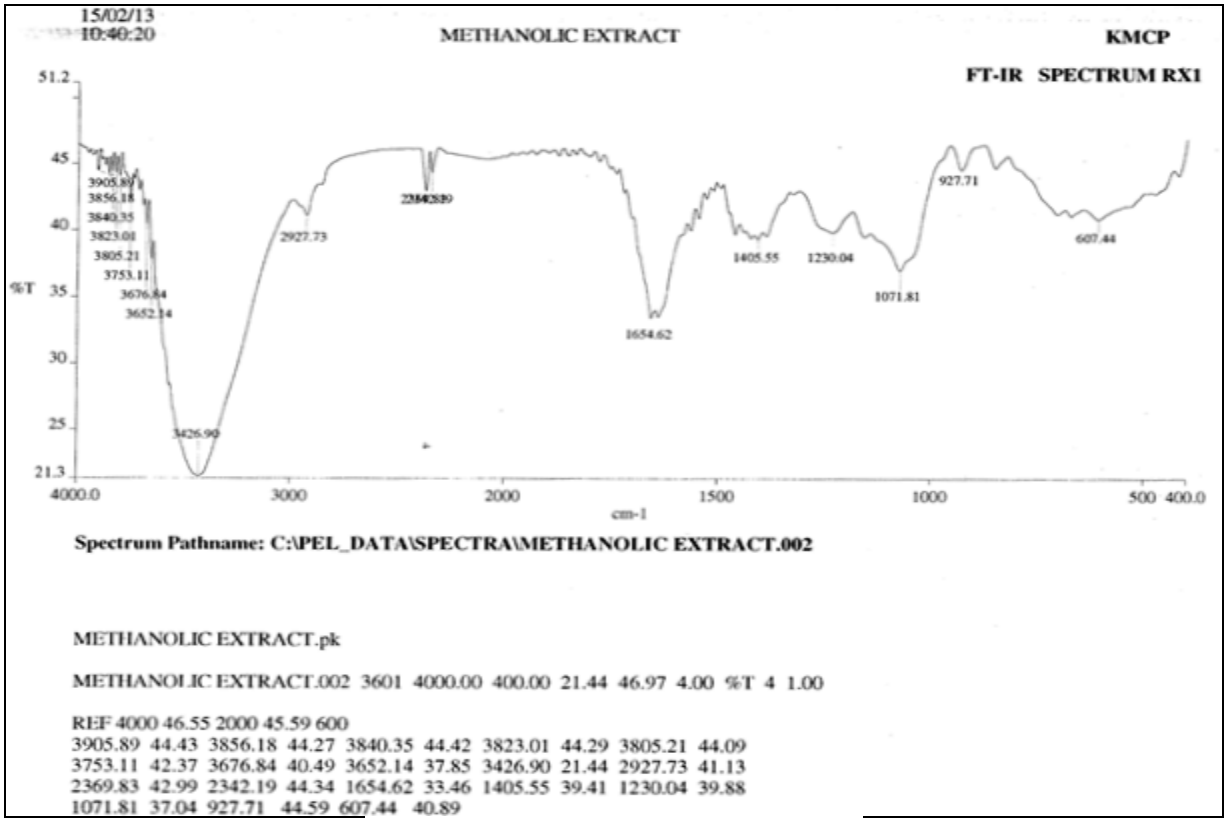

FIG. 3B: FT-IR SPECTRAL ANALYSIS

Legend: FT-IR spectrum for Methanolic extract of *Kappapycus alvarezii* shows similarity in the recorded fig (3a std and 3b sample) when compared with standard carrageenan that confirm the presence of polysaccharides in our extract.

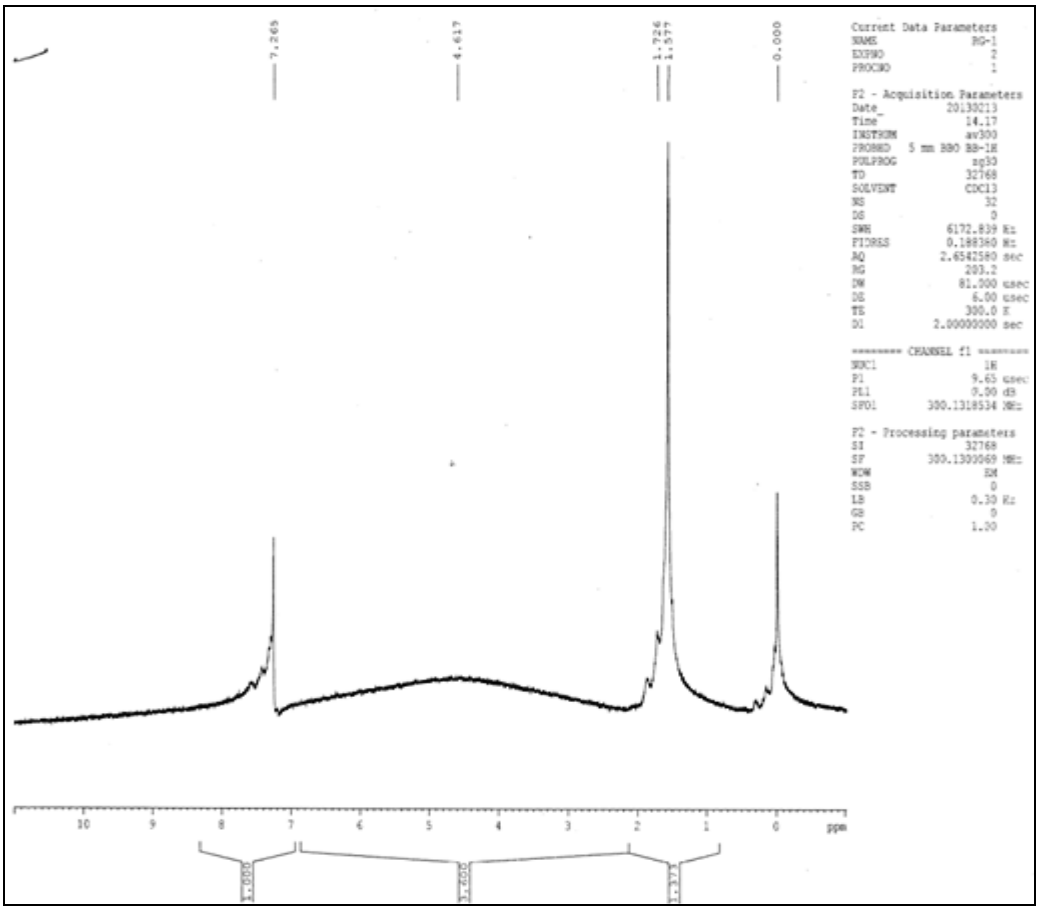

FIG. 4A: H-NMR ANALYSIS (STANDARD)

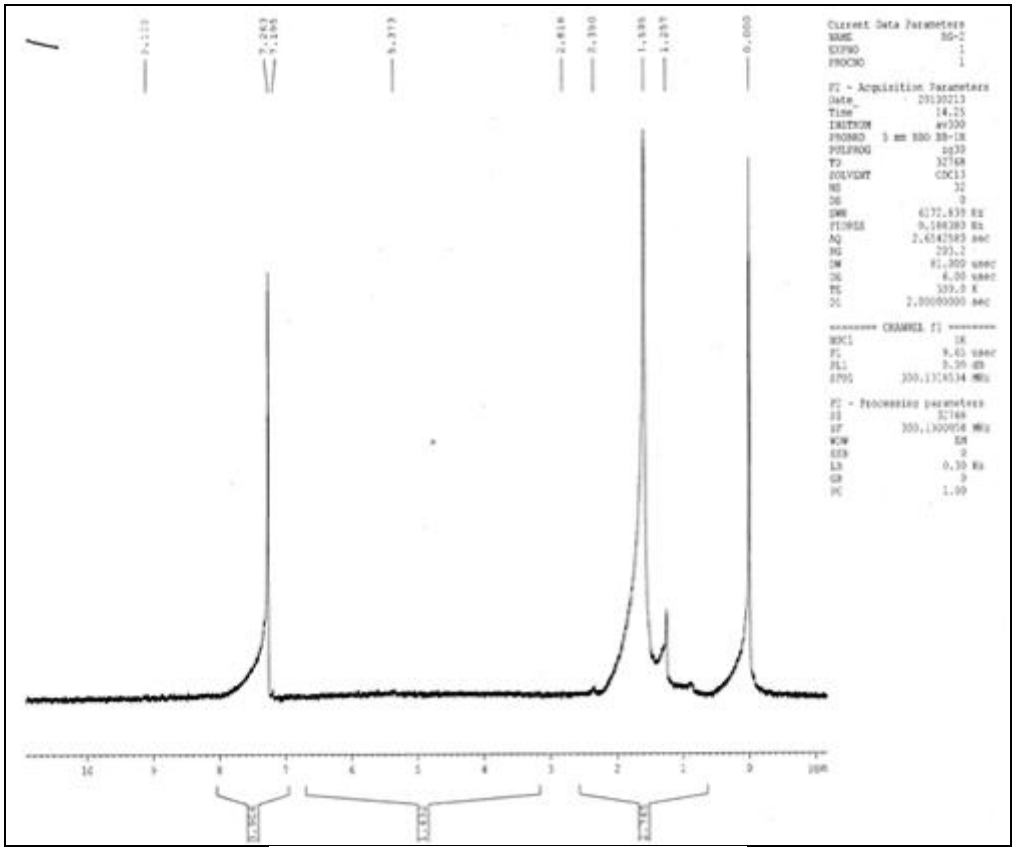

FIG. 4B: H-NMR ANALYSIS (SAMPLE)

Signal at: (CH) H singlet 1.257 aliphatic protons, (OH) H singlet 1.595 alcoholic protons.

Legend: H-NMR studies methanolic extracts showed similarity in fig (4aStd and 4b Sample) that confirmed the presence of polysaccharide in our extract when compared with standard carrageenan.

CONCLUSIONS: Marine organisms possess an inexhaustible source of useful clinical substances for the development of new drugs, among these organisms were find marine algae that are capable of biosynthesizing a broad variety of secondary metabolites used in biotechnology in the discovery of new compounds from marine origin. Seaweeds have been widely used for human consumption in many parts of the world.

Marine algae serve as a source of minerals, vitamins, fatty acids, antioxidants and poly phenol and the crude extracts of *Kappapycus alvarezii* exhibited reducing power and hydroxyl radicals scavenging activity higher than that of standard antioxidants. This antioxidant activity is of particular interest for cosmetic and pharmaco-logical applications. The bioactivity of sulphated polysaccharides like carrageenan depends on the degree and position of sulphatation, the molecular weight (data not given) and the sugar type of glycosidic branching, among other features. Difference in biological activities can be associated with the modification of the carbohydrates.

However, the antioxidants properties extend beyond *K. alvarezii carrageenan*; known antioxidant compounds such as polyphenols, β-carotene, vitamins C and carotenoids present in the algae probably help scavenge free radicals.

Carrageenan is a generic name or polysaccharides extracted from different species of red seaweeds, Carrageenan is commercially important highly sulfated polysaccharides of the galactan group with alternating 1, 3 and 4 linked galactose residue, which fill space between the cellulosic plant structure of red seaweeds [17] acts as an additive in the food industry due to its functional properties which can be used to control moisture, texture to stabilize foods [18].

Kappa carrageenan has 34% 3.6 anhydrous D-galactose groups as part of its repeating structure and 25% ester sulfated group which will easily disperse when heated. This observation supports our results of high carrageenan yield obtained during precipitated by solvent extracts of *K. alvarezii*.

Polysaccharides are important structural components of cell and organelle membrane of higher organisms. Now- a-days marine organism based drugs for inhibiting inflammation and C, H were emerging from different natural sources. There is no doubt that *K. alvarezii*, have a wide range of application.

However, new pharmacological application of bioactive secondary metabolites or applications in the human diet as a functional food with significant amounts of protein, vitamin, mineral, micro element and dietary fiber that improve health are increasing. Multidisciplinary research efforts are necessary for improving the knowledge of the products that can be developed from this resource. These results showed the success of carrageenan crude extraction from *K. alvarezii* providing potential benefits for Industrial application.

ACKNOWLEDGEMENT: The authors are thankful to Mr. P.Sankar (Librarian, Ultra college of Pharmacy, Madurai.) for collection of red algae and the marine sample was identified by Dr. K. Eswaran, Principal scientist at Central Salt and Marine Chemicals Research Institute (CSMCRI), Mandapam, also thanks to Prof. Chandran (Ultra College of Pharmacy) for his valuable support to complete the research work.

REFERENCES:

1. Nisizawa,K. Seaweeds kaiso, Japan Seaweed Association Publication, Kochi, Japan.2002; 23.
2. Lindequist,U. Schweder, T. Marine Biotechnology. In Rehm H.J., Reed G, Biotechnology, W, Wlley-VCH Pub.2001. 10: 441-484.
3. Newman, DJ. Cragg, GM, Snader, KM. Natural products as source of new drugs over the period 1981-2002 .J. Nat. Prod. 2003; 66:1022-1037.
4. Ferraces-Casais, P. Lage Yusty, MA. Quiros ARS and Lopez-Hernandez, J. Evaluation of bioactive compounds in fresh edible seaweeds food anal. Food Anal Method. DOI: 10: 1007/s. 2011. 12161-001-9321-2.
5. Wink. M. Introduction: Biochemistry, role of plant secondary metabolites and their exploitation in biotechnology .wink ed., Sheffield Academic press, Annual Plant Reviews.1999 vol 3, pp1-16.
6. Sangeetha Pugalendren, Babu Sarangam, and Ramasamy Rengasamy. Extraction of R-Phycoerythrin from *Kappaphycus alvarezii* (Doty) Doty ex Silva and analyses of its physico-chemical properties *J. Acad. Indus. Res.* Vol. 1(7) December 2012.
7. Jeyanthi Rebecca. L, Dhanalakshmi. V and Chandra Shekhar. Antibacterial activity of *Sargassum ilicifolium* and *Kappaphycus alvarezii*. Journal of Chemical and Pharmaceutical Research, 2012, 4(1):700-705.
8. Trease G, E and Evans, WC. Pharmacognosy, 13thed London (UK): Bailliere Tindall. 1996.282-396
9. Harper J.B. Phytochemical methods. London, Chapman and Hall Ltd. 1975; 49-188.
10. Kulandaivelu, G. and Gnanam, A. A Laboratory manual on Photosynthesis and chloroplast Genetics. 1987. 8.
11. Chinoy, J.J Singh, Y.D. and Gurumurthi. K. Ind. J. Plant. Physiol.1976; 19: 122-130.
12. Ganesan P.S Chandini S. Kumar N. Bhaskar. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Journal of Food Science and Technology. 2005 10:25-29.
13. Rajasulochana, P Krishnamoorthy and R Dhamotharan. Morphological characteristics and estimation of beta carotene of the experimental red algae. International Journal of Pharmacy and Technology 2011; 3(4): 3726-3734.
14. Association of Analytical Chemists. Official Methods of Analysis of AOAC, Washington 2000.
15. Ghemisis, U.S Determination of vanadium in sampling and analysis of carbon and alloy steels, Reinhold, New York, 1938.
16. Imeson A. Thickening and gelling agents for food .Blackie Academic and Professional publisher Glasgow. 1992; 1-24.
17. Elliason A.C. Carbohydrates in food. Culinary and Hospitality industry publication services. 1996; 561.
18. Ross Kealy. Characterisation of carrageenans. Thesis CHEE4006 individual inquiry. The University of Queenland, Australia 2003; 34.

How to cite this article:
Renuga G, Osman A and Thandapani AB: Evaluation of Marine algae *Kappaphycus alvarezil* as a source of natural preservative ingredient. *Int J Pharm Sci Res* 2013: 4(9); 3548-3555. doi: 10.13040/IJPSR. 0975-8232.4(9).3548-55