A STUDY ON PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITY OF MARINE ALGAE (KAPPAPHYCUS ALVERIZII)

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Abstract

The marine red algae Kappaphycus alvarezii is economically important due to the production of the gelling agent kappa carrageenan, which is used in industrial gums and other products." Carrageen is a smoothening agent used in ice cream, toothpaste, jellies, and medicines. In this study the pharmacological activity of Kappaphycus alverizii was assessed. The seaweed showed (0.996) high antioxidant property compare with standard ascorbic acid (0.160). The high level Anti inflammatory activity was found in seaweed. The anti inflammatory activity of the Bioactive compounds were concentration dependent, with the increasing concentration the activity is also increased. From this study it was concluded that seaweed possessed the elevated amount of radical scavenging capacity. Hence, it could be exploited as one of the potential sources for development pharmaceutical products in order to treat the various diseases such as cancer, coronary heart disease, gastro intestinal diseases, rheumatic diseases, Antiulcer, Digestive system related problems and diabetics.

Keywords: Kappaphycus alvarezi, Phytochemical, Anti oxidant, Anti inflammatory and Diseases

1. INTRODUCTION

Algae (Latin for "seaweed") are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms, such as the giant kelps that grow to 65 meters in length. The US Algal Collection is represented by almost 300,000 accessioned and inventoried herbarium specimens. The largest and most complex marine forms are called seaweeds have a nucleus enclosed within a membrane and plastids bound in one or more membranes (Nabors, 2004; Patrick, 2004). The red algae Kappaphycus and Betaphycus are now the most important sources of carrageenan, a commonly used ingredient in food, particularly yoghurts, chocolate milk and repaired puddings.
**KAPPAPHYCUS ALVAREZII**

**CLASSIFICATION**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
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<tbody>
<tr>
<td>Subkingdom</td>
<td>Biliphyta</td>
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<tr>
<td>Phylum</td>
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<tr>
<td>Class</td>
<td>Florideophyceae</td>
</tr>
<tr>
<td>Order</td>
<td>Gigartinales</td>
</tr>
<tr>
<td>Family</td>
<td>Areschougiaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Kappaphycus</td>
</tr>
<tr>
<td>Species</td>
<td><em>K. alvarezii</em></td>
</tr>
</tbody>
</table>

**English Name:** Red alga

**Similar Species**

*Kappaphycus alvarezii*, *Kappaphycus cottonii*, *Kappaphycus inermis*, *Kappaphycus interme*, *Kappaphycus procrusteanum* and *Kappaphycus striatum*.

*Kappaphycus alvarezii* is a tough, fleshy, firm marine algae ("seaweed") up to 6 feet in length. Its coarse thalli (plural of thallus, a plant body that is not differentiated into root, stem or leaf) are approximately 1/2 inch in diameter. The thalli are heavy, with major axes relatively straight, lacking secondary branches near the tips. It is frequently and irregularly branched, most branches primary. Shiny green to yellow orange, it has a variable form, from a few small branches in shallow water to tangled and gnarled in deeper water, and it may be loosely attached to broken coral or floating, sometimes in large, moving mats. It typically occurs in waters 3 to 50 feet deep. *Kappaphycus species* are among the largest tropical red algae, with a high growth rate (can double in biomass in 15 to 30 days). It was thought until recently that their only method of dispersal was by vegetative fragmentation, which was thought to limit their expansion. *Kappaphycus species* are among the largest tropical red algae, with a high growth rate (can double in biomass in 15 to 30 days). It was thought until recently that their only method of dispersal was by vegetative fragmentation, which was thought to limit their expansion (Huisman, 2007).
Uses:

Granbom et al., (2004) states that, "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." Carrageen is a smoothing agent used in ice cream, toothpaste, jellies, medicines and paint (Granbom et al., 2004 and WHO 2015). Seaweed draws an extra ordinary wealth of mineral and vitamins that can account for up to 36% of its dry mass. It also have much higher oleic and alpha-linoleic acid and EPA content. Based on the above said reason the present study aim to indent that examines isolation of bioactive compounds and its pharmacological activities of brown seaweed.

2. MATERIALS AND METHODS

2.1 Sample Collection

The marine algae Kappaphycus alvarezii was collected from Mallipattinum coastal area at Thanjavur District.

![Kappaphycus alvarezii](image)

**Fig 1: Kappaphycus alvarezii**

2.2 Preparation of Extract

The 20g of fresh algae material was crushed and extracted by cold percolation method using Ethanol sequentially at 24 hrs. The extract was filtered using Whatmann filter paper and concentrated. The extract was put in airtight container and stored in refrigerator which was subjected to following analysis.

2.3 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The extract of Kappaphycus alvarezii was subjected to qualitative test for the identification of various plant constituents by Harborne method (1973).
2.4 ISOLATION OF LIPID (Calzolari, et al., 2009, Zuliani, et al., 2009 and Schuchardt, et al., 2010)

5g sample was taken in a test tube. 10 ml of phosphate was added to grind well using mortar and pestle. Add 2ml of 80% phenol and 2 ml of 80% chloroform in a grinded sample allow to centrifugation process at 3000 rpm for 15 minutes. The supernatant was removed and take a pellet in a tube add Proteinase k to digest the protein in a sample. After it was mixed with 2 ml of 80 % ethanol centrifuge at 3000 rpm for 15 minutes and the supernatant was discarded the pellet was stored in 4° C for further use.

2.5. ISOLATION OF OMEGA- 3 - FATTY ACID (Calzolari, et al., 2009, Zuliani, et al., 2009 and Schuchardt, et al., 2010)

The stationary phase was prepared as silica slurry with solvent (EtOAc) or buffer at 1: 2 and applied to a glass plate. (0.25 mm thickness for analytical separations and 2 – 5 mm thickness for preparative separations are prepared). After application of the adsorbent, the plates are air – dried for 10 – 15 minutes and then oven - dried for 10 – 15 minutes at 100°C – 110°C. The samples are spotted using capillary tubes at 1.5 cm distances between them for preparative TLC, the sample is applied as a band across the layer rather than as a spot. The organic phase is separated from the acidified filtrate (pH 11-12). It is extracted with chloroform (3 xs), condensed by evaporation and used for chromatography. The omega- 3 - fatty acid spots were separated using the solvent mixture chloroform, glacial acetic acid, methanol and water in the ratio of 16: 8:3:2. The color and Rf value of the separated omega- 3 - fatty acid were recorded both under ultra violet and visible light after spraying with anisaldehyde and sulphuric acid.

2.5. ISOLATION OF DHA FROM OMEGA- 3 - FATTY ACID (Calzolari, et al., 2009, Zuliani, et al., 2009 and Schuchardt, et al., 2010)

The stationary phase is prepared as silica slurry with solvent (EtOAc) or buffer at 1: 2 and applied to a glass plate. (0.25 mm thickness for analytical separations and 2 – 5 mm thickness for preparative separations are prepared). After application of the adsorbent, the plates are air – dried for 10 – 15 minutes and then oven - dried for 10 – 15 minutes at 100°C – 110°C. The sample was wetted with half diluted chloroform and lixiviated with Ethanol for 24 hrs at RT. The organic phase is separated from the acidified filtrate (pH11-12). It is extracted with chloroform, condensed by evaporation and used for chromatography. The sample of DHA was isolated by using a solvent such as Chloroform, Diethyl ether, methanol and acetic acid.
in the ratio of 4: 3: 2: 1. The color and \( R_f \) value of the separated DHA was recorded both under ultra violet and visible light.

2.6. *In-Vitro* Screening For Anti-Inflammatory Activity of DHA by HRBC Method (Gandhidasan *et al.*, 1991).

**Procedure**

The 2ml blood was centrifuged and the packed cells were washed with isosaline and 10% suspension was made with Isosaline. The drug samples were prepared by suspending the residues in hot water. The assay mixture contained the drug, 1 ml phosphate buffer, 2 ml hypo saline, 0.5 ml HRBC suspension, hydrocortisone sodium was used as the reference drug. Instead of hypo saline 2 ml of distilled water was used as a control. All the assay mixture were incubated at 370C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm. The percentage haemolysis was calculated by the following formula.

**Calculation**

The percentage of HRBC membrane stabilization was calculated using the formula,

\[
\text{Percentage protection} = \frac{100 - \text{Optical density of sample}}{\text{Optical density of control}} \times 100
\]

**DETERMINATION OF ANTIOXIDANT PROPERTY BY POWER REDUCING ASSAY** Yildrim *et al.*, 2000.

**Procedure**

1 ml of sample was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferric chloride (0.5ml, 0.1%) and absorbance measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid standard.

**Calculation**

\[
\text{Percentage scavenging activity} = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100
\]
Where $A_{control}$ is the absorbance of the control. $A_{test}$ is the absorbance in the presence of the sample.

1. RESULTS AND DISCUSSION

In this study the DHA was successfully isolated from the selected sample and their pharmacological potential was analyzed. The results are described below.

**PHYTOCHEMICAL STUDIES OF SEAWEED (Kappaphycus alvarezii)**

The preliminary phytochemical investigation revealed the presence of Saponins, Carbohydrates, Protein, tannins, Gum and flavonoids, as showed in Table-1 and fig 2. The results showed that of *Kappaphycus alvarezii* demonstrated the presence of all phytocompounds tested, except the absence of Alkaloids, Carbohydrates, Reducing Sugar, Tannins, Flavanoids, and Gum in above extracts of *Kappaphycus alvarezii*. Thus the preliminary screening test may be useful in the detection of the bioactive compounds and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

**Table: 1 PHYTOCHEMICAL STUDIES OF SEAWEED (Kappaphycus alvarezii)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Tests</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Reducing Sugars</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Gum and Mucilage</td>
<td>+</td>
</tr>
</tbody>
</table>
ISOLATION OF OMEGA 3 FATTY ACID BY THIN LAYER CHROMATOGRAPHY

The Omega 3 fatty acid was isolated by Thin layer Chromatography. The Rf value is 0.64 %. Fatty acids are the simplest of the biopolymers in that they can consist simply of a carboxylic acid functional group with a hydrocarbon chain. The selected sample contains high amount of Omega 3 fatty acid which is widely used for the medicine to treat the Ulcer, cardio problems, cancer, inflammation and developmental disorders.

Fig 3: ISOLATION OF DHA BY THIN LAYER CHROMATOGRAPHY

The DHA was isolated from the selected sample. The sample is rich in polyunsaturated fatty acids (PUFAs). An essential omega-3 fatty acid, Docosahexaenoic acid (DHA), was detected. The Rf value of the selected sample shows 4.6 respectively. This feature of these newly isolated Fatty acids is potentially useful in commercial mass culture in local areas or as a medicine preparation.

INVITRO ANTI INFLAMMATORY ACTIVITY

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by
reducing inflammation as opposed to opioids, which affect the central nervous system. Here the isolated bioactive compounds DHA possess the good anti-inflammatory activity and the inhibition rate is 60% at 0.5ml of concentration than the seaweed extracts which shows 58% of activity. (Table 3)

Table 3 In Vitro Study of Anti Inflammatory Activity by HRBC Method

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Percentage of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard Diclofenac</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>Seaweed Extract</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>DHA</td>
<td>66</td>
</tr>
</tbody>
</table>

HRBC method was selected for the in vitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The result indicted that the biopolymer has significant anti-inflammatory property. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the fatty compounds present in the sample.

Jose Estevez et al., 2004 suggested that significant quantities of 2-O-methyl- and 3-O-methyl-L-galactose units are also present in *Kappaphycus alverizii*. A great tendency to retain Ca$^{2+}$ and Mg$^{2+}$, in spite of massive treatments with Na$^+$ and K$^+$ salts, was observed. The complexation between agarans and agarans–κ-carrageenans through divalent cations and the possible zipper-type carbohydrate–carbohydrate interactions would be two complementary mechanisms of interactions.

*K. alvarezzi* showed vitamin A activity of 865 μg retinal equivalents/100 g of sample. It contained a higher quantity of unsaturated fatty acids, in which relative percentage of oleic acid, cis-heptadecanoic acid, and linoleic acid utility of *K. alvarezzi* (Eucheuma) for various nutritional products, including antioxidant for use as health food or nutraceutical supplement (Mohamed Fayaz et al., 2005).
4. In vitro Antioxidant activity

In this present study, we have evaluated the free radical scavenger activity of DHA and seaweed extract by power reducing method due to the presence of high amount of bioactive compounds.

A concentration dependent scavenging activity was observed in crude extract of seaweed and DHA with maximum activity of 85% and 91% respectively (table. 4). They had relatively higher reducing power than other rice’s due to the presence of high flavonoid and phenolic compounds.

Table 4 In Vitro Study of Antioxidant Activity by Power Reducing Method

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Percentage of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Seaweed Extract</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>DHA</td>
<td>91</td>
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</table>

Natural antioxidants that are present in herbs, Rice and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices, rice and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In this present study, we have evaluated the free radical scavenger activity of selected sample due to the presence of elevated amount of flavonoid.

Reactive oxygen species generated in the human body cause oxidative damage and responsible for many degenerative diseases such as coronary heart diseases, atherosclerosis, diabetes, ageing and cancer. Kris-Etherton et al., (2002) analyzed that the concentration of total phenolics in the grain has been positively associated with the antioxidant activity, with potential beneficial effects on health, such as reduction of oxidative stress, aid in the prevention of cancer in the control of blood lipids and related diseases, which may help in the prevention of cardiovascular problems, and in the prevention of the complications of diabetes. Through this study a definite dosage formulation for consumption of DHA and formulated products selected seaweed Kappaphycus alverizii which will enhance health promotion, when taken as fortified foods or dietary supplements. Hence, it could be exploited as one of the potential sources for development pharmaceutical products in order to treat the various diseases such as cancer, coronary heart disease, gastro intestinal diseases, rheumatic diseases, Antiulcer, Digestive system related problems and diabetics. The further study will
be carried out for the preparation of Supplement and Medicine by using the bioactive compounds and seaweeds.

Reference


