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# IN VITRO ANTIOXIDANT SCREENING OF FUCOIDON EXTRACTED FROM KAPPAPHYCUS ALVAREZII (RED SEAWEED)

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## ABSTRACT

Objective: The present study was carried out to evaluate the antioxdidant potential and toxicity effect of polysaccharide-fucoidon from the red seaweed Kappaphycus alvarezii. Fucoidon a polysaccharide contain fucose and sulphate as major compounds. Interestingly, various studies reported that the presence of sulphated content of the sample play a significant role in pharmacological activities. In this study fucoidon extract from Kappaphycus alvarezii were tested for different phytoconstituents and the in vitro antioxidant activity was studied by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, reducing power activity, hydrogen peroxide scavenging activity, superoxide scavenging activity and nitric oxide scavenging activity. Results: The highest free radical scavenging activity of DPPH assay, super oxide radical scavenging activity, reducing power assay, hydroxyl radicals assay and nitric oxide radical scavenging activity, was found to be 70.91±4.96 µg/ml, 83.21±5.82µg/ml, 0.78±5.46µg/ml, 67.50±4.72µg/ml, 76.19±5.33µg/ml, at highest concentration of methanol extract of 80 µg/ml respectively in this study. Conclusion: The present study concluded the in vitro antioxidant activity of fucoidon extracted from Kappaphycus alvarezii which might be due to the presence of the antioxidant compounds.Hence ,fucoidon have the scope of being used us natural antioxidants in treating many human diseases.

**KEYWORDS:** Fucoidon, Antioxidant**,** *Kappaphycus alvarezii*, Scavenging activity.

## 1. INTRODUCTION

Oxidation is essential to many living organisms for production of energy for biological processes. The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced due to the oxidation of cell and leads to cell death and tissue damage. Free radicals are responsible for aging and causing various human diseases such as atherosclerosis, diabetes, cancer, hypertension, alzheimer's disease, parkinsonism and cirrhosis.[1]

Antioxidants dealing with an important role in the prevention and treatment of a variety of diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. [2]

The antioxidants in the human diet are of great interest as possible protective agents to help human body to reduce oxidative damage. To prevent lipid oxidation food industries have long using synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as preservatives in food products which are restricted due to their carcinogenic effects and has led to increased interest in antioxidant substances from natural resources. [3]

Seaweeds are widely consumed and have been valued as an edible and medical resource. Many studies have found that some species of seaweeds are having therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects. [4]

*Kappaphycus alvarezii* is commonly known as red seawed. The seaweed , Kappaphycus alvarezii, is one of the world's most economically important vegetable crops. Seaweed growers inoculate compost beds with spawn, which is grain colonized with a specific commercial strain. Seaweed spawn is generally rye grain or millet that has been heat sterilized and inoculated with mycelium from an axenic culture of a particular commercial strain.[5]

*Kappaphycus alvarezii* (syn.k.cottonii;Eucheuma cottoni) is a species of red algae one of the most important commercial source of carrageenans,a family of gel forming, viscosifying polysaccharides. Farming method affect the character of the carrageenan that can be extracted is from the seaweed. This alga grows to two meteres long and is green or yellow in colour. Its very fast growing,known to double its biomass in 15 days. Different carrageenan types in

differ in composition and conformation,resulting in a wide range of rhelogical and functional properties. Carrageenan are used in a variety of commercial applications as gelling,thickening and stabilizing agents,especially in food product such as fozen desserts,chocolate milk,cottage cheese,whipped cream,instant products,yogurt,jellies,pet foods,and sauces.Aside from these function carrageenans are used in pharmaceutical formulation, cosmetics and industrial applications such as mining. It has been proved by research workers that kappaphycus alvaezii has significant anticancer activity. Kappaphycus has beed used in a herbal medicine for anti-inflammatory, diuretic,choleretic and hemostatic properties.[6]

**Scientific Classification**

Empire :Eukaryota
Kingdom :Plantae
Subkingdom :Biliphyta
Phylum :Rhodophyta
Subphylum :Eurhodophytina
Class : Florideophyceae
Subclass :Rhodymeniophycidae
Order :Gigartinales
Family : Solieriaceae
Genus :Kappaphycus

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

All chemicals were procured from Ponmani Scientific Chemicals Suppliers, Tiruchirappalli, Tamilnadu, India and were of analytical grade.

### 2.2. Sample

A sample of, *Kappaphycus alvarezii* collected from a coastal area of rameswaram Island, Tamilnadu, India.

### 2.3. Samples preparation

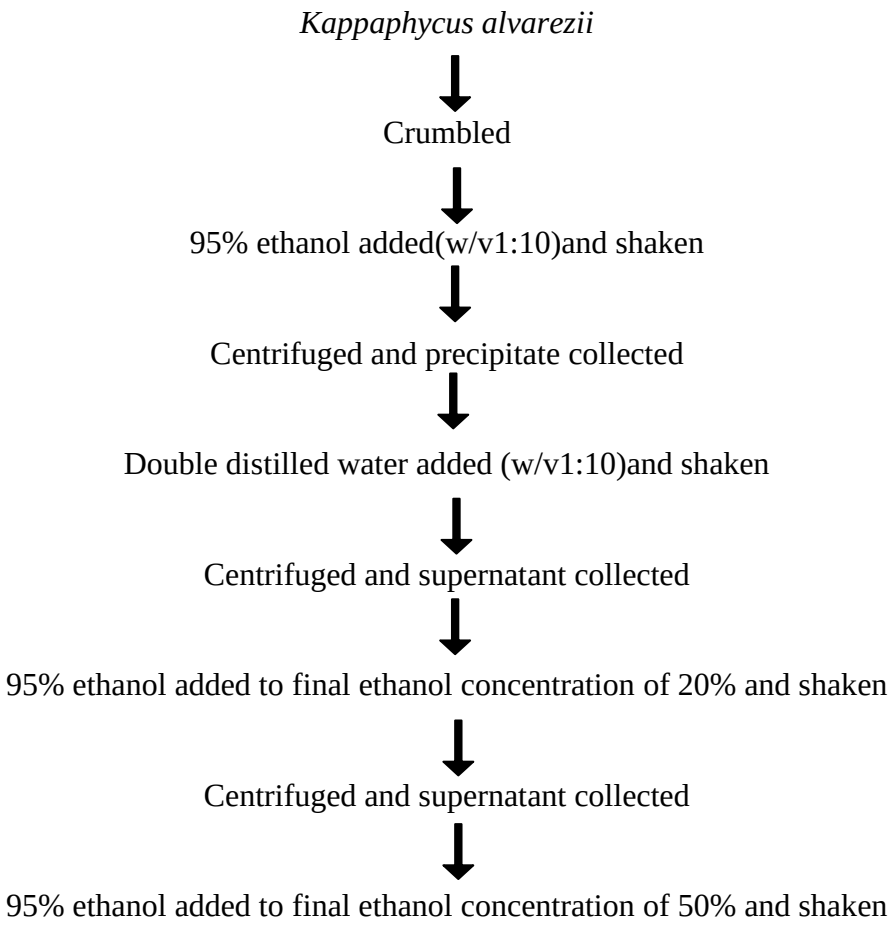
A sample of, Seaweed *Kappaphycus alvarezii* was washed with fresh water soon after collection in order to remove salt and sand. It was then dried at $50^0$C and kept in plastic bags at $4^0$C until use.

### 2.4. Extraction of fucoidan

The fucoidon was extracted as describe by Cheng-Yuan Wang et, al, 2015. The dried alga sample was ground and mixed with 95% ethanol (w/v ¼ 1:10), shaken for 1 hour at room temperature to remove pigments, proteins, and lipid , then centrifuged at 970g for 10 minutes. The residue was collected, mixed with double-distilled water (w/v ¼ 1:10) and placed in a water bath maintained at $40^0$C for 15 minutes with shaking. The mixture was centrifuged at 3870g for 10 minutes and the supernatant was collected. Ethanol (95%) was added into the supernatant to give a final ethanol concentration of 20% in order to precipitate alginic acid and pigment The mixture was centrifuged at 9170g for 30 minutes, the supernatant was collected, and 95% ethanol was added until a final ethanol concentration of 50% was reached in order to obtain fucoidan. The final ethanol-precipitated fucoidan was then recovered by centrifugation at 9170g for 30 minutes, dried at $40^0$C, and milled, which yielded purified fucoidan preparation (PFP).[7]

**Fig:1 - Flowchart for extraction of fucoidan from *Kappaphycus alvarezii***

**EXTRACTION OF FUCOIDAN:**



↓

Centrifuged and precipitate collected

↓

400 hot air-dried

↓

Purified form of fucoidon

## 2.5. EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY OF FUCOIDON

The prepared Fucoidon was stored in a refrigerator until use. Doses such asc20,40 ,60 ,80 µg/ml where chosen for *in vitro* antioxidant activity.

### a. Determination of DPPH scavenging activity

Different aliquots of 0.2 to 1 ml of sample extract solutions were taken in different test tubes. To these entire tubes methanol was added and made up to 1ml. To this 4m1 of methanolic DPPH was added and shaken well.

The mixture was allowed to stand at room temperature for 20 min. The control contains only methanol and DPPH. The readings were noted at 517 nm against methanolic blank.

The change in absorbance of the samples was measured. Free radical scavenging activity was expressed as the inhibition percentage calculated by using the formula Percentage of antioxidant activity = [A - B/A] x 100.Where, 'A' is absorbance of control 'B' is absorbance of sample.[8]

### b. Determination of Reducing power activity

1 ml of fucoidon extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferric cyanide (2.5 ml). The mixture was incubated at 50˚c 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 cloride (0.5ml, 0.1%) and absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid standard.

Percentage inhibition scavenging activity was calculated by (A control x A test/A control) x100.Where A control is the absorbance of the control. A test is the absorbance in the presence of the sample.[9]

**c. Determination of superoxide scavenging activity**

Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the fucoidon (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts.

All the tube where the initial optical density was measured at 560 nm in a spectro photometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity. [10]

**d. Determination of hydroxyl radical scavenging activity**

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. Percentage inhibition of H2O2 radical scavenging activity was calculated by (A control x A test/ A control) x 100.Where A control is the absorbance of the control. A test is the absorbance in the presence of the sample.[11]

**e. Determination of Nitric oxide scavenging activity**

The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of fucoidon (50mg) and incubated at 25°C for 30 minutes. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the extracts. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer. [12]

**Statistical analysis**

The determinations were conducted in triplicate and results were expressed as Mean ± SD.

## 3. RESULT

Table 1 and figure 2 shows the DPPH scavenging effect increased with the increasing concentrations of *Kappaphycus alvarezii* powder extract as compared to standard ascorbic acid and highest DPPH scavenging activity of fucoidon extracted from *Kappaphycus*

*alvarezii* as compared to ascorbic acid was observed as 70.91 % inhibition at 80 µg/ml concentration which indicates the DPPH scavenging effective of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid.

**Table 1 DPPH Radical scavenging activity of Fucoidon at different concentrations**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| Fucoidon | 12.28±0.85 | 28.19 ±1.97 | 46.37 ±3.24 | 70.91 ±4.96 | 42.18 |
| Standard (Ascorbic acid) | 25.6 ± 2.04 | 61.26 ± 4.90 | 88.98 ± 7.11 | 99.34 ± 7.94 | 34.91 |

Note: Values were expressed as Mean ± SD for triplicates

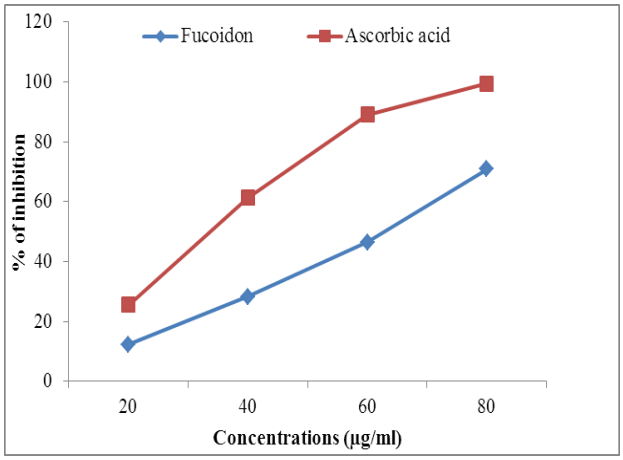



**Fig 2 DPPH Radical scavenging activity of Fucoidon at different concentrations**

### 3.3. Superoxide scavenging activity

Table 2 and figure 3 showed the superoxide scavenging effect increased with the increasing concentrations of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid. Powder extract as compared to standard ascorbic acid and highest superoxide scavenging effect of *Kappaphycus alvarezii* was observed as 83.21.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid.

**Table 2 Superoxide Radical scavenging activity of Fucoidon at different concentrations**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| Fucoidon | 14.64±1.02 | 32.50±2.27 | 67.50±4.72 | 83.21± 5.82 | 50.46 |
| Standard (Ascorbic acid) | 31.25 ± 2.50 | 64.23 ± 5.13 | 89.54 ± 7.16 | 98.51 ± 7.88 | 31.62 |

Note: Values were expressed as Mean ± SD for triplicates.

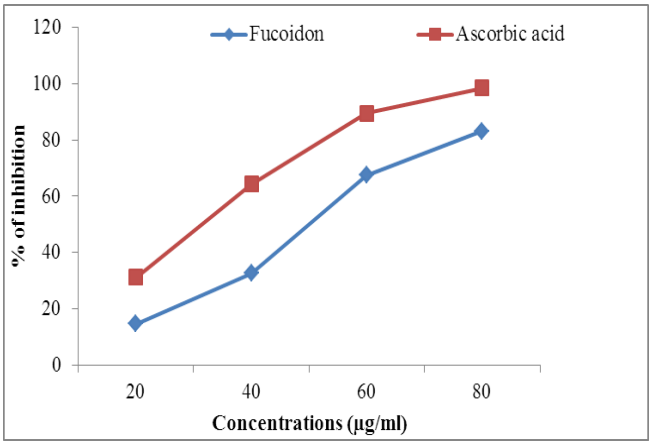



**Fig 3 Superoxide Radical scavenging activity of Fucoidon at different concentrations**

### 3.2. Reducing power activity

Table 3 and figure 4 shows the Reducing power effect increased with the increasing concentrations of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid. Powder extract as compared to standard ascorbic acid and highest reducing power activity of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid was observed as 0.78 % inhibition at 80 µg /ml concentration which indicates the Reducing power effective of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid.

**Table 3 Reducing power assay of Fucoidon at different concentrations**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) |
|---|---|---|---|---|
| Fucoidon | 0. 13±0.96 | 0. 33±2.34 | 0. 53±3.71 | 0. 78±5.46 |
| Standard (Ascorbic acid) | 0.41 ± 0.03 | 0.71 ± 0.05 | 0.89 ± 0.07 | 0.98 ± 0.08 |

Note: Values were expressed as Mean ± SD (Optical density) for triplicates

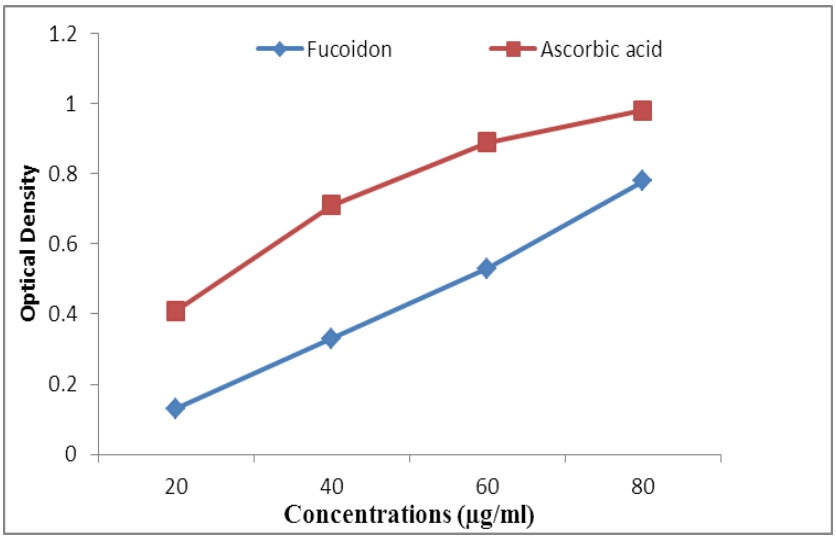



**Fig 4 Reducing power assay of Fucoidon at different concentrations**

### 3.4. Hydroxyl Radicals scavenging activity

Table 4 and figure 5 showed the hydrogen peroxide scavenging effect increased with the increasing concentrations of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid. Powder extract as compared to standard ascorbic acid and highest hydrogen peroxide scavenging effect of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid was observed as 67.50.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid.

**Table 4 Hydroxyl Radicals assay of Fucoidon at different concentrations**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| Fucoidon | 17.91±1.25 | 29.16±2.04 | 45.41±3.17 | 67.50±4.72 | 62.13 |
| Standard (Ascorbic acid) | 32.21± 2.51 | 56.45± 4.40 | 78.65±6.13 | 92.75±7.2 | 35.26 |

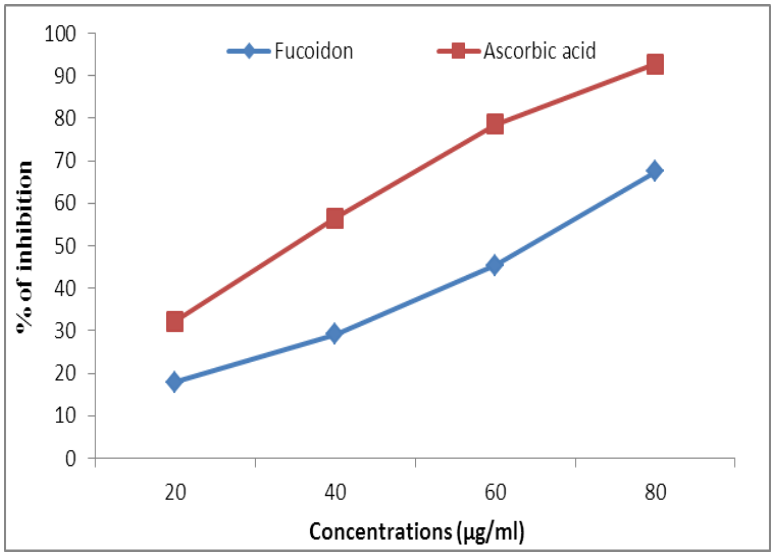



**Fig 5 Hydroxyl Radicals assay of Fucoidon at different concentrations**

### 3.5. Nitric oxide scavenging activity

Table 5 and figure 6 showed the Nitric oxide scavenging effect increased with the increasing concentrations of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid. Powder extract as compared to standard ascorbic acidand highest Nitric oxide scavenging effect of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid was observed as 76.19.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of fucoidon extracted from *Kappaphycus alvarezii* as compared to ascorbic acid.

**Table 5 Nitric oxide assay of Fucoidon at different concentrations**

| Parameters | 20 (µg/ml) | 40 (µg/ml) | 60 (µg/ml) | 80 (µg/ml) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| Fucoidon | 15.71±1.09 | 33.80±2.36 | 57.14±3.99 | 76.19±5.33 | 54.23 |
| Standard (Ascorbic acid) | 26.21 ± 2.04 | 59.62± 4.65 | 84.23 ± 6.56 | 96.45 ± 7.52 | 35.88 |

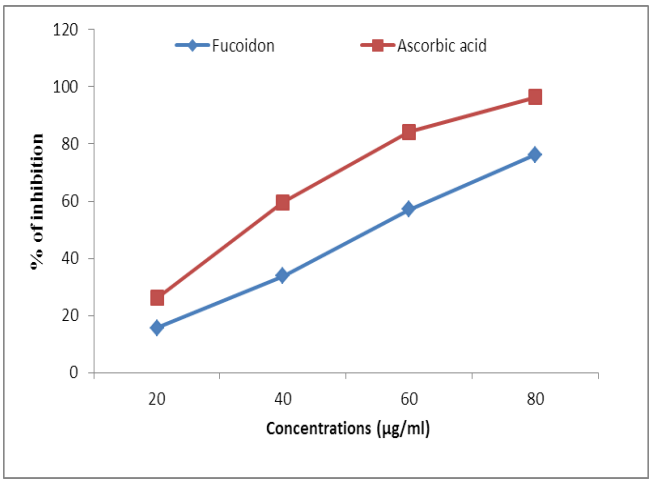



**Fig 6 Nitric oxide assay of Fucoidon at different concentrations**

## DISCUSSION

The presence of secondary metabolites like tannins, saponin, carbohydrates, glycosides, alkaloids, flavonoids, terpenoids, steroids, polyphenols and anthraquinones of *Kappaphycus alvarezii* suggests that the plant might be of medicinal importance.[13] Due to the presence of flavonoids and phenol suggests that the plant might have an antioxidant, anti-allergic, anti-inflammatory antimicrobial, anticancer activity. [14] The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and antibacterial activities and can and in wound healing and burns.[15] Saponins and glycoside are also very important classes of secondary metabolites as some are cardio active and used in treatment of heart conditions.[16]

The phenolic and flavonoids are widely distributed secondary metabolites in plants having antioxidant activity and have wide range of biological activities as anti-apoptosis, anti-aging, anti-carcinogen, anti inflammation, atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.[17] This study also proved that many dietary polyphenolic and flavanoid constituents derived from *Kappaphycus alvarezii* are more effective antioxidants than ascorbic acid and thus might contribute significantly to the protective effects in vivo.[18]

The present study was also revealed the in vitro antioxidant properties of *Kappaphycus alvarezii* which might be due to the presence of phenolic and flavonoid compounds in methanolic extract.[19] DPPH radicals react with suitable reducing agents and then electrons become paired-off and the solution loses colour steriochiometrically with the number of electrons taken up . Such reactivity has been widely used to test the ability of compounds of *Kappaphycus alvarezii* extracts to act as free radical scavengers .Though the extracts showed good DPPH scavenging activity but it was less effective than standard Ascorbic acid. This activity is due to presence of phenolic and flavonoid components in the plant extracts.[20]

The Reducing antioxidant power assay, a yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals causes the conversion of the ferric cyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls Prussian blue spectroscopically, a higher absorbance indicates a higher reducing power.[21]

Reducing power of activity in the *Kappaphycus alvarezii* extracts indicated that some components in the extract were electron donors that could react with the free radicals to convert them into more stable products to terminate radical chain reaction.[22]

Superoxide anion radical is generated by four electron reduction of molecular oxygen in to water. This radical also formed in aerobic cells due to electron leakage from the electron transport chain super oxides are generated from molecular oxygen of oxidative enzymes and as well as non-enzymatic reactions such as auto oxidation by catecholamines.[23] The studies also proved the super oxide free radical scavenging activity of fucoidon extract of *Kappaphycus alvarezii* was noticed significant reduction of the super oxide anions.[24]

Hydrogen peroxide is a biologically relevant, non radical oxidizing species may be formed in tissues through oxidative processes. Hydrogen peroxide which in turn generate hydroxyl radicals (•OH) resulting in initiation and propagation of lipid peroxidation. The ability of the extracts to quench (•OH) seems to be directly related to the prevention of the lipid peroxidation. Appears to be moderate scavenger of active reactive oxygen species.[25]

Nitric oxide is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling inhibition of platelet aggregation and regulation of cell mediated toxicity .It is a diffusible free radical that plays many roles as an effectors molecule

in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. [26]

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

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