



Extraction of natural bioactive nutrients from *Kappaphycus alvarezii* and analyses of its nutraceutical potential

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Abstract

The present research focused to identify the nutritive compounds present in the seaweed *Kappaphycus alvarezii*. 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, β -carotene 6.5 mg/g.f.wt and total carotenoid 7.9 mg/g.f.wt. *k. alvarezii* extracts revealed macromolecules contents such as carbohydrate contents of 29mg, Protein 18.7mg and fiber content 3.7mg /100 g of dry wt. Micronutrients were estimated that calcium content was 35.2mg/100mg and iron content found to be more in 1.14 mg, phosphorus content of 18.7 mg, magnesium 20.6mg /100 mg of d.wt, sulphate 20.2mg, sodium 22.4mg, potassium 13.4mg. The results show that, the presence of 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.

Keywords: *Kappaphycus alvarezii*, micronutrients, carotenoid, vitamin, carrageenan

1. Introduction

Kappaphycus alvarezii is one of the most commercially important species of red algae in India. The algae are morphologically tough, fleshy, firm and coarse thalli, with axes and branches 1 – 2 cm diameter, and grow up to 2 m tall. It can be found as flat reef 1 to 17 m deep underwater, or loosely attached to broken coral, or as unattached. Red tropical seaweed *Kappaphycus alvarezii* (*K. alvarezii*) fascinates biopolymer for its wall cell polysaccharides. Commercial cultivation of *K. alvarezii*, originated in Philippines in 1960 [1], since then countries like Japan, Indonesia, Tanzania and South Africa are cultivating this species on a large scale [2]. Farming techniques have undergone several innovations since it was first introduced. Today seaweed farming is a viable alternative source of income for small scale fishermen [3], After acclimatization and laboratory culture, the alga was introduced in the sea under confined conditions that employed a novel bag technology, initially in the Gujarat coast of India and later in Mandapam, Tamil Nadu, southern India [4]. In India, the demand from the phycocolloid industry is great, but the present production from natural habitats is very low and not sufficient to cater the needs for local industry. This gap between the demand and supply can be bridged through mariculture practices and by cultivating the useful species on a commercial scale.

The marine red alga *Kappaphycus alvarezii* is economically important due to the production of the gelling agent kappa carrageenan, which is used in industrial gums and other products [5]. The algae are economically important due to the extraction of kappa carrageenan for the production of agar,

gelatin and other nutraceutical and pharmaceutical products. Carrageen is a soothing agent used in ice cream, toothpaste, jellies, medicines and paint. The *K. alvarezii* economically important red tropical seaweed, which is highly demanded for its cell wall polysaccharide, is the most important source of kappa carrageenan. It is easily accessible, in huge amounts, for food and pharmaceutical applications [6]. The *K. alvarezii* is effortlessly accessible in huge amount for food and pharmaceutical applications [7]. The seaweed edible film showed the possible uses in various industries including food industries, pharmaceutical, cosmetic and toiletries industries and also agricultural industry. Antioxidants are effective in protecting the body against damage by reactive oxygen species.

The negative effects of oxidative stress may be mitigated by antioxidants. Marine algae extracts have been demonstrated to have strong antioxidant properties [8]. Phenolic compounds play an important role as antioxidants.

The present investigation focused the possibility of utilizing the marine red algae *Kappaphycus alvarezii* and their active principles in biological effects, including antioxidant, and nutraceutical potential.

2. Materials and methods

2.1 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 obtained seaweed has to be cleaned completely before using for experiment. The seaweed

was washed under running tap water to eliminate all the unwanted foreign particles. 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.

2.2 Extracts of samples

The samples were extracted by using two solvents of methanol and Acetone by the method described as [9]. 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.

2.3 Analysis of macro & micronutrients potentials

Crude samples were subjected for phytochemical screening according to the standard methods as described by Trease and Evans [10] and Harper [11]. Carotenoid content was determined by [12] modified method, Dissolve 0.05 g of dried powdered sample in 2 ml 80% acetone and centrifuge at 5000 rpm for 10 min. Take supernatant and volume made up to 5 ml. Read the absorbance of solution at 480 and 510 nm. The carotenoid content was calculated by the formula as follows: $7.6 [E_{480} - 1.49 E_{510}]$. Vitamin C was analysed by dichlorophenol indophenols method by Chinoy and Singh [13] and polyphenol was analysed by spectrophotometric method [14] and quantitative analysis on the amount β -carotene was performed according to the modified procedure [15]. Macromolecules such as carbohydrate was analysed by Anthrone method [16], protein was measured by lowrey's method [17] 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 standard methods of analysis of AOAC [18] association of analytical chemicals.

3. Results and discussion

Marine organisms are a rich source of structurally novel and biologically active metabolites.

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. *Kappaphycus* species are suitable for industries to extract the phycocolloids (agar-agar, carrageenan, etc). The demand for carrageenan seaweeds is a derived demand influenced primarily by the market for carrageenan products. Refined or semi-refined carrageenan has been widely used in dairy, meat, pet food, water gels and other products [19].



Fig 1: Shows the sample of *K. alvarezii* (red algae)

The principal pigments found in Rhodophyta or red seaweeds are phycoerythrin and phycocyanin and the primary polysaccharides are agars and carrageenan. Red algae are ecologically significant as primary producers [Figure 1] providers of structural habitat for other marine organisms which is highly demanded for its nutraceutical and pharmaceutical applications. Marine algae are known to produce a variety of compounds and some of them have been shown to possess biological activity of potential medicinal values in the last three decades the discovery of metabolites with biological activities from seaweeds has increased significantly.

Table 1: Phytochemical Screening of *Kappaphycus alvarezii* crude extracts

Phytochemicals	Methanol Extract	Acetone Extract
Pigments	+	+
Fibre	+	+
Vitamin	+	+
Carbohydrates	+	+
Proteins	+	+
Fat	-	-
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;

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 (Table1) showed that contained carbohydrate, protein, gum, mucilage, fat, phenols and nutrients found to be plentiful as reported by many investigators.

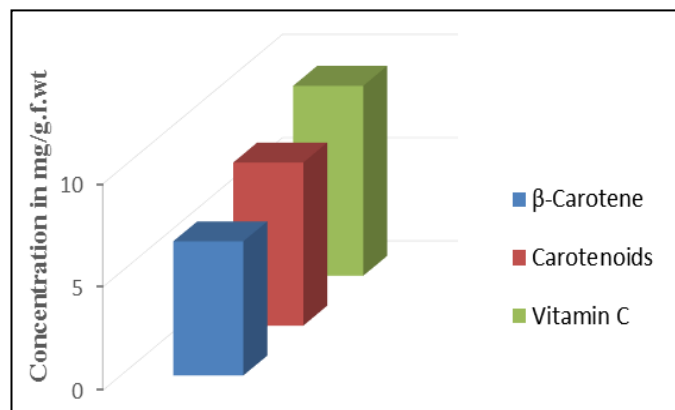


Fig 2: Analysis of nutrients in *K. alvarezii*

Legends

Figure 2 showed preparation of natural antioxidants through classical biochemical techniques to get high yield. X – indicates the quantity of vitamin C, carotenoids and β-carotene nutrient found in extracts sample.

The powder was then used for the estimation of parameters such as bioactive molecules, antioxidants and minerals. Antioxidant potential of the red algae (*Kappaphycus alvarezii*) was determined by estimation of vitamin C, carotenoids and β-carotene. The quantity of vitamin C was high than carotenoids and β-carotene. Seaweed is a rich source of several vitamins, including vitamin A (in the form of carotenoids and vitamin C).

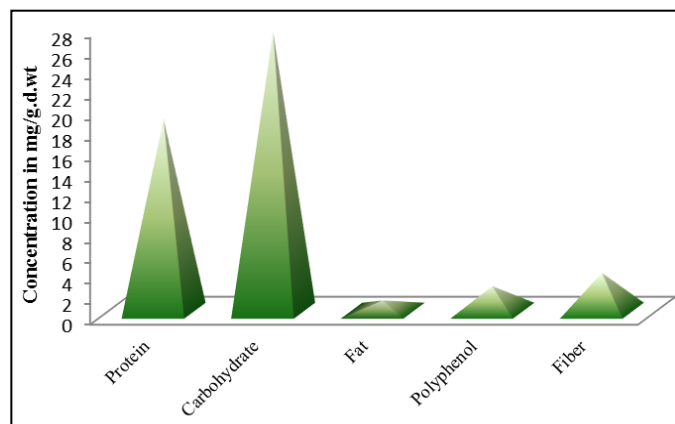


Fig 3: Estimation of macromolecules in *K. alvarezii*

Legends

Figure 3 represented the presence of various nutritive macromolecules in extracts of dried samples.

Based on experimental results the carbohydrates were high as 29gm than protein 18.78gm when compared to other macromolecules like fiber poly phenol, vitamin and fats. Hence the species can serve as functional food with vital nutritional and biological value. Seaweed may also contain chemicals that benefit cholesterol levels, and it may have anti-inflammatory and antioxidant properties.

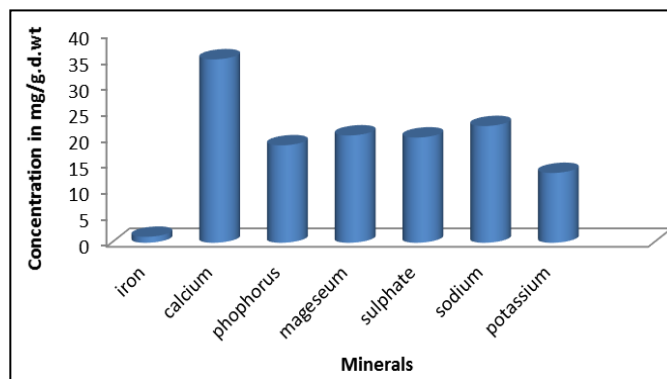


Fig 3: Determination of Micronutrient in *K. alvarezii*

Legends

Figure 4 indicated the contents of mineral nutrient present in the acid digested samples. Results were represented five replicated experiments

Micro nutrient metals such as iron, phosphorus, calcium, sulphate sodium potassium and magnesium [Figure 4]. The primary metabolites produced by these organisms may be potentially bioactive compounds of interest in the pharmaceutical industry. Results of this study suggested that the utility of *Kappaphycus alvarezii* proved to be a promising area of pharmaceutical study [19]. Seaweed is also a rich source of several minerals, including calcium, magnesium, potassium, copper, and iron. Nutrients and phytochemicals found in seaweed in food products as a way to reduce the risk of cardiovascular disease.

Carrageenan is a soluble fiber product derived from red seaweed and a food additive that has been consumed for hundreds of years around the world. Carrageenan is a versatile product, used in a variety of foods such as ice cream and other dairy products, syrups and lunch meats. Polysaccharides are well known additives in the food industries as had the capabilities of gel forming and thickening agent which are commonly extracted from seaweeds in the form of carrageenan. In addition, carrageenan is used to improve the sustainability for profile dietary supplements and non-food applications like personal care, pharmaceuticals and industrial uses. Derived from nature, carrageenan replaces animal-based products, like gelatin in soft capsules in pharmaceuticals, vitamins and dietary supplements and petrochemicals in paints and varnishes, cosmetics and lotions, reducing their VOC (volatile organic content) levels [20]. Carrageenan also improves the manufacturing process efficiency and stability of toothpaste. When carrageenan is added to foods, it safely and efficiently stabilizes food. It is often used as a thickening agent much in the same way ingredients such as flour, cornstarch and tapioca are used to thicken or bind other ingredients. Carrageenan is a permitted additive for use in organic food in many countries.

4. Conclusion

Our results suggest that seaweeds have been widely used for

human consumption in many parts of the world. Marine algae serve as a source of minerals, vitamins, fiber, antioxidants and phenol and the crude extracts of *Kappaphycus alvarezii* exhibited reducing power and hydroxyl radicals scavenging activity higher than that of standard antioxidants. Carrageenan is derived from red seaweed that is cultivated in an environmentally friendly and sustainable manner. Most red seaweed is produced in Southeast Asia by small family farms without the need for fresh water or arable land. Carrageenan has a long history as a safe food stabilizer that thickens and extends shelf-life efficiency, helping to provide healthy and nutritious food to a hungry world. Different bodies of scientific experts have reviewed all the science relevant to the way we consume carrageenan and continue to conclude that carrageenan is safe for use in foods.

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