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Pharmacological importance of sulphated polysaccharide carrageenan from red seaweed *Kappaphycus alvarezii* in comparison with commercial carrageenan

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ABSTRACT

Pharmacological properties of native carrageenan (κ) extracted from *Kappaphycus alvarezii* and commercial carrageenan (Sigma-Aldrich) were evaluated using *in vitro* antioxidant, anticancer and antidiabetic studies. Phytochemical analysis of native and commercial carrageenans showed the presence of alkaloids, saponins, steroids, gums & mucilages and carbohydrate. Both native and commercial carrageenans exhibited better antioxidant activities such as total antioxidant capacity (87 ± 0.47 and 82.6 ± 0.47 μg A.A/g), hydroxyl radical scavenging activity (61.4 ± 0.27 and 58.66 ± 0.31 μg/ml), nitric oxide radical scavenging activity (80.42 ± 0.22 and 73.66 ± 0.22 μg/ml), DPPH radical scavenging activity (56.26 ± 0.20 and 53.67 ± 0.082 μg/ml) and reducing power assay (46.57 ± 0.32 and 42.54 ± 0.27 μg/ml) at the maximum concentration of 100 μg/ml carrageenans. These results indicated that native carrageenan from *K. alvarezii* possessed better antioxidant potential in comparison with commercial carrageenan. Anticancer activities of both carrageenans showed excellent inhibition on the growth of breast, colon, liver and osteosarcoma cell lines at the maximum concentration of 150 μg/ml. Native carrageenan exhibited an excellent anticancer activity on colon carcinoma cell lines (67.66 ± 0.168%) with the IC_{50} value of 73.87 μg/ml and commercial carrageenan possessed a potent inhibition on the growth of breast cancer cell lines (67.33 ± 0.077%) with the IC_{50} value of 123.8 μg/ml. These results clearly indicated the beneficial effect of native and commercial carrageenans as anticancer agents being a free radical scavenger. Anti-diabetic property of both carrageenans showed inhibition effect on α- glucosidase enzyme. The inhibitory effect depends on concentration of carrageenans and it was recorded that maximum (74.49 ± 1.05 and 67.42 ± 0.63) inhibitory effect of α- glucosidase enzyme at 500 μg/ml concentration.

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1. Introduction

All higher organisms including humans have an efficient antioxidant network system. It showed their relevance in the prevention of various diseases, in which free radicals are implicated [1]. The term “free radicals” mostly refers to reactive oxygen species (ROS), sometimes called as active oxygen species, which are various forms of activated oxygen, including superoxide ions (O_2^-) and hydroxyl radicals (OH), as well as non-free radical species such as hydrogen peroxide (H_2O_2) ions [2]. In living organisms, various ROS are formed in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes, macrophages and perioxisomes. Endogenous sources of most of the oxidants are produced by cells. Exogenous sources of free radicals are formed by tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides [2]. Cellular metabolism in higher animals is remarked by ongoing formation of pro-oxidants, which is to be balanced by antioxidant defense systems. When balance is interrupted the oxidative stress is occurred by free radicals and it can result in atherosclerosis, cataract formation, aging, carcinogenesis, chronic inflammation, ischemia, diabetes, septic and haemorrhagic shock, neurodegenerative disorders, etc. The negative effects of oxidative stress can be reduced by antioxidants [3]. The term antioxidant’ refers to the

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activity of numerous vitamins, minerals and other phytochemicals to protect the damage caused by ROS [4]. Antioxidant defense system scavenges and minimizes free radicals formation. Antioxidants in biological systems have multiple functions, including defending against oxidative damage and participating in the major signalling pathways of cells. One major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species. Natural antioxidants are being extensively studied for their capacity to protect organisms and cells against the deleterious effects of oxidative stress [5]. Many clinical and epidemiological studies have suggested that the intake of dietary antioxidants has an important role in the prevention of the oxidative stress-related diseases.

In biomedicine, much attention has been paid to natural antioxidants and their association with health benefits [6]. Marine environment contains infinite number of different species of plants and animals. These creatures provide a vast storehouse of chemical compounds, which are unknown to the terrestrial environment (National Oceanic and Atmospheric Administration). Marine algae's have valuable sources of structurally diverse bioactive compounds and SPS (sulphated polysaccharides). SPS are carbohydrate polymers of marine algal species, mainly found in Rhodophyta. Rhodophyceae or Red seaweed species have been reported to display antitumor [7] heavy metal chelation [8], nitrate reductase [9] and *in vivo* [10] and *in vitro* [11] antioxidant activities. Many studies have shown that some marine polysaccharides from red seaweeds have antioxidant, anticoagulant, antiviral, anticancer and immunomodulating activities [12,13]. Carrageenan, one of the sulphated polysaccharides of red algae is mainly localized in the cell wall of the algae. It was widely employed as a gelling agent in many industrial applications. However, there are very few records about antioxidant, anticancer and antidiabetic capacity of sulphated polysaccharide especially carrageenans are available. Therefore, considering the importance of the above, in the present study *in vitro* antioxidant, cytotoxicity and antidiabetic activities of native carrageenan from *K. alvarezii* was evaluated in comparison with commercial carrageenan (Sigma-Aldrich).

2. Materials and methods

2.1. Experimental samples

Native carrageenan from *K. alvarezii* was extracted according to the standard methodology [14], it was previously characterized as kappa (κ) carrageenan, which was compared with commercial carrageenan (*K. alvarezii* – C1263, type III κ-carrageenan; Sigma Aldrich product, Mumbai). Dried seaweed (20 g) was soaked in 700 ml distilled water for two hours and then soaked in boiled water for 30 min. Boiled sample was ground well using a blender with 3 g sodium hydroxide pellets and subjected to prolonged boiling with constant stirring for four hours. The boiled sample was mixed with 10 g NaCl and filtered through a nylon cloth held over a stainer. The residue was washed using 300 ml of hot distilled water until the carrageenan was removed. The resultant mixture was added gradually to 700 ml of 85% isopropanol and stirred continuously to precipitate the carrageenan. Finally the precipitated carrageenan was dried, powdered and kept in airtight container until further use.

2.2. Qualitative and phytochemical analysis of native and commercial carrageenans

The preliminary qualitative and phytochemical characteristics such as Alkaloids, Glycosidase, Saponins, Phenolics, Flavonoids, Terpenoids, Steroids, Tannins, Carboxylic acid, Quinons, Coomarins, Resins, Pholobatannins, Leucoanthocyanins, Cardiac glycosidase, Anthaquinone, Carbohydrates, Protein, Lipid, Gums and mucilages were analyzed in both native and commercial carrageenans by following the standard methodologies [15–21].

2.3. Total antioxidant property

Total antioxidant property of both native and commercial carrageenans was evaluated by the method of Prieto et al. [22]. In brief, 0.3 ml each of both native and commercial carrageenans at different concentrations of 12.5, 25, 50 and 100 μg/ml were taken individually in 15 ml test tubes and 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. Then the tubes were incubated at 95 °C for 90 min. After incubation, the absorbance of the reaction mixture was measured at 695 nm using UV–vis spectrophotometer (UV2301II model). The total antioxidant activity was expressed as the number of gram equivalent of ascorbic acid and the calibration curve was prepared using various concentrations of ascorbic acid (50,100, 150, 200, 250, 300, 350, 400, 450 and 500 μg/ml). Butylated hydroxy anisole (BHA) was used as standard antioxidant (Barreira et al. [23]).

2.4. *In vitro* radical scavenging assay

2.4.1. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}/Ascorbate/EDTA/H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao [24]. The reaction mixture containing $FeCl_3$ (100 μM), EDTA (104 μM), H_2O_2 (1 mM), 2-deoxy- D-ribose (2.8 mM) and different concentrations (12.5, 25, 50 and 100 μg/ml) of native and commercial carrageenans and the final volume was made up to 1 ml with potassium phosphate buffer (20 mM, pH 7.4), further the reaction mixture taken in the test tubes were incubated for 1hr at 37 °C. The free radical damage imposed on the substrate, deoxyribose (TBARS) was measured by the method of Yuan and Walsh [25]. 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added to the test tubes and incubated at 100 °C for 30 min. After cooling, absorbance was measured at 535 nm against a control containing deoxyribose and buffer. The percentage of scavenging was determined by comparing the result of the test and control using the following formula

Radical scavenging (%) = AC – AT/AC × 100

AC- Absorbance of control

AT- Absorbance of test

2.4.2. Nitric oxide radical scavenging assay

Nitric oxide radical scavenging assay was performed by the modified method of Gulcin [26]. Nitric oxide radicals generated from sodium nitroprusside solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. 2 ml of sodium nitroprusside (10 mm) was mixed with 1 ml of different concentrations (12.5, 25, 50 and100 μg/ml) of both native and commercial carrageenans in phosphate buffer (pH 7.4). The mixture was incubated at 25 °C for 150 min. After incubation, 1 ml of sulphanilic acid reagent (0.33% sulphanilamide in 20% acetic acid) was added and allowed to stand for 5 min. Then, 1 ml of 0.1% napthyl ethylene diamine dihydrochloride was added and incubated at room temperature for 30 min. Absorbance was read at 540 nm and the percentage scavenging was calculated by following the above formula.

2.4.3. DPPH (2, 2-diphenyl –1-picrylhydrazyl) assay

The free radical scavenging activity of carrageenans was measured by 1-1- Diphenyl-2-picryl-hydrazyl (DPPH) using a

modified method of Blois [27] and Sanjivkumar et al. [28]. DPPH was used as a reagent, which evidently offers a convenient and accurate method to titer the oxidizable groups of natural or synthetic antioxidants. 1 ml of DPPH solution (0.1 mM solution of DPPH in methanol) was taken in a sterile test tube. To this, 3 ml of different concentrations (12.5, 25, 50 and100 μg/ml) of both native and commercial carrageenans were added and allowed to stand for 10 min. After 10 min, the absorbance was measured at 517 nm. The percentage scavenging activity was calculated by following the above formula.

2.4.4. Reducing power assay

Reducing power of the carrageenans was determined by the modified method of Yamaguchi et al. [29] and Sanjivkumar et al. [28]. Reaction mixture containing different concentrations (12.5, 25, 50 and 100 μg/ml) of both native and commercial carrageenans, 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferric cyanide. The mixture was incubated at 50 °C for 20 min. After that, 2.5 ml of TCA (10% w/v) was added and centrifuged for 10 min at 2000 rpm. The supernatant was collected, to this 1 ml of distilled water and 0.1% (W/V) of ferric chloride solution were added and the absorbance was measured at 700 nm. The percentage reducing power was calculated by following the above formula.

2.5. Anticancer activity of native and commercial carrageenans against cancer cell lines

2.5.1. Cell culture maintenance

Anticancer assay was performed against four different human carcinoma cell lines such as breast (MCF7) carcinoma, colon (HT-29) carcinoma, liver (Hep G2) carcinoma and osteosarcoma (MG63). Cell lines were obtained from National Centre for Cell Science, (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37 °C, at 5% CO_2, 95% air and 100% relative humidity. The cultures were maintained by passaging weekly, and the culture medium was changed twice a week.

2.5.2. Anticancer activity – MTT assay

The cell lines for breast carcinoma (MCF7), colon carcinoma (HT-29), liver carcinoma (Hep G2) and osteosarcoma (MG63) were plated separately in micro well plates with DMEM medium containing 10% FBS at 1×10^5 cells/well. The plates with cells were incubated for 24 h under 5% CO_2 and 95% air at 37 °C. After incubation, the plates with the cell lines were washed with PBS and then serum free medium was added and kept for 1 h in the incubator. Then, serum free medium in the plates was removed and the control plates with individual cell lines received serum free medium and treatment plates received various concentration of (10, 25, 50, 100, 250 μg/ml) of both native and commercial carrageenans dissolved in distilled water. The cultures were again incubated as above. After 24 h of incubation, 100 μl of 5 mg/ml MTT solution [5 mg/ml in phosphate buffer saline (PBS)] was added in to each well and the cultures were further incubated for 4 h at 37 °C in a CO_2 incubator and then 100 μl of DMSO was added and the forming crystals were dissolved by gently pipetting. Spectrophotometrical absorbance of the purple blue formazan dye was measured in microplate reader at 570 nm. The assay was performed in triplicate for each cell lines. The results were compared with positive control (cyclophasphamide treated cell lines). Nonlinear regression graph was plotted between percentage cell inhibition and Log concentration and IC_{50} were determined using GraphPad Prism 7 software.

The percentage cell viability (%) = AT/AC x 100

The percentage cell inhibition (%) = AT/AC x 100

AT- Absorbance of test sample

AC- Absorbance of control

2.5.3. In vitro *antidiabetic activity – inhibition of α- glucosidase [30]*

The α-glucosidase inhibitory activity was determined by premixing α- glucosidase (0.07 Units) with different concentrations (100–500 μg/ml) of native and commercial carrageenans. Then 3 mM *p*-nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37 °C for 30 min and the reaction was terminated by addition of 2 ml of sodium carbonate. The α-glucosidase inhibitory activity was recorded by measuring the *p*-nitrophenyl release from *p*-nitrophenyl glucopyranoside at 400 nm. A standard drug acarbose was used as a positive control. The percentage α-glucosidase inhibitory activity was calculated by the following formula

Percentage Inhibition (%) = OD of Control – OD of Sample/OD of control × 100

The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of α glucosidase activity under assay condition.

2.6. Statistical analysis

The data obtained in the present study were expressed as Mean ± SD and were analyzed using one way ANOVA at 5% significance level. Further a multiple comparison test-Tukey's test was conducted to compare the significant differences among the parameters using computer software Statistica 6.0 (Statosoft, UK).

3. Results

The present study was designed to examine the *in vitro* antioxidant, anticancer and antidiabetic activities of native carrageenan from *K. alvarezii* in comparison with commercial carrageenan.

3.1. Phytochemical screening to determine the purity of carrageenans

Carbohydrate, protein, gums and mucilages, sugar derivative of saponins, flavonoids and steroids were observed in both native and commercial carrageenans. It confirmed that, native carrageenan was equally good and possessed better purity like commercial carrageenan (Table 1).

Table 1
Phytochemical screening of native and commercial carrageenans.

Sl.No	Tests	Phytochemical characterization	
		Native carrageenan	Commercial carrageenan
1.	Alkaloids	+	+
2.	Glycosidase	–	–
3.	Saponins	+	+
4.	Phenolics	–	–
5.	Flavonoids	+	+
6	Terpenoids	–	–
7.	Steroids	+	+
8.	Tannins	–	–
9.	Carboxylic acid	–	–
10	Quinons	–	–
11.	Coomarins	–	–
12.	Resins	–	–
13.	Pholobatannins	–	–
14.	Leucoanthocyanins	–	–
15.	Cardiac glycosidase	–	–
16.	Anthaquinone	–	–
17	Carbohydrates	+	+
18.	Gums and mucilages	+	+

+: present; –: absent.

3.2. Total antioxidant activity

The result on total antioxidant potential of both native and commercial carrageenans is represented in Fig. 1a. Concentration dependent antioxidant activity was observed in both native and commercial carrageenans. The maximum antioxidant activity of 87 ± 0.47 and 82.6 ± 0.47% was observed at the highest concentration (100 μg/ml) of both native and commercial carrageenans respectively and the minimum antioxidant activity of 58.6 ± 1.69 and 55.3 ± 1.24% was observed at the lowest concentration (12.5 μg/ml) of both native and commercial carrageenans respectively. Native carrageenan from *K. alvarezii* possessed highest antioxidant capacity when compared with commercial carrageenan. The result of both native and commercial carrageenans was compared with standard drug BHA, which exhibited only 73.5 ± 0.08% antioxidant activity at 100 μg/ml concentration. Total antioxidant potential of experimental carrageenans was significantly higher than the control group.

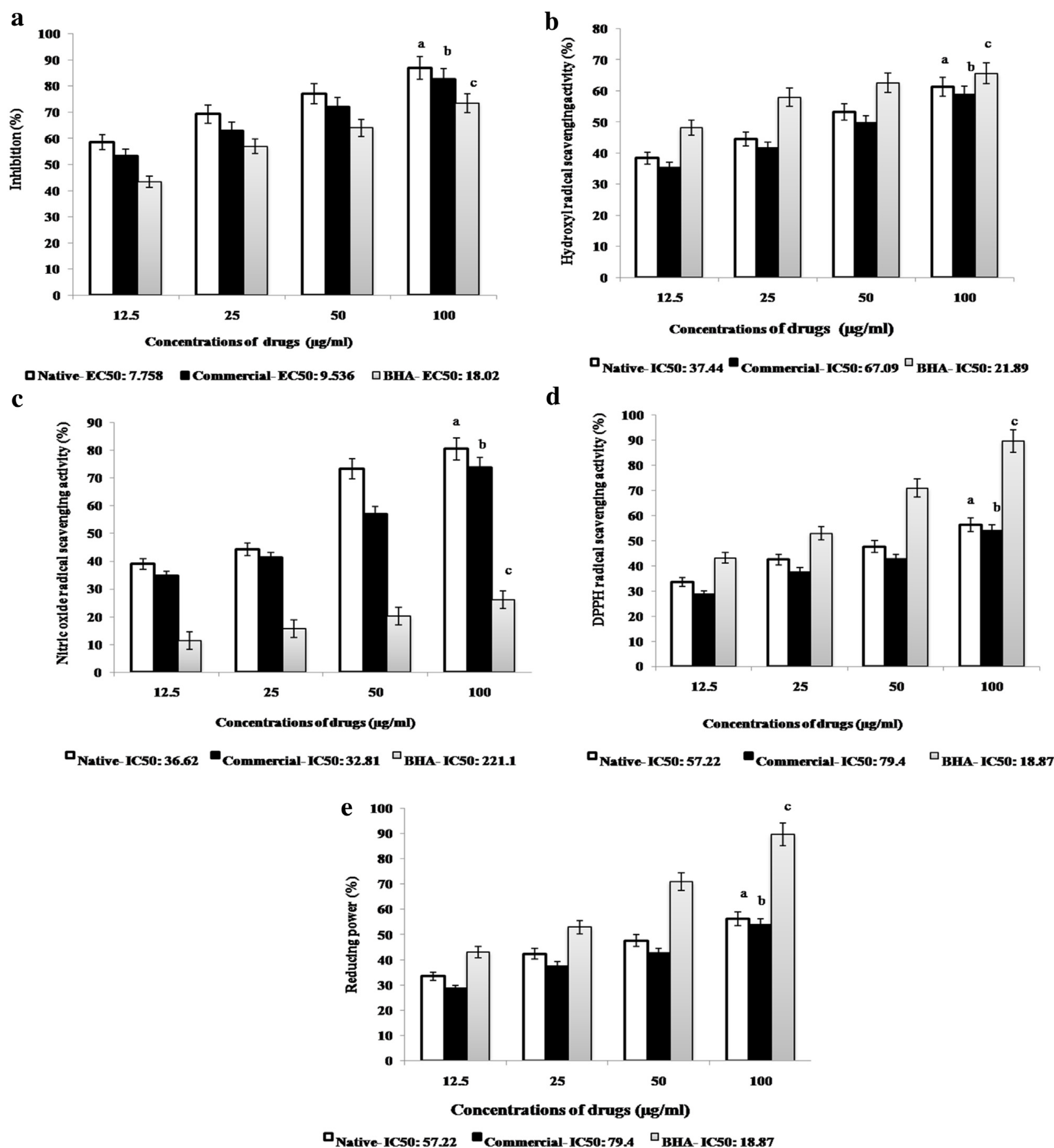


Fig. 1. Antioxidant properties such as total antioxidant (a), hydroxyl radical scavenging (b), nitric oxide radical scavenging (c), DDPH radical scavenging (d) and reducing power (e) of native and commercial carrageenans at different concentrations in comparison with BHA.
Each value is the Mean ± S.D of triplicate analysis, bars represented in different superscript letters are statistically significant (One way ANOVA test: $P < 0.005$; further *post hoc* multiple comparison with Tukey's test) EC_{50}.

3.3. Hydroxyl radical scavenging activity

The *in-vitro* hydroxyl radical scavenging effect was increased with the increasing concentrations of carrageenans and both carrageenans displayed maximum radical scavenging activity (native carrageenan: 61.4 ± 0.27 and commercial carrageenan: 58.66 ± 0.31%) at the highest (100 μg/ml) concentration. Similarly, both carrageenans exhibited minimum radical scavenging activity (native carrageenan: 38.44 ± 0.40 and commercial carrageenan: 35.34 ± 0.32%) at the lowest (12.5 μg/ml) concentration (Fig. 1b). The results showed that both native and commercial carrageenans exhibited potent scavenging activity, but it was lower (65.67 ± 0.47%) than the standard drug BHA at 100 μg/ml concentration. The 50% inhibitory concentration (IC_{50}) value of both native and commercial carrageenans was determined as37.44 and 67.09 μg/ml, respectively.

3.4. Nitric oxide radical scavenging activity

Native carrageenan from *K. alvarezii* exhibited better nitric oxide (NO) radical scavenging activity when compared with commercial carrageenan (Fig. 1c). The NO radical scavenging capacity was exhibited with concentration dependent and it showed maximum NO activity of 80.42 ± 0.22% and 73.66 ± 0.22% at 100 μg/ml concentration of both native and commercial carrageenans respectively. Minimum NO scavenging activity was recorded at the lowest concentration (12.5 μg/ml) of both carrageenans (native carrageenan: 38.93 ± 0.28 and commercial carrageenan: 34.62 ± 0.094%). Both native and commercial carrageenans displayed excellent nitric oxide (NO) radical scavenging activity than the standard drug BHA (26.1 ± 0.30% at 100 μg/ml concentration). The IC_{50} value of the native and commercial carrageenans was determined as 36.62 and 32.81 μg/ml, respectively.

3.5. DPPH (2, 2-diphenyl −1-picrylhydrazyl) radical scavenging activity

The result on DPPH radical scavering assay showed that the native carrageenan from *K. alvarezii* possessed better activity (56.26 ± 0.20%) than the commercial carrageenan (53.66 ± 0.082%) at the highest (100 μg/ml) concentration. DPPH radical scavering activity was increased with increasing concentration of carrageenans (Fig. 1d). At the lowest concentration, both native

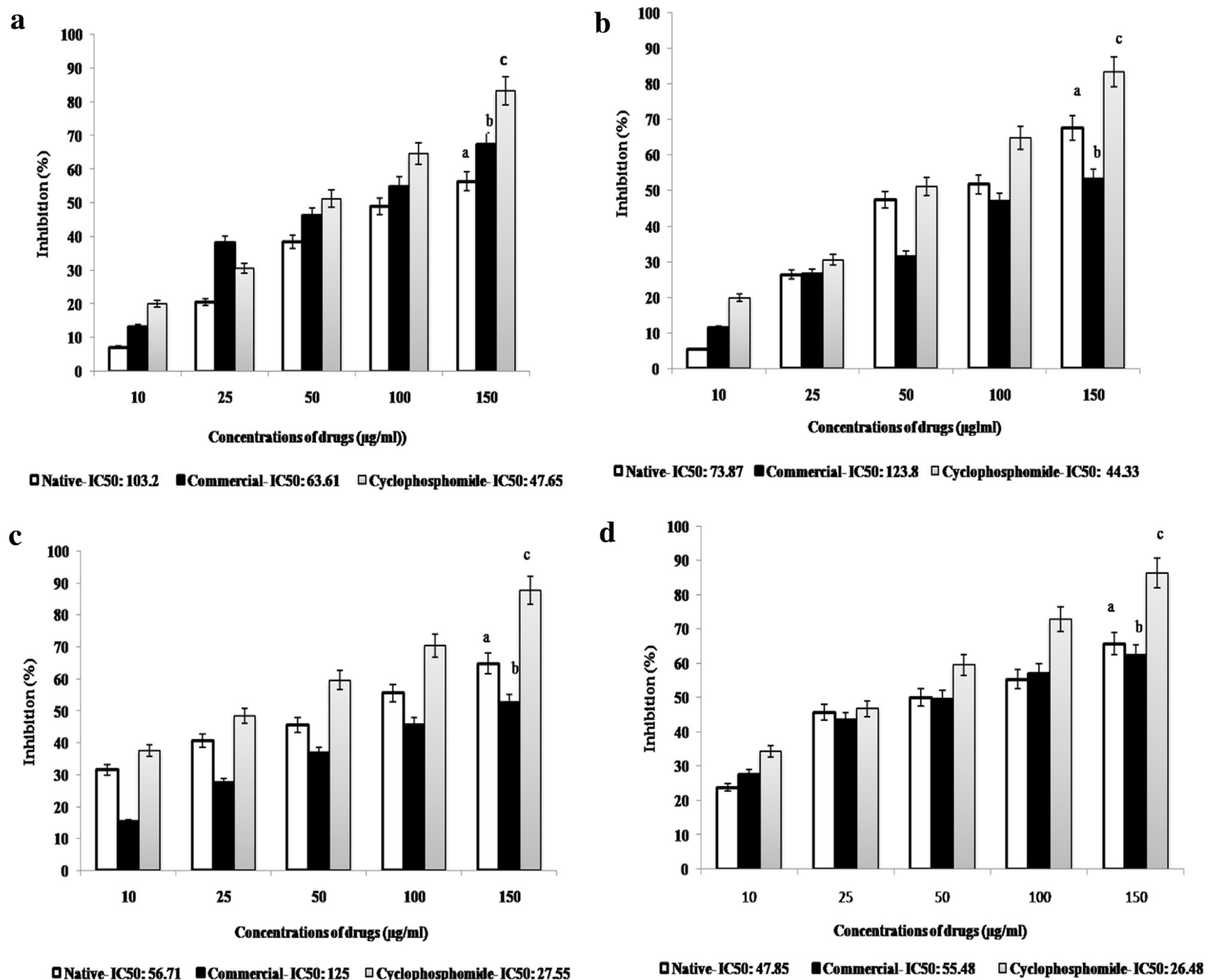
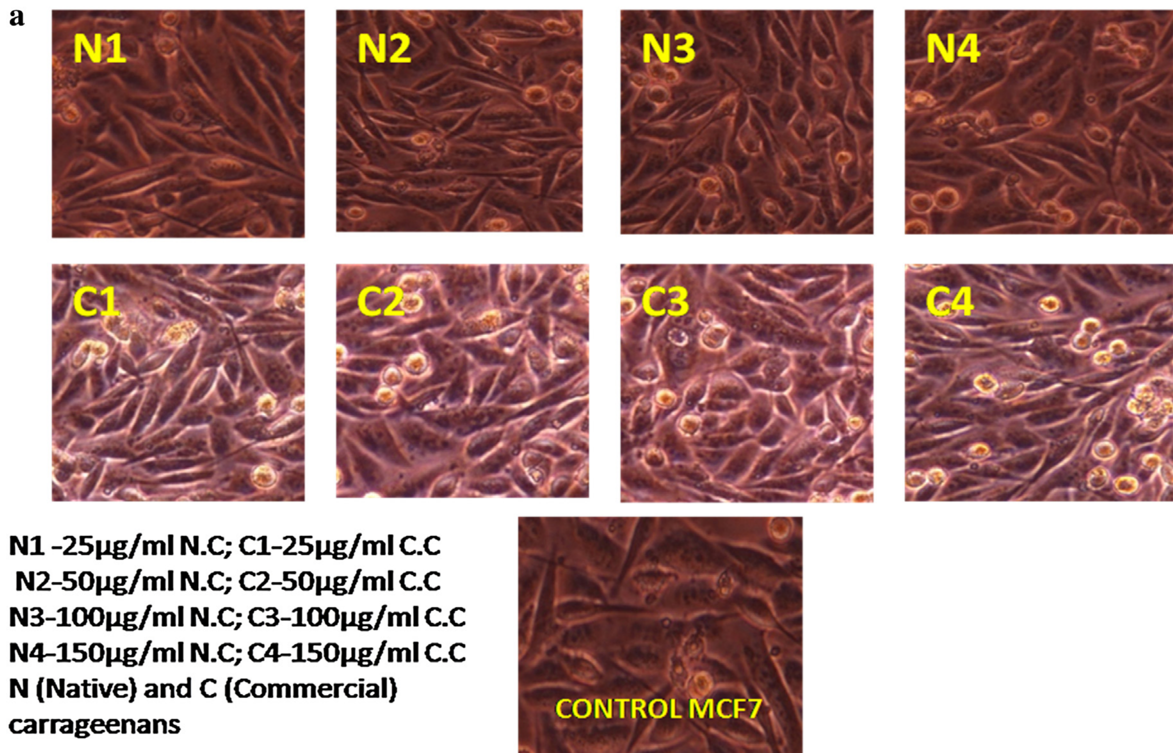


Fig. 2. Anticancer activities of native and commercial carrageenans on (a) Breast cancer (MCF-7), (b) Colon cancer (HT-29), (c) Liver cancer (Hep G2) and (d) Osteosarcoma cells line (MG63) at different concentrations.
Each value is the Mean ± S.D of triplicate analysis, bars represented in different superscript letters are statistically significant (One way ANOVA test: $P < 0.005$; further *post hoc* multiple comparison with Tukey's test).



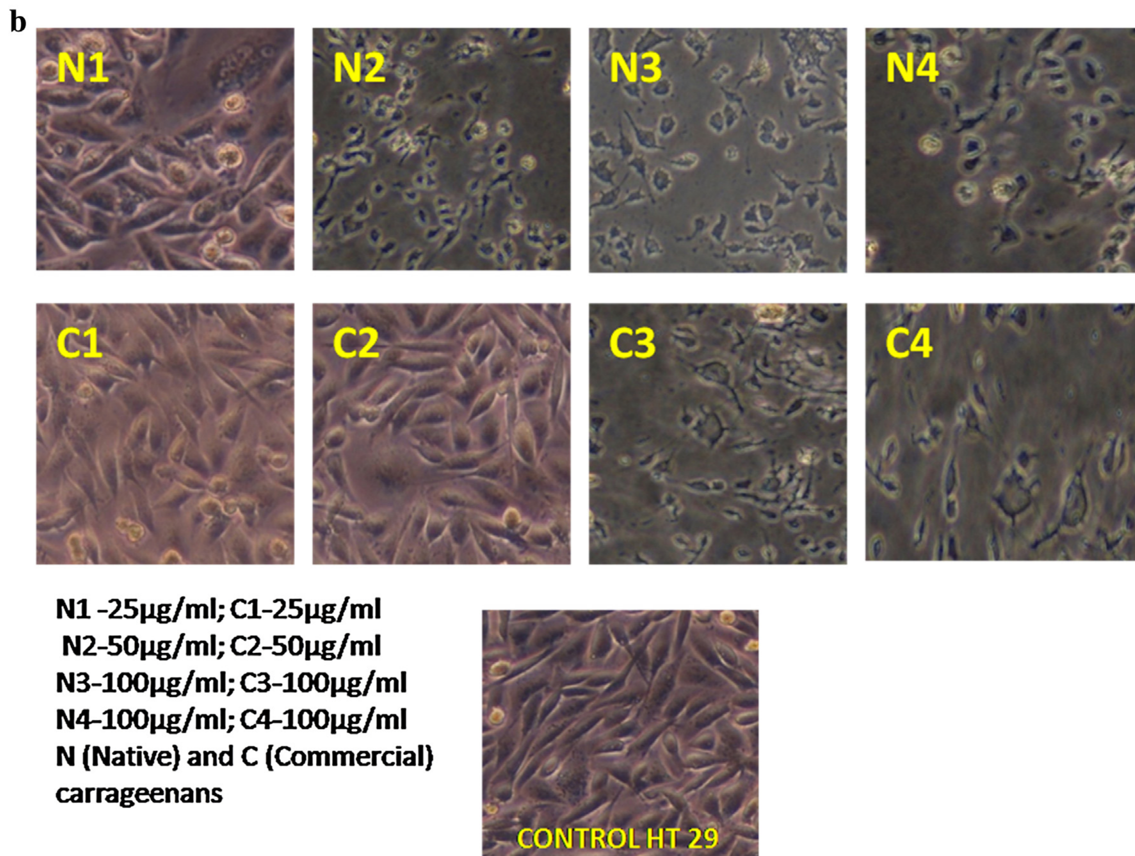


Fig. 3. Cell viability of (a) MCF-7 and (b) HT-29 cell lines treated with Native and Commercial carrageenans.

(33.52 ± 0.31%) and commercial (28.50 ± 0.22%) carrageenans possessed minimum radical scavenging activity. The IC_{50} value recorded was 57.22 and 79.4 μg/ml respectively for native and commercial carrageenans. The result indicated that the DPPH radical scavenging activity of native and commercial carrageenans was lower than the standard drug BHA (89.6 ± 0.06%) at the concentration of 100 μg/ml.

3.6. Reducing power assay

The reducing power effect of carrageenans was increased with increasing concentration (Fig. 1e). Commercial carrageenan showed lower (42.54 ± 0.27%) reducing power effect when compared with native carrageenan (46.57 ± 0.32%) at the highest concentration (100 μg/ml) and the same trend was observed at the lowest (12.5 μg/ml) concentration (commercial carrageenan: 22.71 ± 0.21 and native carrageenan: 29.31 ± 0.27%). The IC_{50} value of native and commercial carrageenans was recorded as 139.4 and 175.5 μg/ml, respectively. The reducing power effect of both carrageenans was compared with the standard BHA, the result displayed that the native and commercial carrageenans exhibited potent activity at maximum concentration, but it was low when compared with the standard drug BHA (71 ± 0.49% at 100 μg/ml concentration).

The results obtained from the *in vitro* antioxidant studies demonstrated that, native carrageenan obtained from *K. alvarezii* proved to be a better source of antioxidants in comparison with commercial carrageenan.

3.7. Anticancer activity of native and commercial carrageenans

Anticancer activities of native and commercial carrageenans were assessed by MTT assay. The results obtained are summarized below.

3.7.1. Anticancer activity against breast cancer cell line (MCF-7)

Result on treatment of various doses of native and commercial carrageenans on breast cancer (MCF-7) cell line showed percentage cell viability and percentage cell inhibition. Decrease in cell viability and increase in cell inhibition were observed in dose dependent manner when compared with (untreated) control cells (Fig. 2a and 3a). Significant ($P < 0.05$) reduction in cell viability was observed from 100 to 150 μg/ml concentration of native (50.93 ± 0.174 to 43.63 ± 0.257%) and commercial carrageenans (45.04 ± 0.698 to 32.67 ± 0.077%) and significant growth inhibition were also noted in the same concentration of native (49.07 ± 0.174 to 56.37 ± 0.257%) and commercial (54.96 ± 0.698 to 67.33 ± 0.077%) carrageenans. Commercial carrageenan showed excellent anticancer activity against breast carcinoma cell line, when compared with native carrageenan, whereas the standard drug cyclophosphamide treated cancer cell lines showed the growth inhibition of 64.68 ± 1.012 and 83.42 ± 1.023% at 100 and 150 μg/ml concentrations respectively (Fig. 6). Both native and commercial carrageenans possessed significant inhibition effects against MCF-7 cell proliferation with the IC_{50} values of 103.2 and 63.61 μg/ml, respectively. It was comparatively higher than the IC_{50} value (47.65 μg/ml) of the standard drug cyclophasphamide (Fig. 3).

3.7.2. Anticancer activity against colon cancer cell line (HT-29)

The anticancer activity of both native and commercial carrageenans on colon cancer cell line (HT-29) possessed dose dependant inhibitory activity. The inhibition rate observed at different concentrations (10 to 150 μg/ml) of native and commercial carrageenans treated groups is followed as 5.30 ± 0.215 to 67.67 ± 0.168% and 11.38 ± 0.128 to 53.29 ± 0.120%, respectively. The control group exhibited 100% cell viability. Native carrageenan possessed admirable cell inhibition against colon cancer cells, when compared with commercial carrageenan (Fig. 2b and b). The IC_{50} value of the standard drug cyclophosphamide was found to be 44.33 μg/ml; whereas, it was observed as 73.87 and 123.8 μg/ml for native and commercial carrageenans respectively.

3.7.3. Anticancer activity against liver cancer cell line (Hep G2)

Native and commercial carrageenans reduced the numbers of viable Hep G2 cells in a dose-dependent manner. Commercial carrageenan possessed moderate activity, when compared to native carrageenan. At the minimum concentration (10 μg/ml), both native and commercial carrageenans possessed 31.5 ± 0.66 and 15.19 ± 0.73% growth inhibition (Fig. 2c). Native carrageenan showed strongest inhibition of 64.81 ± 0.87% at 150 μg/ml concentration, whereas the commercial carrageenan showed only 52.66 ± 0.56% inhibition at the same concentration. However, when the concentration level increased, simultaneously the percentage inhibition of cells was also increased. The IC_{50} value of both native and commercial carrageenans was determined as 56.71 and 125 μg/ml, respectively, but it was higher than the IC_{50} value (27.55 μg/ml) of the standard drug cyclophasphamide.

3.7.4. Anticancer activity against osteosarcoma cancer cell line (MG63)

Native and commercial carrageenans have promising cytotoxic activity against MG63 cells in *in-vitro*, when compared with the standard drug cyclophasphamide (Fig. 2d). Commercial carrageenan showed cytotoxic activity and cell inhibition of 27.6 ± 1.30% at 10 μg/ml, whereas it increased to 62.35 ± 1.60% at 150 μg/ml on MG63 cells. Similarly, the native carrageenan possessed strong inhibition of 23.4 ± 0.92% at 10 μg/ml, but it increased to 65.70 ± 1.62% at150 μg/ml concentration. The IC_{50} value of the standard drug cyclophasphamide, the native and commercial carrageenans was observed as 26.48, 47.85 and 55.48 μg/ml respectively.

The results on cytotoxic effect of both native and commercial carrageenans evidenced that both carrageenans were equally good, however native carrageenan was excellent to possess the inhibition on colon carcinoma (HT-29), liver carcinoma (Hep G2) and osteosarcoma carcinoma (MG63) cell lines. Similarly, commercial carrageenan exhibited splendid inhibition on breast carcinoma (MCF-7) cell lines.

3.8. In vitro *antidiabetic activity (inhibition of α- glucosidase)*

In vitro antidiabetic activity of native and commercial carrageenans against α-glucosidase enzyme showed a dose-dependent inhibitory activity. At a concentration of 100 μg/ml, both native and commercial carrageenans showed 27.61 ± 0.69 and 24.55 ± 0.68% inhibition of α-glucosidase, whereas at the same concentration, the α- glucosidase inhibitory activity of standard drug acarbose was found to be 50.73 ± 1.39%. When the concentration of both native and commercial carrageenans as well as the standard drug increased to 500 μg/ml, the α-glucosidase inhibitory activity was also significantly increased to 74.49 ± 1.05, 67.42 ± 0.63% and 79.56 ± 1.22, respectively. In comparison, native carrageenan possessed better α- glucosidase inhibitory activity than commercial carrageenan (Fig. 4). The ^{IC50} value determined for both native and commercial carrageenans was 222.8 and 270.3 μg/ml, respectively. At the same time, the IC_{50} value of standard drug acarbose against α- glucosidase was found to be 106 μg/ml.

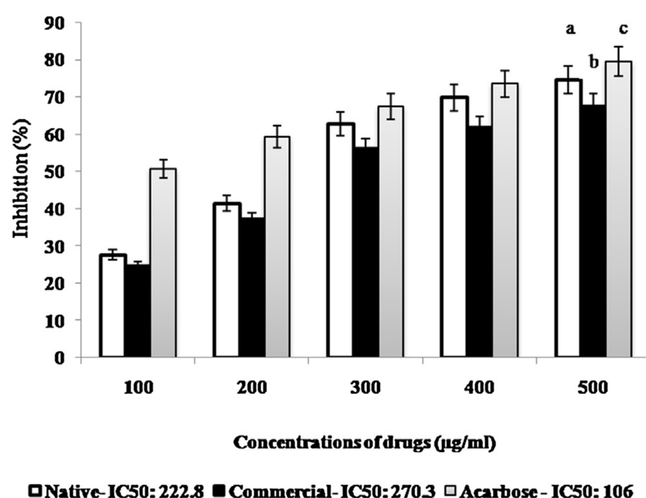


Fig. 4. *In vitro* antidiabetic activity (inhibition of α-glucosidase enzyme) of different concentrations of native and commercial carrageenans in comparison with acarbose.
Each value is the Mean ± S.D of triplicate analysis, bars represented in different superscript letters are statistically significant (One way ANOVA test: $P < 0.005$; further *post hoc* multiple comparison with Tukey's test).

4. Discussion

Natural defense mechanism of an organism is overwhelmed by an excessive generation of reactive oxygen species (ROS), there by oxidative stress occurs. Consequently, cellular and extracellular macromolecules (proteins, lipids, and nucleic acids) suffer through oxidative damage, causing tissue injury [31]. Antioxidants are substances that can delay or prevent oxidation of cellular oxidizable substrates [32]. The most common synthetic antioxidants used at present are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) [33]. Seaweeds are considered to be a rich source of natural antioxidants [34]. Seaweed polysaccharides have been demonstrated to play an important role as free-radical scavengers and antioxidants for the prevention of oxidative damage in living organisms [32]. Badarinath et al. [35] suggested that, antioxidant activity of different products were not able to conclude based on a single antioxidant assay. Several *in vitro* assays should be carried out to evaluate the antioxidant capacity. Because, each test products were varying in different aspects; so, it is difficult to compare them with only one assay, comparison among different *in vitro* methods should be followed to evaluate the accurate antioxidant capacity. Realizing the fact, in the present study *in vitro* antioxidant activity of native carrageenan from red seaweed *K. alvarezii* and commercial carrageenan were evaluated by total antioxidant, hydroxyl radical scavenging, nitric oxide radical scavenging, DPPH radical scavenging and reducing power assays.

In the present study, phytochemical investigation of red algal polysaccharide of native and commercial carrageenans showed the presence of gums and mucilages, carbohydrate, protein, sugar derivatives of saponins, steroid and flavonoids. The additive and synergistic effects of phytochemicals in seaweeds have been proposed as being responsible for their potent antioxidant and anti-cancer activities [10]. These phytochemicals may be effective in combating or preventing diseases due to their antioxidant effect [36]. The total antioxidant potential is a relevant tool for investigating the relationship between dietary antioxidants and pathologies induced by the oxidative stress [37]. Rafiquzzaman et al. [38] reported that the alkali treated carrageenan from seaweed *Hypnea musciformis* showed highest total antioxidant capacity (3.8 mg Ascorbic Acid equivalent) at 2 mg/ml concentration. In the present study also, native carrageenan from *K. alvarezii* and commercial carrageenan possessed an excellent total antioxidant capacity (ascorbic acid equivalent of 0.87 and 0.82 mg) in 100 µg/ml concentration, but standard antioxidant BHA showed moderate (0.73 mg of ascorbic acid equivalent) antioxidant capacity at the same concentration. Rocha de Souza et al. [39] stated that there is always a positive correlation between sulphate content of the carrageenan polysaccharide and its antioxidant capacity. Similarly, Mahendran and Saravanan [40] pointed out that sulphated polysaccharide from green seaweed Caulerpa racemosa possessed tremendous antioxidant capacity (80.24 ± 0.56%) at 1000 µg/ml concentration. These findings suggested that native and commercial carrageenans from *K. alvarezii* showed relevant antioxidant activity than carrageenans from other seaweeds. This may be due to the chemical structure of carrageenans which varies according to the specific algal species [41].

Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology [42]. This radical has a capacity to join nucleotides in DNA and cause strand breakage that contributes to carcinogenesis, mutagenesis and cytotoxicity [43]. Hydroxyl radical was measured by the deoxyribose method [44]. Ferric-ascorbate- EDTA-H_2O_2 (Fenton reaction) generates hydroxyl radical, which react with deoxyribose to produce thiobarbituric acid reactive substances (TBARS) and upon heating with TBA will form pink chromogen. The hydroxyl quenchers reduce TBARS production and formation of pink chromogen by competing with deoxyribose. The scavenging activities of native and commercial carrageenans against hydroxyl radicals were significantly determined with the concentration of the polysaccharides. Similarly, Huamao et al. [45] also stated that κ-carrageenan oligosaccharides from *K. striatum* possessed relevant antioxidant activity in *in-vitro* system. Shonima et al. [46] suggested that polysaccharide isolated from *Ulva fasciata* exhibited an excellent hydroxyl radical scavenging activity and the IC_{50} value was found to be 70 µg/ml concentration. But, sulphated polysaccharide isolated from green seaweed *C. racemosa* showed tremendous radical scavenging activity (68.29 ± 1.03%) only at 1000 µg/ml concentration. Likewise, Sokolova et al. [47] portrayed that, carrageenan polysaccharides (lambda, iks-, kappa-, kappa/beta and kappa/iota) from red algae *Gigartinaceae* sp and *Tichocarpaceae* sp displayed moderate scavenging effect to hydroxyl radicals, the capacity of carrageenans to scavenge hydroxyl radicals was significantly determined with the polysaccharide concentration except commercial κ- carrageenan. Commercial sample of iota-carrageenan exhibited the highest (21%) inhibition percentage at the concentration of 0.5 mg/ml, and the scavenging capacity of commercial lambda-carrageenan was same to that of lambda carrageenan from *C. armatus* with the concentration of 0.5 mg/ml. Invariably, in the present study the maximum (61.4 ± 0.27%) scavenging activity was shown by the native carrageenan of *K. alvarezii* at 100 µg/mg concentration, however the commercial carrageenan displayed 58.66 ± 0.31% scavenging activity at the same concentration. Native carrageenan from *K. alvarezii* exhibited more prominent effect in hydrogen peroxide scavenging in comparison with commercial carrageenan. The results of this study suggested that, the scavenging actions of these compounds are different, with acetylated and sulfated polymers being effective in scavenging the hydroxyl radical. Rocha de Souz et al. [39] also stated that the carrageenan polysaccharides, including lambda (*Gigartina acicularis*, *G. pisillata*), κ- (*K. alvarezii*) and iota (*E. spinosa*) (Sigma Aldrich) exhibited hydroxyl radical scavenging activity. But the iota carrageenan had a high inhibitory effect on hydroxyl radicals with the IC_{50} value of 0.281 ± 0.072 in relation to the lambda

(0.357 ± 0.120) and κ- (0.335 ± 0.016) carrageenans. Rafiquzzaman et al. [38] reported that, carrageenan extracted from *Hypnea musciformis* showed dose-dependent hydroxyl radical scavenging activity ranging from 7.8 to 50.7% (0.5–2 mg/ml^{-1}). Zhou et al. [48] proposed that, λ-carrageenan fractions (CF1, CF2 & CF3) from *Chondrus ocellatus* also exhibited hydroxyl radical scavenging activity at high concentration with the IC_{50} value over 0.1 mg/ml. This concluded that, native (κ) carrageenan from *K. alvarezii* possessed maximum hydroxyl radical scavenging activity than commercial (κ) and other type of carrageenans from various seaweeds. This could be due to the nutritional effect and the life stages of the seaweeds, as well as the extraction procedures of the carrageenans from the selected seaweeds [48].

The toxicity and damage caused by nitric oxide (NO) and O_2 are multiplied as they react to produce reactive peroxy nitrite, which lead to serious toxic reactions with biomolecules such as protein, lipids, and nucleic acids. High concentration of NO has deleterious effect and therefore, it is necessary that the production of NO be tightly regulated [49]. When NO is produced by macrophages, the nitric oxide radical can be converted into peroxynitrites, which will cause diverse chemical reactions in a biological system including nitration of tyrosine residue of protein, triggering lipid peroxidation, inactivation of aconites, inhibition of mitochondrial electron transport and oxidation of biological thiol compound [50]. Matloub et al. [51] reported that polysaccharides isolated from marine algae *Dictyopetris membraceae*, *Corallina officinalis* and *Petrocladia capillraceae* possessed 77–82% nitric oxide scavenging activity at 100 μg/ml concentration. But, Sokolova et al. [47] proposed that, at the concentration of 0.25 mg/ml, iks-carrageenan was active in scavenging nitric oxide (37.26%), because it contains three sulphate groups per disaccharide unit and a 3,6-anhydrogalactose unit, so it was the most effective in nitric oxide scavenge. But low sulphated kappa/beta- and commercial kappa-carrageenans showed lowest nitric oxide scavenging activity. Likewise in the present study, the nitric oxide radical scavenging activities of the native and commercial carrageenans were depending upon the concentration of the polysaccharide. Both native and commercial carrageenans inhibited the nitric oxide released from the sodium nitroprusside. The maximum (80.42 ± 0.22%) scavenging activity was shown by native carrageenan of *K. alvarezii* at 100 μg/ml concentration, whereas commercial carrageenan showed 73.66 ± 0.22% inhibition at the same concentration. Comparatively it was higher than that of the standard drug. Similarly, antioxidant actions of carrageenans against reactive oxygen/nitrogen species depend on polysaccharide concentration and such fine structural characteristics as presence of a hydrophobic 3,6-anhydrogalactose unit, amount and position of sulphate groups, and an oxidant agent, on which sample antioxidant action is directed. The present findings revealed that abundant quantity of sulphate content in the seaweed *K. alvarezii* was reflected in both native and commercial carrageenans, due to the highest sulphate content, these carrageenans possesses highest nitric oxide radical scavenging activity than other carrageenans.

DPPH have been used extensively as a free radical to evaluate reducing substances [52]. A freshly prepared DPPH solution exhibits a deep purple color. This purple color generally fades when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless product (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. In the present study DPPH free radical scavenging activity of native and commercial carrageenans was recorded as 56.26 ± 0.20% and 53.66 ± 0.082% respectively at 100 μg/ml concentration, however lower than the standard drug (89.6 ± 0.54%). Similarly, Rafiquzzaman et al. [38] reported the DPPH free radical scavenging activity of carrageenan from *H. musciformis*, which was varied between 15.01 to 45.09% (0.5–2 mg/ml). In accordance with these Matloub et al. [51] suggested that sulphated polysaccharides extracted from seaweeds *Enteromorpha intestinalis* and *Corallina officinalis* exhibited 50 and 53% scavenging activity respectively at 100 μg/ml concentration. Similarly, John Peter Paul [53] showed that fucoidan from *Padina distromatica hauck* exhibited 40.43 ± 2.80% DPPH scavenging activity at 100 μg/ml concentration. Zhou et al. [54] suggested that, λ-carrageenan fractions (CF1, CF2, CF3) from *C. ocellatus* also possessed scavenging activity against DPPH, among them CF3 had the strongest scavenging activity on DPPH, while CF1 had the weakest effect. In the present study, DPPH radical scavenging properties of native and commercial carrageenans were comparatively similar with other carrageenans, mainly due to the elements which are common in all types of carrageenans.

Reducing capacity is considered as a significant indicator of potential antioxidant activity of a compound [55]. The reducing power assay is used to evaluate the ability of an antioxidant to donate electrons or hydrogen. The presence of reductants (i.e. antioxidants) causes the reduction of the $Fe3^{+}$/ferricyanide complex to the ferrous form. Rafiquzzaman et al. [38] stated that, the reducing power of carrageenans extracted from *H. musciformis* represented 1.1 mg/ml ascorbic acid at 2 mg/ml concentration. Sokolova et al. [47] also pointed out that kappa/iota- and kappa/beta-carrageenans were the most active reducing agents, whereas lambda- iks- and kappa-carrageenans were less effective reducing agents with the value of approximately 84.39, 66.61 and 58.50 mmol of ascorbic acid equivalents/g. Likewise, in the present study, native carrageenan from red algae *K. alvarezii* (46.57 ± 0.32%) and commercial carrageenan (42.54 ± 0.27%) possessed moderate reducing power at 100 μg/ml concentration, when compared to the standard drug (71.01 ± 0.49). There was a stable increase in reductive potential of the carrageenans was observed with increase in the concentration. Natural antioxidants are believed to break free-radical chain reactions by donating an electron or hydrogen atom to free radicals. Therefore, the reducing power of a compound is a significant indicator of its potential antioxidant activity. Similarly, Costa et al. [56] showed that heterofucans from the seaweed *S. filipendula* expressed reducing power (42%) as percentage activity of ascorbic acid at 0.1 mg/ml concentration. The same authors suggested that the heterofucan SF-1.0 v and SF-1.50 v showed considerable reducing power at 0.1 mg/ml concentration. At the highest concentration (0.5 mg/ml), SF-1.0 v possessed activity, which is similar to that found in vitamin C. Likewise, Costa et al. [57] also pointed out that sulphated polysaccharide from *Graffenrieda caudate* expressed reducing power as 42% inhibition shown by 0.2 mg/ml of ascorbic acid at 100 μg/ml concentration. It concludes that both native and commercial (κ) carrageenans from *K. alvarezii* possessed moderate reducing power; which may be due to the characteristic quality of carrageenans and its algal source [57].

The present study is the first attempt to report the antioxidant (total antioxidant potential, hydroxyl radical, nitric oxide, DPPH and reducing capacity) properties of native carrageenan from *K. alvarezii* in comparison with commercial carrageenan. The compounds like steroids and flavonoids identified in *K. alvarezii* have been described as a strong antioxidant agent [58]. Rocha de Souz et al. [39] reported that commercial κ- carrageenan (*K. alvarezii*) from Sigma Aldrich possessed moderate hydroxyl radical scavenging activity, this is similar to our findings too. Similar works were also done by several authors on carrageenan from various sources like *Hypnea musciformis, K. striatum, Gigartinaceae* sp, *Tichocarpaceae* sp, *C. armatus, Gigartina acicularis, G. pisillata, E. spinos, Chondrus ocellatus, Dictyopetris membraceae, Corallina officinalis,*

Petrocladia capillraceae, Enteromorpha intestinalis, Corallina officinalis, S. filipendula and *Graffenrieda caudate.* But the native carrageenan from the seaweed *K. alvarezii* is the most predominant one and exhibited better activity than carrageenans from other seaweeds.

Antioxidants are frequently used with the goal of preventing cancer. Cancer is a broad term for a class of diseases characterized by abnormal cells that grow and invade healthy cells in the body. It is the second leading cause of death next to heart diseases. Breast cancer is the leading cause of death among women in many countries [59]. This is the second most common cancer overall, with nearly 1.7 million new cases diagnosed worldwide in 2012. Colorectal cancer is the third most common cancer in the world, with nearly 1.4 million new cases diagnosed in 2012 [60]. Dietary habits can affect the development of colorectal cancer. Liver cancer is the fifth most common cancer in men and the eighth most common in women [61]. One of the report says that 711000 cases of primary liver cancer occurred worldwide in 2007; it is the third leading cause of cancer death worldwide with an estimated 680000 deaths occurred during 2007. Bone cancer or osteosarcoma was prevalently found in younger age group than many other cancers, 15% of female and 17% of male patients were aged under 15 were diagnosed osteosarcoma [62]. Therefore, in the present study *in vitro* studies on anticancer activity of native and commercial carrageenans against human breast (MCF-7), colon (HT-29), liver (Hep G2) and osteosarcoma (MG63) cell lines were determined though MTT assay.

Most toxicologists believed that *in vitro* toxicity testing methods can be more useful than *in vivo* toxicology studies in living animals due to more time and cost-effective methods. MTT (3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. When cells die, they lose the ability to convert MTT into formazan, thus color formation serves as a useful and convenient marker of only the viable cells. In the present study, native carrageenan from *K. alvarezii* and commercial carrageenan exhibited *in vitro* anticancer activities at various concentrations and the maximum inhibition was observed in 150 μg/ml concentration against breast (MCF-7), colon (HT-29), liver (Hep G2) and osteosarcoma (MG63) cell lines. With increase in concentration, both carrageenans documented reduced percentage of cell viability. Similarly, several studies were reported that carrageenan polysaccharides possess antiproliferative activity against cancer cell lines [63]. Carrageenans may block the interactions between cancer cells and the basement membrane by antimetastatic activity, thus it inhibits tumor cell proliferation and tumor cell adhesion to various substrates [64].

Ahna et al. [65] reported that, sulphated polysaccharide (CPs) isolated from *E. cava* showed the cell inhibitory activity against the growth of FM3A (mouse mammary carcinoma cell line) and B16F10 (colon carcinoma cell lines) at the concentration of 9.4–75 μg/ml, compared to the non-treated control cells. Similarly, in the present study at 150 μg/ml concentration, both native (56.37 ± 0.257 and $67.67 \pm 0.168\%$) and commercial (67.33 ± 0.077 and $53.29 \pm 0.120\%$) κ- carrageenan possessed maximum cell inhibition on the growth of breast (MCF-7) and colon (HT-29) carcinoma cell lines and induction of apoptosis mediated through both the mitochondrial mediated and death receptor-mediated pathways. As well, their results indicated that both carrageenans induced apoptosis via the death receptor-mediated pathway through both the direct and indirect activation of caspase- 3 in HT-29 cells. It indicated that both native and commercial carrageenans are a potentially useful therapeutic agent for colon cancer by simultaneous activation of different apoptotic factors and pathways [66] Similarly, Luo et al. [67] recorded that intratumoral injection of λ-carrageenan (50 mg/kg) had a significant antitumor activity in mice, but in *in-vitro* studies, 0.25-1.0 mg/ml of λ-carrageenan (24 h) failed to show cell inhibition against 4T1 breast cancer cell lines and cell viability was maintained above 80%, which indicated a low cytotoxicity of λ-carrageenan to tumor cells. Likewise, iota carrageenan at 1000 μg/ml concentration showed 75.4% viability of HCT116 (colon carcinoma) cells and no cytotoxicity was observed [68].

In an another study, Kim et al. [69] revealed that, fucoxanthin (0.01% in drinking water) from *Hijikia fusiforme* possessed a chemopreventive potential against preneoplastic aberrant crypt foci (ACF) in the colon of (B6C3F1) mice by providing it for 7 weeks. Laminarin from *Laminaria digitata* induces apoptosis in HT-29 colon cancer cells, as well as the involvement of the ErbB signalling pathway revealed that laminarin induced cell death in a dose-dependent manner [70]. Kotake-Nara et al. [71] recorded that fucoxanthin isolated from *U. pinnatifida* revealed a remarkable inhibitory activity against the viability of human colon cancer cell lines (HT-29, DLD-1, and Caco-2) through apoptosis, which was evidenced by DNA fragmentation. Moghadamtousi et al. [72] also exhibited that, fucoidan (20 μg/ml) extracted from brown seaweed *F. vesiculosus* demonstrated a dose-dependent antiproliferative effect and induction of apoptosis through both the mitochondrial-mediated and death receptor-mediated pathways.

Ariffin et al. [73] have observed that iota carrageenan was non-cytotoxic to normal and cancer liver cell lines even at 2000 μg/ml. But in the present study, both native and commercial κ-carrageenans ^{respectively} possessed 35.19 ± 0.87 to $47.34 \pm 0.56\%$ viability of liver carcinoma Hep G2 cell line at 150 μg/ml concentration. Similarly, Yuan et al. [74] reported that carrageenan oligosaccharides from *K. striatum* showed significant inhibition on the growth of transplantable sarcoma S180 in – bearing mice. Zhou et al. [48] proposed that low molecular λ-carrageenan from *C. ocellatus* possessed anticancer activity on H-22 (mouse liver cell line). They also suggested that antitumor activity of carrageenan might be closely related to their molecular weight. Likewise, in the present study, both native and commercial κ- carrageenans inhibited the cell viability of human liver carcinoma Hep G2 (68.50 ± 0.66 and $84.81 \pm 0.73\%$) and MG63 osteosarcoma (76.6 ± 0.92 and $72.4 \pm 1.34\%$) at 10 μg/ml concentration itself. κ-carrageenans with lower molecular weights have better antioxidant properties and may be promising for cancer prevention [75].

Nisizawa [76] suggested that carrageenan play an important role on clearing the digestive system, protecting the stomach surface membrane, and also preventing the effects of potential carcinogens on the intestine. Yuan et al. [77,78] pointed out that, consumption of $200\,\mu g\,g^{-1}$ carrageenan per day showed the uppermost antitumor activities. Hu et al. [79] recorded that carrageenan oligosaccharide from the red seaweed *K. striatum* orally administrated to mice for 14 days inoculated with S180 tumor cell suspension resulted in growth inhibition of transplantable sarcoma cells, increased macrophage phagocytosis, enhanced antibody production, increased lymphocyte proliferation, stronger NK cell activity, and elevated levels of IL-2 and TNF-α. These results suggested that the studied oligosaccharides exert their antitumor effects by promoting the immune system.

This attempt was made to examine the anticancer activity of native and commercial kappa carrageenans on human carcinoma cell lines (breast (MCF-7), colon (HT-29), liver (Hep G2) and osteosarcoma (MG63). Because, iota carrageenan failed to show anticancer activity against carcinoma cell lines [68]. But Yuan et al. [74] and Zhou et al. [48] documented antitumor activity of carrageenan oligosaccharides from *K. striatum* and low molecular λ

carrageenan on mice carcinoma cell lines (HCT116 colon and H-22 liver), but nobody has focused on human cell lines. Thus, the present findings clearly emphasized that the native and commercial carrageenans are effectively active against various carcinoma cell lines.

Dumelod et al. [80] proposed that λ-carrageenan incorporated into a meal reduced the blood glucose level in human subjects. The concept being that is, the soluble fiber decreases the amount of carbohydrates that reach the bloodstream by delaying their absorption in the small intestine. They portrayed that the average blood glucose level of subjects were significantly lower after consuming the carrageenan meal than consuming the regular meal (P < or = 0.05 at 15, 45, and 90 min). Similarly, Masako Fuwa et al. [81], proposed that κ-carrageenan-supplemented rice i.e. CP (adding carrageenan during cooking) had significantly lower blood sugar level after 15 and 30 min than CG (i.e. carrageenan gel or in the combination of [CG-CaCl2] to pre cooked rice). But they failed to report the mechanism behind in it. In recent approach, postprandial hyperglycemia was controlled by inhibiting the carbohydrate hydrolyzing enzymes such as α- glucosidase and α-amylase in the digestive system [82]. α-Glucosidase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation [83]. The principal factor acting on α-glucosidase activity is hydrogen scavenging because α- glucosidase provides hydrogen to catalyze the hydrolysis of the α-(1,4)-glucosidic linkage [84]. Inhibitors like acarbose and meglitol, which are currently in clinical use for inhibit α- glucosidase and α-amylase [85]. In the present study, *in vitro* α- glucosidase assay suggested that native and commercial κ- carrageenans inhibit α-glucosidase enzyme (27.61 ± 0.69 to 74.49 ± 1.05% and 24.55 ± 0.68 to 67.42 ± 0.63%) with increasing concentrations (100–500 μg/ml). It was quit lower than the standard drug acarbose (50.73± 1.39% to 79.56 ± 1.22%). Likewise, John Peter Paul [53] proposed that sulphated polysaccharide fucoidan exhibited 70.43% inhibition of α- glucosidase enzyme at 100 μg/ml concentration itself. Vishnu Kiran and Murugesan [86] also reported that silver nanoparticles from seaweed *Halymenia poryphyroides* exhibit *in-vitro* α- glucosidase inhibitory activity (52.10%) at 400 μg/ml concentration. From the study, it could be proved that blood glucose lowering capacity of carrageenans only based on its α- glucosidase inhibitors. *In-vitro* α- glucosidase activity of native carrageenan from *K. alvarezii* and commercial carrageenan could prove useful in the prevention and management of metabolic disorders like diabetes.

On the basis of the above results, it can be concluded that native carrageenan from *K. alvarezii* (κ) and commercial carrageenan (κ) possess significant antioxidant, anticancer and antidiabetic activities on *in vitro* models. Throughout the study, native carrageenan exhibited better pharmaceutical activity in comparison with commercial carrageenan, because native polysaccharides do not always exist alone, it conjugate with other components such as amino acid and nucleic acids residues. Sometimes the polysaccharide conjugates act as a whole in isolation [87]. This may be the reason behind the surplus medicinal properties of native carrageenan. Wu et al. [88] also suggested that, native crude polysaccharides always exhibited notable antioxidant activity, but after further fractionation, the final purified form of polysaccharide showed moderate or low activity due to loss of certain elements through industrialization and refining process.

5. Conclusion

This study has exposed a novel function of (κ) carrageenans as antioxidant agents against human breast carcinoma, colon carcinoma, liver carcinoma and osteosarcoma cell lines and also serves as an efficient inhibitor for the enzyme α-glucosidase. It provides a strong evidence for the use of carrageenan in folklore treatment as multi therapeutic agent. Further *in vivo* studies are also needed for understanding their mechanism of action as antioxidants.

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