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# An Investigation on the Antioxidants, Antifungal and Antibacterial of the *Kappaphycus Alvarezii*

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## ABSTRACT

This paper presents the details of investigations carried out on the various aspects such as antioxidants, antifungal and antibacterial of *Kappaphycus alvarezii*. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Antioxidant potential of the red algae (*Kappaphycus alvarezi*) was determined by estimation of vitamin C, vitamin E, selenium and magnesium. The compositions of vitamin C, vitamin E, selenium and magnesium were found to be 0.123gm, 0.243gm, 0.0012gm, 0.0245gm per 100 gm respectively. It was noted that *Kappaphycus* is exhibited more antioxidant against vitamin E and magnesium. It was observed that *k. alvarezii* was highly active against *Aspergillus fumigatus* as compared to other test organisms in the case of anti-fungal whereas it was shown maximum activity against *Staphylococcus aureas* in the case of anti-bacterial.

**Keywords:** Red algae, *Kappaphycus*, antioxidants, antifungal; antibacterial

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## INTRODUCTION

Fresh seaweeds have long been used in food diets, as well as traditional remedies. In Asian countries such as in China, Japan and Korea, seaweeds serve as an important source of bioactive natural substances. Many metabolites isolated from marine algae possess bioactive effects. The discovery of metabolities with biological activities, from macroalgae, has increased significantly in the past three decades, on the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants. Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Some seaweeds have the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. In the marine ecosystems seaweeds are directly exposed and are susceptible to ambient microorganisms such as bacteria, fungi and viruses. Antioxidants are effective in protecting the body against damage by reactive oxygen species. In Japan, various kinds of edible seaweeds have been traditionally consumed as additives and seasonings with different food stuffs. They have multiple therapeutic benefits such as suppression against some types of cancer. However, natural antioxidants are not limited to terrestrial sources. Some of the seaweeds are consider to be a rich source of antioxidants. Examples include, chlorophylls, carotenoids, tocopherol derivates such as vitamin E and related isoprenoided that are structurally related to plant – derived antioxidant were found in some marine organisms. During the course of antioxidant activity screening of seaweeds commonly found in various seasons.

Antioxidants are effective in protecting the body against damage by reactive oxygen species. There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) that are commonly used in lipid containing food [1]. Many natural antioxidants have already been isolated from different kinds of plant, such as oilseeds, cereal crop, vegetables, leaves, roots, species and herbs [2]. Among natural antioxidants, phenolic antioxidants are in the fore front as they are widely distributed in the plant kingdom. Plants contain diverse group of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid datives and flavonoids. Reactive oxygen species (ROS) is generated in living organisms during metabolism [3]. Excess amounts of ROS may be harmful because they can initiate biomolecular oxidants which lead to cell injury and death and create oxidative stress which results in numerous diseases and disorders such as cancer, stroke, mycocardial infraction, diabetes, septic and haemorrhagic shock alzhemer's and parkinsons diseases. The negative effects of oxidative stress may be mitigated by antioxidants. Marine algae extracts have been demonstrated to have strong antioxidant properties [4, 5]. Some of the seaweeds are considered to be a rich source of antioxidants) [6].

In general, from the critical review of literature, it has been observed that the most studies on the nutrient contents of seaweeds have concerned fresh plants. Little is known of

the effects of processing by drying or canning. The present investigation aims at on the investigation on antioxidants, antifungal and antibacterial aspects of *Kappaphycus sp.*

## MATERIALS AND METHODS

Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Algae samples were cleaned at epiphytes and necrotic parts were removed. Samples were 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 estimation of antioxidants, antifungal and antibacterial of *Kappaphycus sp.* This powder was stored in cold conditions in an airtight container and analysis was carried out within three months of processing. Material methods for the estimation of antioxidants, antifungal and antibacterial of *Kappaphycus sp.* are presented below.

### Antifungal assay

Antifungal activity of the algal extract residues was tested against five human pathogenic fungi, namely, *Aspergillus fumigatus, Candida albicans, Epidermophyton sp., Microsporum canis* and *Trichophyton verrucosum.* Disc diffusion method was adopted for antifungal assay. Hi-Media sterile paper discs (SD 067) (6 mm diameter) were impregnated with the extract of fraction residues dissolved in ethanol to give a concentration of 700 µg/disc. The test fungi were maintained on potato dextrose agar (PDA) plates. The medium had the following composition.

### Potato dextrose agar medium (PDA)

Commercially obtained PDA MO 96 (Hi-media) was used. The medium contained

| | |
|---|---|
| Potato infusion from 200 g potatoes | |
| Dextrose | 20. 0 g |
| Agar | 18.0 g |
| Sterile distilled water | 1000 ml. |

Prepared medium was sterilized by autoclaving at 1.1 kg/cm$^2$ pressure (121$^o$C) and used. Seeding of PDA plates with the desired test fungi was done with actively growing cultures with the help of a sterile cotton swab. The impregnated discs were firmly placed on these seeded plates, incubated at 29±1$^o$C for 48 hrs and observed for zones of inhibition around the discs.

### Antibacterial assay

Clinical isolates of *Bacillus megaterium, Citrobacter sp., Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris* and *Vibrio cholera* were used as the test

organisms. For the assay of fraction residues, *Staphylococcus aureus* was used as the test organism. The bacteria were subcultured and routinely maintained on both nutrient agar and Muller-Hinton agar. However, for inoculum preparation and assay of antibacterial activity, Muller-Hinton agar was used.

**Nutrient agar**

| | |
|---|---|
| Beef extract | 3.0 g |
| Peptone | 5.0 g |
| Sodium chloride | 5.0 g |
| Agar | 15.0 g |
| pH | 7.2 |

The above were dissolved in 1.0 L distilled water and sterilized at 121$^{o}$C (1.1 kg/cm$^{2}$ pressure) for 15 minutes.

**Muller-Hinton Agar**

| The medium contained in 1.0 L of water | |
|---|---|
| Casein hydrolysate (enzymic) | 17.5 g |
| Beef infusion | 30.0 g |
| Soluble starch | 1.5 g |
| Agar | 20.0 g |

The final pH of the medium after sterilization at 1.1 kg/cm$^{2}$ (121$^{o}$C) for 15 minutes was adjusted to 7.4±0.2 (at 25$^{o}$C).

**Bioassay**

Disc diffusion method was adopted for the determination of antibacterial activity of the extract residues. From the stock cultures of various test organisms, inoculum was prepared by sub-culturing each of the organism on Muller-Hinton agar at 37$^{o}$C. Seeding of Muller-Hinton agar plates were done using the 24 hr culture with a cotton swab under aseptic conditions. The discs loaded with extract residues were aspetically placed on top of the seeded medium and gently pressed to ensure contact. The plates were then incubated at 37$^{o}$C. After overnight incubation, the plates were observed for zones of inhibitions.

**Antioxidants**

The property of antioxidant was evaluated by estimating the various vitamins and metals. The procedure is given for typical vitamins and metals.

**Estimation of Vitamins**

Vitamin content of dry, powdered sample of the alga was estimated by HPLC. The dried sample, 100 mM perchloric acid: acetonitrile (2:1 v/v) solution was added and left in a water bath at 50$^{o}$C for 30 minutes. The resulting solution was centrifuged at 6000 X g and the upper layer was used for HPLC analysis. HPLC system (SCHIMADZU) equipped with UV detector was used under the following analytical conditions for the estimation of nicotinic acid, vitamin B1, vitamin B6 and vitamin B12.

| | | |
|---|---|---|
| Column | : | STR ODS-11 (4.6*150) |
| Mobile | : | Acetonitrile /100 mM, Sodium phosphate buffer (pH 2.1) 0.8 mM Sodium octane sulfonate (9:1 v/v) |
| Flow rate | : | 1.0 ml/min |
| Temperature | : | 40$^{o}$C |
| Detection | : | UV/210 nm |

**Estimation of heavy metals**

100 ml of water was added to 20 ml of bromine in a glass- stoppered bottle. The stopper was inserted into the bottle, and shook. It was allowed to stand for 30 minutes, and used the supernatant layer.

**Procedure**

An accurately weighed quantity of the powder was transferred to equivalent 3 mg of iodide, to a nickel crucible. 5 g of sodium carbonate, 5 ml of 50% (w/v) sodium hydroxide solution, and 10 ml of alcohol, was added after taking care that the entire specimen was moistened. The crucible on a steam bath was heated to evaporate the alcohol, then dried the crucible at about 100°C for about 30 minutes to prevent spattering upon subsequent heating. The crucible with its contents was transferred to a furnace heated to about 500°C, and heated the crucible for about 15 minutes. The crucible was cooled, added 25 ml of water, covered the crucible with a water glass, and boiled gently for about 10 minutes. The solution was filtered, and washed the crucible with boiling water, collecting the filtrate and washings in a beaker. Phosphoric acid was added until the solution is neutral to methyl orange, then added 1 ml excess of phosphoric acid. Added excess of Bromine water, and boiled the solution gently until colorless and then for 5 minutes longer. A few crystals of salicylic acid was added, and cooled the solution to about 20°C. 1 ml of phosphoric acid was added and about 0.5 g of potassium iodide, and titrated the liberated iodine with 0.005 N sodium thiosulfate VS, adding starch solution when the liberated iodine color has nearly disappeared. The quantity was calculated, in µg, of iodide in the portion of powder taken by the formula: 105.8*V*N/0.005, in which V is the volume, in ml, of sodium thiosulfate consumed, and N is the normality of the sodium thiosulfate solution used.

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## RESULTS AND DISCUSSION

### Antioxidants

Antioxidant potential of the red algae (*Kappaphycus alvarezi*) was determined by estimation of vitamin C, vitamin E and heavy metals such as seleium and magnesium. Results obtained for various antioxidants of *Kappaphycus sp*. are shown in Table 1. Figure 1 shows the graphical representation of various antioxidants available in the species. From Table 1 and Figure 1, it can be observed that the compositions of vitamin C, vitamin E, Selenium and Magnesium are 0.123gm, 0.243 gm, 0.0012 gm, 0.0245 gm per 100 gm respectively. From the studies, it can be noted that *Kappaphycus* is exhibited more antioxidant against vitamin E and magnesium. Bin et al [7] measured the methanol-chloroform extract of the marine red alga, *Rhodomela confervoides* for antioxidant activity using the alpha, alpha-diphenyl-beta-picryhydrazyl radical-scavenging assay and the beta-carotene-linoleate bleaching assay systems and compared with those of the positive controls of butylated hydroxytoluene, galli acid and ascorbic acid. It was observed that significant associations between the antioxidant potency and the total phenolic content, as well as between the antioxidant potency and the reducing power were found for the tested fractions and sub-fractions. Further, their result suggested that the phenolic compounds might be major contributors to the antioxidative activities of *R. confervoides*. Fayaz et al [8] carried out studies on chemical composition, iron bioavailability and antioxidant activity of *Kappaphycus alvarezii* (Doty). In the case of antioxidant activity, the yields of extractables obtained from *K. alvarzzi* using various solvents such as n-hexane, acetone, ethyl acetate, ethanol were analyzed. Among the different individual solvents used, ethanol extracted the most constituents in successive extractions. In the case of chloroform:methanol, the yield of extrables was comparatively high, 9.48%(1:1) and 7.8%(2:1). The polyphenol content in the ethanol extract was maximum (4.83%) followed by the chloroform:methanol extract (3.32%), whereas individual solvent extracts using ethyl acetate, n-hexane, and acetone showed less than 1.5% polyphenol. Acetone and hexane extracts showed moderate activity at all the concentrations. The extracts of chloroform:methanol mixture showed comparatively higher activities 56.4%, 61.24% and 75.8% and 82.5% at 1:1 and 2:1 concentration respectively at 750 and 1000 ppm levels. This is mainly due to the presency of ascorbic acid and polyphenols which are hydrophilic antioxidants. Xiao et al [9] determined antioxidant activity (AA), total phenolic content and reducing power of the crude extract, fractions and subfractions derived from a red alga, *polysiphonia urceolata*. Their result showed that the crude extract and the ethyl acetate-soluble fraction exhibited higher antioxidant activity than butylated hydroxytoluene in the alpha-alpha-diphenyl-beta-picrylhydrazyl assay model, at all of four concentration levels tested, while in the beta-carotene-linoleate assay system, the crude extract and the ethyl acetate-soluble fraction exhibited similar or in most cases, higher AA than gallic acid and ascorbic acid at the same concentrations. Chew et al [10] studied the total phenolic content (TPC) and antioxidant activity (AOA) of 50% aqueous methanol extracts of the marine algae, Padina antillarum, Caulerpa racemosa and *Kappaphycus alvarezii.* P. antillarum was found to have the highest TPC, 2430±208mg gallic acid equivalents (GAE) per 100g dried sample and ascorbic acid equivalent antioxidant capacity (AEAC), 1140±85mg AA/100g. C. racemosa and *K. alvarezii* displayed lower TPC and AEAC. C. racemosa

had 144±22 mg GAE/100g dried sample of TPC and 14.3±2.0 mg AA/100g of AEAC, while *K. alvarezii* had 115±35mg/100g dried sample of TPC and 37.8±16.8 mg AA/100g of AEAC.

**Table 1: Various Chemical constituents of *Kappaphycus sp.***

| S.No. | Chemical constituent | Composition | SD |
|---|---|---|---|
| 1 | Antioxidants | | |
| 2 | Vitamin C | 0.123gm/100mg | 0.123±0.02 |
| 3 | Vitamin E | 0.243gm/100mg | 0.243±0.015 |
| 4 | Selenium | 0.0012gm/100mg | 0.0012+0.03 |
| 5 | Magnesium | 0.245gm/100mg | 0.245±0.02 |

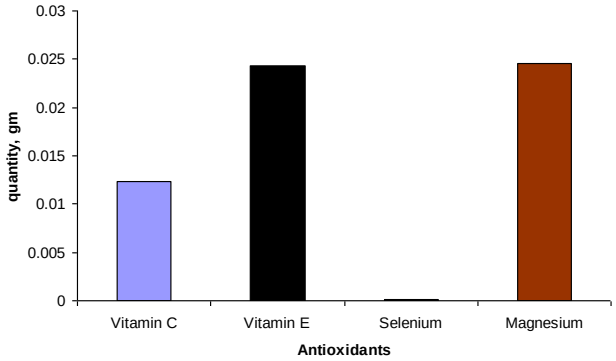


**Figure 1: Plot of antioxidants available in *Kappaphycus sp.***

## Anti-fungal and anti-bacterial activity

The chloroform: methanol (2:1v/v) extract residue of *k.alvarezii* was highly active against *Aspergillus fumigatus* as compared to other test organisms (Figure 2). Activity of the algal extract was 65% against *microsporum canis* and 40.4% against both *Epidermophyton sp*. and *Candida albicans*. The algal extract had no effect on the growth of *Trichophyton verrucosum*.

The chloroform:methanol (2:1 v/v) extracts of the experimental alga was prepared as described earlier and tested at a concentration of 700 µg/disc by disc diffusion method against eleven pathogenic bacteria (Figure 3). The extract residues of the algae recorded maximum activity against *Staphylococcus aureas* with an inhibition zone of 6.1 cm. The extract residues did not show any effect on the growth of *Proteus vulgaris*. Thirty one to fifty five percent of maximum activity was observed against remaining pathogens. A wide range of compounds were isolated and shown to be responsible for the antimicrobial activities of marine algae. Despite the isolation of a number of antimicrobial compounds from marine algal sources, none seems to have reached the level of clinical trials and the search for compounds from marine alga having clinical potential continues. The difference in the activity levels of our study in comparison with the literature may be due to different solvent systems, environmental factors and type of bioactive compound.

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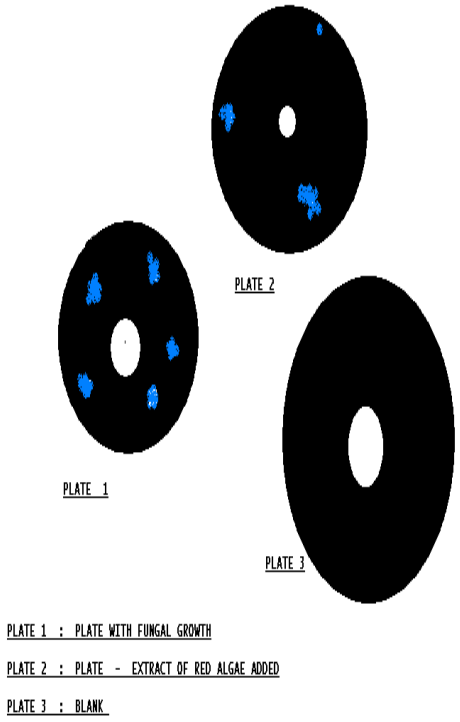


**Figure 2: Inhibition activity of *K. alvarezii* against fungal mixture**

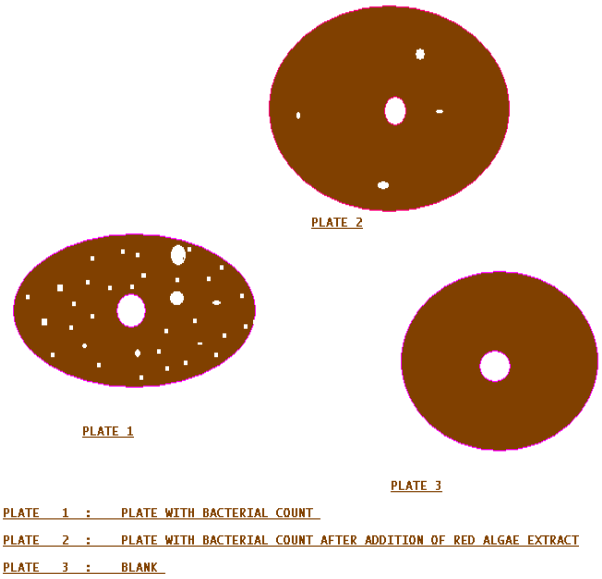


**Figure 3: Inhibition activity of *K. alvarezii* against bacterial mixture**

## CONCLUSION

Details of investigations carried out on the various aspects such as antioxidants, antifungal and antibacterial of *Kappaphycus alvarezii*. have been presented in this paper. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Antioxidant potential of the red algae (*Kappaphycus alvarezi*) was determined by estimation of vitamin C, vitamin E, selenium and magnesium. The compositions

of vitamin C, vitamin E, selenium and magnesium were found to be 0.123gm, 0.243gm, 0.0012gm, 0.0245gm per 100 gm respectively. It was noted that *Kappaphycus* is exhibited more antioxidant against vitamin E and magnesium. It was observed that *k. alvarezii* was highly active against *Aspergillus fumigatus* as compared to other test organisms in the case of anti-fungal whereas it was shown maximum activity against *Staphylococcus aureas* in the case of anti-bacterial. Results of this study suggested that the utility of *K. alvarezii* proved to be a promising area of pharmaceutical study. The differences between our results and the results obtained in the previous studies may be due to several factors. Because of the intra specific variability, occasionally related to seasonal variations as observed in the literature and at the same time test materials have trace impurities. From the overall study, it can be concluded that the *K. alvarezii* may serve as functional food with vital nutritional and biological values.

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