Profiling of Phytochemical and Antioxidant Potentials of Red Seaweed Kappaphycus alvarezii.

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Abstract

Seaweeds are one of the most important living resources of the marine environment and possess valuable bioactive compounds which are nontoxic to human health. Due to their wide applications they are utilised in pharmacological research. The present study focused on the Phytochemical analysis and screening for antioxidant potentials of the seaweed extracts of *Kappaphycus alvarezii*. In the present study the red seaweed *Kappaphycus alvarezii* was collected and using Soxhlet apparatus the selected seaweed was extracted with two different solvents, namely chloroform and methanol. Qualitative method of Phytochemical screening was performed and the antioxidant potentials of the seaweeds was evaluated using DPPH and ABTS assay. The results of the present study show some promising antioxidant activity. Further research on *Kappaphycus alvarezii* can pave the way for it to be utilized as an alternative choice as an effective therapeutics with minimized side effects.

Keywords: Antioxidant activity, Seaweed, Free radical, DPPH, ABTS, *Kappaphycus alvarezii*

Introduction

Oxygen is a highly reactive atom capable of forming free radicals which have the ability to disturb the structure and function of the cell. Free radicals are unpaired electrons which cause them to seek out and capture electron from other substance. Free radical damage is stabilized by antioxidants which posses desired redox reaction with physical and chemical compatability, non-toxic and effective at low concentration. Seaweeds are exposed to free radicals and strong oxidizing agents due to reaction between sunlight and oxygen. However, seaweed's structural component does not experience any oxidative damage (Matsukawa *et al.*, 1997). This hypothesized that seaweeds are capable to generate essential defence mechanisms against oxidation. Therefore seaweeds are considered as an important source of antioxidant substances that may also correspond to protect human body against reactive oxygen species (Plaza *et al.*, 2008). Reactive oxygen species (ROS) is generated in living organisms during metabolism (Cavas *et al*, 2005). 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 infarction, diabetes, septic and haemorrhagic shock, Alzheimer's and Parkinson's diseases. The negative effects of oxidative stress may be mitigated by antioxidants.

Kappaphycus alvarezii is a species of red algae. It is one of the most important commercial sources of Carrageenans, a family of gel-forming, viscosifying polysaccharides. *Kappaphycus* has been used in herbal medicine for its anti-inflammatory, diuretic, cholretic and hemostatic properties. In India, the use of spice is

common; *K. alvarezii powder* has been indicated as an ingredient for the preparation of spice to enhance the nutritional quality because of the presence of ash, protein, Vitamin E and crude fiber content.

The earlier study reveals the red algal species Kappaphycus alvarezii inherit compounds which includes amino acids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, starch and fatty acids (Division Rhodophyta)that has bactericidal or bacteriostatic property which are non-toxic in the nature and used as rich source of antioxidants for consumer as a healthy food on the basis of nutraceutical property. K. alvarezzi has nutritive and antioxidant property.

The present study aims to screen for phytoconstituent profiling and antioxidant potentials of *Kappaphycus alvarezii.*

Materials and Methods

Sample Collection

The seaweed K.alvarezii was collected from the Gulf of Mannar, Tamil Nadu, India. The collected seaweed samples were washed with seawater and then in fresh water and extraneous matters were removed. After that they were brought to the laboratory in sterile plastic bags. The samples were rinsed with fresh water and sterile distilled water, shade dried, cut into small pieces and powdered in a lab mixer grinder. The powdered samples were then stored in a freezer for further study.

Extract Preparation from seaweed Powder

The powdered sample of 5g was extracted in Soxhlet apparatus using chloroform and methanol (200 ml) as solvents for 8h at a temperature maintained not more than the boiling point of the solvent. The extracts were filtered using Whatman No.1 filter paper and kept under a hot air oven (40°C) for solvent evaporation. The residues obtained were stored in a freezer at -20°C.

Phytochemical screening

Phytochemical screening of the extract was carried out according to the standard method as described by Trease and Evans (1989).

(i) Test for Saponins

Each of the extract was separately stirred in a test tube, foaming which persisted on warming was taken as an evidence for the presence of saponins.

(ii) Test for Cardiac Glycoside

Small portion of the extract was hydrolyzed with dilute hydrochloric acid for a few hours in a water bath and then the hydrolysate was tested with Legal's reagent, borntrager's reagent and Baljet reagent to detect the presence of various sugars.

(iii) Test for Alkaloids

To a few ml of filtrate, 1 ml of Mayer's reagent (potassium mercuric iodide solution) was added. White or cream-coloured precipitate indicates the presence of alkaloids.

(iv) Test for Flavonoids

The extract was treated with a small piece of magnesium ribbon; this was followed by drop wise addition of concentrated hydrochloric acid. Colours ranging from orange to red indicate flavones, red to crimson indicate flavonols, crimson to magenta indicate flavonones.

(v) Test for Tannins

For tannins identification, one mL of ferric chloride (5% $FeCl_3$) was added to 1 mL of the algal extract. Formation of dark blue or greenish black color indicates the presence of tannins.

(vi) Test for Terpenoids

Two or three granules of tin metal were added in 2 ml thionyl chloride solution. Then the extract was added into test tube and warmed. The formation of pink colored indicates the presence of tri terpenoids.

(vii) Test for steroids

The extract was dissolved in chloroform and equal volume of concentrated sulphuric acid was added. Bluish red to cherry red in the chloroform layer and green fluorescence in the acid layer indicates the steroidal components in the tested extract.

(viii) Test for phenol

The extract was dissolved in 5 ml of distilled water and to this, few drops of neutral 5 % ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

(ix) Test for Coumarin

For coumarins identification, 1 mL of 10 % NaOH was added to 1 mL of algal extract. Formation of yellow colour indicates the presence of coumarins.

DPPH Assay

The effect of the extracts on DPPH radical was estimated according to the procedure described by Gadow *et al.* (1997). Two mL of 6 X 10^{-5} M methonolic solution of DPPH was added to 50 µl of a methonolic solution (10 mg ml^{-1}) of the extract and absorbance measurement was commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 minutes at room

temperature. Methanolic solutions of pure compound [quercetin] were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the extract was calculated from the absorbance value at the end of 16th minute as per the following formula. The percentage inhibition of the DPPH radical by the extract was calculated according to the formula of Yen and Duh (1994).

$$\text{Inhibition Percentage} = [(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidants at t = 16 min. All determinations were performed in triplicate.

ABTS radical cation decolorization assay

The free radical scavenging capacity of the extracts was tested using ABTS radical cation decolorization assay (Re *et al.*, 1999). ABTS was dissolved in water to get 7 mM concentration. ABTS radical cation (ABTS*$^{+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The free radical was stable for more than two days, when stored in the dark at room temperature. For the study of the test extracts, the ABTS*$^{+}$ solution was diluted with absolute ethanol to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30°C. Reagent blank reading was taken (A_0). After addition of 2.0 mL of diluted ABTS*$^{+}$ solution ($A734\ nm$ = 0.700(±0.02) to 20 µL of test sample, the absorbance was read at 30°C exactly six minutes after initial mixing (A_t). Appropriate solvent blanks were run in each assay. The percentage inhibition of absorbance at 734 nm was calculated using the following formula and decrease of the absorbance between A_0 and A_t.

$$PI = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$is the absorbance of the antioxidant at t = 6 min. All determinations were carried out in triplicates.

Result and Discussion

Fig.1. Antioxidant potentials of Methanol and Chloroform extracts of *Kappaphycus alvarezii* using DPPH assay.

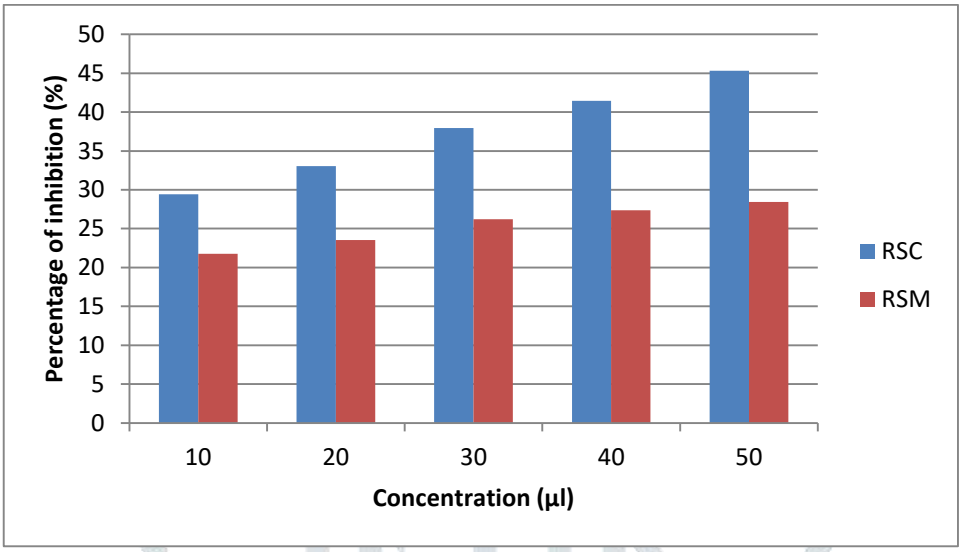

Fig.2. Antioxidant potentials of Methanol and Chloroform extracts of *Kappaphycus alvarezii* using ABTS assay.

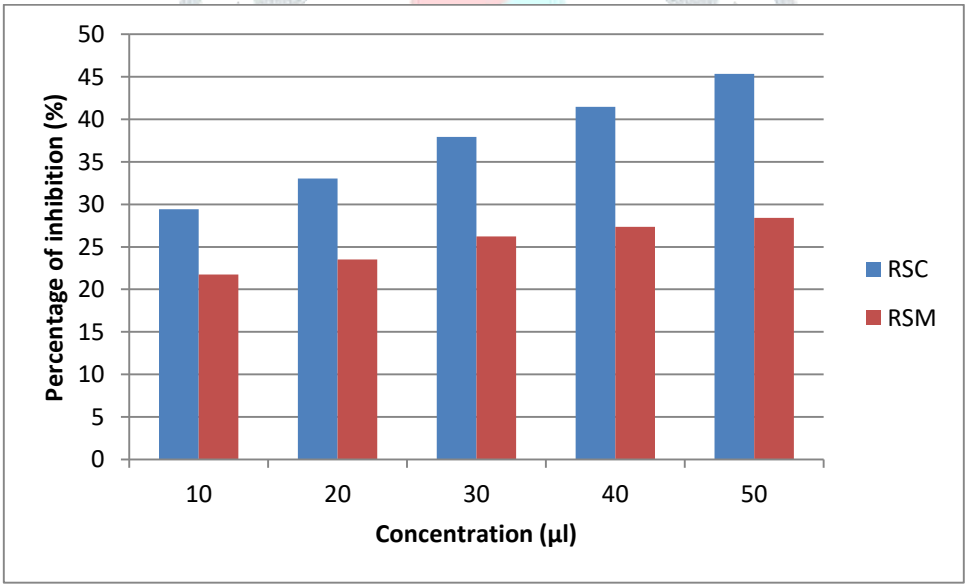

Table.1. Phytochemical screening of methanol and chloroform extracts of *Kappaphycus alverzii*.

Phytochemicals	***Kappaphycus alverzii***	
	Chloroform	Methanol
Alkaloids	+	+
Phenol	+	+
Flavanoids	-	-
Saponins	-	+
Steroids	+	+
Tannins	-	+
Terpenoids	+	+
Coumarin	+	-
Cardiac glycosides	+	-
Amino acid	+	+

The phytochemicals were screened in *Kappaphycus alvarezii* extracts using standard procedures. The methanol and chloroform extracts were assessed for the presence or absence of nine major phytochemicals. It was observed that the following phytoconstituents namely Alkaloids, Phenols, Terapioids, Amino acids and Steroids were present in both Chloroform and methanolic extracts of *Kappaphycus alvarezii*. The phytoconstituents namely coumarin and cardiac glycosides were found only in chloroform extract of *Kappaphycus alvarezii* whereas Saponins and Tannins were found only in methanolic extracts. These Phytoconstituents exhibit a wide range of therapeutic properties evidenced based on the literature. Our present finding evidenced that the methanolic extract of *Kappaphycus alvarezii* showed increased activity for alkaloids and terpenoids compared to chloroform extracts.

In the present antioxidant study, it was observed that there was ascending trend in percentage reduction of DPPH with increasing concentration of extracts. Although Methanol extract of *K.alvarezii* showed least scavenging activity at lesser concentrations, when the concentration was increased it showed the highest DPPH scavenging potential. The IC50 values were calculated using regression analysis and were observed to be 1.8±0.0152µg/ml, 2.4±0.01µg/ml respectively for Methanolic and Chloroform extract of *Kappaphycus alvarezii*. The results of the present study reveal that there is a strong correlation between antioxidant activity

and phenolic content. It is believed that the antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors, and free radical quenchers and phenolics can also act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals.

Conclusion

Study reveals the extracts of *K.alvarezii might be* utilized as a natural source of antioxidant, supplement in food and pharmaceutical industry. Further study is must to isolate and characterize the specific bioactive compound present in it responsible for antioxidant activity.

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