Original Article

In vitro radical scavanging activities of *Chrysaora quinquecirrha* nematocyst venom

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ABSTRACT: The venom of *Chrysaora quinquecirrha* (sea nettle) contains several toxins that have bioactivity in mammals. In our study we aimed to extract proteins from *Chrysaora quinquecirrha* and to test the antioxidant potential of both crude protein and purified fractions. Proteins extracted from sea nettle nematocyst venom were purified through Sephadex G-100 column chromatography. The molecular weight of purified proteins was determined by gel filtration and SDS-PAGE and was found to be 105, 65, and 9 kDa for Frc-1, Frc-2, Frc-3, respectively. The *in vitro* antioxidant potential of *Chrysaora quinquecirrha* was evaluated in different systems *viz*. radical scavenging activity by DPPH reduction, superoxide radical scavenging activity in PMS/NADH-NBT system, hydroxyl radical by Fe^{3+}-Ascorbate-EDTA-H_2O_2 system and nitric oxide (NO) radical scavenging activity in sodium nitroprusside/ Greiss reagent system. Frc-3 displayed the maximal antioxidant activity and found to have different levels of antioxidant properties in the models tested. In scavenging hydroxyl radicals, its activity was intense (IC_{50} = 50.8 µg/mL) while in scavenging NO radical, it was moderate (IC_{50} = 381.4 µg/mL).

Keywords: Antioxidant peptide, *Chrysaora quinquecirrha*, DPPH radical, superoxide radical, hydroxyl radical, nitric oxide radical

1. Introduction

Oxidative-free radicals are byproducts of the normal reactions within our body. These reactions include the generation of calories, the degradation of lipids, the catecholamine response under stress, and the inflammatory processes (*1*). If the balance between oxidative-free radical production and eradication is maintained, the harmful effects of free radicals would be minimized in the body. However, if the unwanted free radicals are not eradicated efficiently, oxidative stress would occur. Oxidative stress, caused by reactive oxygen or free radicals, has been shown to be associated with the progression of many diseases including cancer, heart disease, and depression, among others (*2-4*). An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases (*5*). The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases (*6*). Various antioxidant compounds are identified in many natural sources including some protein compounds. Proteins of jellyfish (*7*) and protein hydrolysates from different sources, such as milk protein (*8*), maize zein (*9*), egg-yolk (*10*), porcine proteins (*11*), yellow stripe trevally (*12*), yellowfin sole frame (*13*), mackerel (*14*), have been found to possess antioxidant activity.

The jellyfish, *Chrysaora quinquecirrha*, a cnidarian of the class Scyphozoa, the order, Semaeostomeae and the family Pelagiidae is distributed widely in the Bay of Bengal, India. The *Chrysaora quinquecirrha* has two types of tentacles, fishing and mesenteric tentacles. These tentacles have been shown to contain many types of stinking organelles or nematocyst (*15*). It has been reported that venom from the fishing tentacles nematocyst is considerably more lethal than that of mesenteric tentacles nematocyst (*16*). Research on *Chrysaora quinquecirrha* has indicated the presence of numerous compounds, including various amines and large molecules such as proteins or peptides (*16*) as its components. Jellyfish nematocyst has been venom found to possess myotoxic (*17*), neurotoxic (*18*), apoptotic (*19*), cytotoxic (*20*), cardiotoxic (*21*) and antimicrobial activity (*22*). Regarding jellyfish derived peptides, to the best of our knowledge there are only a few reports dealing with their antioxidant properties (*7*). In this study we isolated two proteins and a peptide from fishing tentacles nematocyst of *Chrysaora quinquecirrha* and demonstrated its antioxidant potential.

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2. Materials and Methods

2.1. *Chemicals*

Sephadex G-100, 2-deoxy ribose, 1,1-diphenyl-2-picryl-hydrazil (DPPH), ethylene diamine tetra acetic acid (EDTA), hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), sodium nitroprusside and trolox were purchased from M/s. Sigma Chemical Co., St. Louis, MO, USA. Protein molecular weight maker was purchased from Genei (India). All other chemicals and solvents were of analytical grade and were obtained from Himedia chemicals, Mumbai, India.

2.2. *Specimen collection and protein purification*

Nettles were collected during the summer months in the Bay of Bengal near Pazhayar, Tamilnadu, India. The fishing tentacles were excised manually *in vivo* and nematocyst were isolated by the method described by Rice and Powell (*23*). The nematocyst mass was suspended in phosphate buffered saline and observed under microscope to confirm the presence of nematocyst. When required, approximately, 1 mg of freeze dried nematocysts were resuspended in 10 mL of double distilled water, and then subjected to 8 × 30 sec sonication cycles at 4°C with 1 min rest between cycles. The venom preparation was aliquoted, lyophilized and stored at -20°C. Crude proteins extracted from nematocyst were purified by Sephadex G-100 gel filtration and 3 mL fractions were collected and their absorbance, at 280 nm was determined by an ultraviolet detector. Protein concentration was measured by the method of Lowry *et al.* (*24*).

The molecular weight of the proteins were estimated by Sephadex G-200 gel filtration chromatography according to the method of Andrews (*25*) by using the parameter $K_{av} = (V_e - V_o) / (V_t - V_o)$. A standard curve was determined by chromatographing phospholipase, bovine serum albumin, ovalbumin, carbonic anhydrase, soyabean trypsin inhibitor and lysozyme calculating K_{av} for each, and plotting native MW *vs.* K_{av}. Reducing SDS-PAGE analysis was conducted (*26*) on crude nematocyst extracts and chromatography fractions using a Bio-Rad Mini-PROTEAN II electrophoresis system. Samples containing low amounts of protein were concentrated by TCA precipitation prior to analysis. Protein samples were separated on 10-15% polyacrylamide gels and protein bands were visualized with Coomassie brilliant blue R-250 (*27*).

2.3. *Radical scavenging assays*

The antioxidant activity of the proteins were determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blios (*28*). A measurement of superoxide anion scavenging activity of proteins was performed based on the method described by Nishimiki *et al.* (*29*). Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compound (proteins) for hydroxyl radical generated by Fe^{3+}-Ascorbate-EDTA-H_2O_2 system (Fenton reaction) according to the method described by Kunchandy and Rao (*30*). The nitric oxide radical inhibition activities of proteins were measured by the method described by Garrat (*31*). All the tests were performed for six times.

2.4. *Statistical analysis*

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) by using statistical package of social science (SPSS) version 10.0 for windows. The values are mean ± SD for six experiments in each group. *P* values < 0.05 were considered as level of significance.

3. Results

Nematocyst preparation yielded cleanest preparation with no visible ruptured nematocyst (Figure 1). Three major protein peaks appeared in the elution of Sephadex G-100 (Figure 2). The eluted volume of the first peak was 48 mL (Frc-1) and the eluted volume of the second

Figure 1. Light microscopic views of the nematocysts prepared suspensions of *Chrysaora quinquecirrha* species.

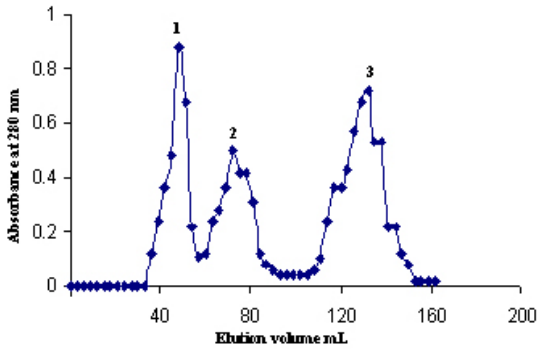

Figure 2. Chromatogram of crude nematocyst venom by Sephadex G-100 size-exclusion chromatography. Protein concentration was monitored at 280 nm. Three major protein peaks were obtained at the flow rate of 25 mL/h.

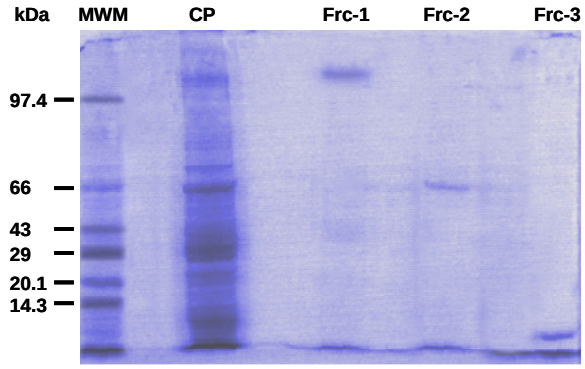

Figure 3. SDS-PAGE analysis of *Chrysaora quinquecirrha* nematocyst proteins purified by gel filtration. Protein bands were stained with Coomasie Blue. *Lane* 1, molecular weight marker (MWM); *lane* 2, crude protein; *lanes* 3, 4, and 5, Frc-1, 2, and 3, respectively.

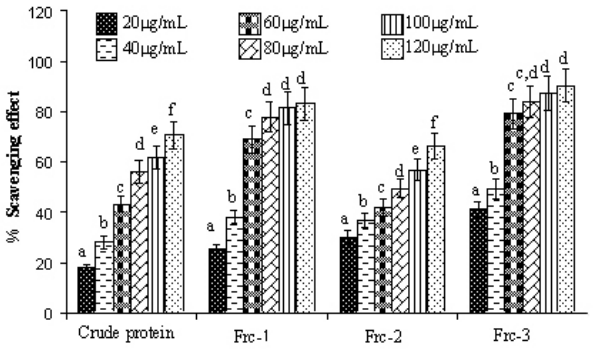

Figure 4. Scavenging effect of protein samples on DPPH radical. The superscripts a-f in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

Table 1. IC_{50} values of protein samples

Assays	Crude protein (µg/mL)	Frc-1 (µg/mL)	Frc-2 (µg/mL)	Frc-3 (µg/mL)
DPPH radical scavenging assay	78.4 ± 1.80	59.7 ± 1.53	82.0 ± 1.68	53.8 ± 0.82
Superoxide radical scavenging assay	80.04 ± 2.40	63.93 ± 1.82	76.58 ± 1.20	55.02 ± 0.63
Hydroxyl radical scavenging assay	65.2 ± 0.75	52.1 ± 0.61	73.2 ± 1.65	50.8 ± 0.95
Nitric oxide radical scavenging assay	539.3 ± 50.01	441.9 ± 19.56	400.1 ± 9.72	381.4 ± 0.95

peak (Frc-2) and third peak (Frc-2) was 72 and 132 mL, respectively. The estimated native molecular masses of the other major peaks (Frc-1, 2, and 3) were calculated as 105, 65, and 9 kDa.

Figure 3 shows the bands of five protein samples. Marker lane shows bands of protein markers. Lane 2 shows crude proteins (CP) and more than 8 proteins were seen after staining of SDS-PAGE gel. Frc-1, Frc-2, and Frc-3 lanes show the purified protein. The molecular weight of these proteins were 105, 65, and 9 kDa, respectively.

The results obtained by DPPH assay are shown in Figure 4. *Chrysaora nematyocyst* proteins exhibited powerful DPPH radical scavenging activity. CP at a concentration from 20-120 µg/mL showed a scavenging effect on the DPPH radical from 18.0-70.7%. The results were found to be statistically significant ($P < 0.05$). IC_{50} values of all experiments are shown in Table 1. IC_{50} of CP, Frc-1, Frc-2, and Frc-3 was found to be 78.4, 59.7, 82.0, and 53.8 µg/mL, respectively.

The superoxide radical-scavenging activities of proteins are shown in Figure 5. The protein samples scavenge superoxide anion radical in a dose dependent manner. Frc-3 at a concentration from 20-120 µg/mL showed a scavenging effect on the superoxide anion radicals from 43.9 to 92.6%. The results were found to be statistically significant ($P < 0.05$).

Figure 6 shows the % scavenging effects on hydroxyl radical of CP, Frc-1, Frc-2, and Frc-3. IC_{50} of Frc-1 and Frc-3 was 52.1 and 50.8 µg/mL, respectively. Frc-1 at a concentration from 20-120 µg/mL, the % scavenging effect was from 50.7-88.0% and for Frc-3 at a concentration from 20-120 µg/mL, the % scavenging

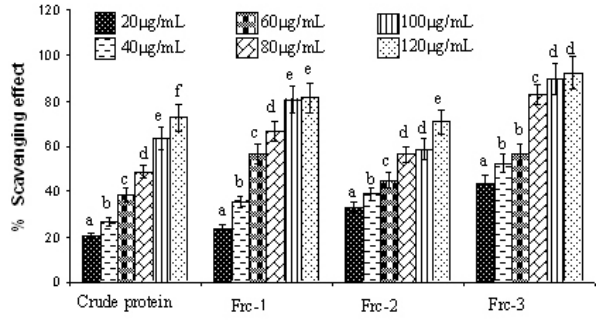

Figure 5. Scavenging effect of protein samples on superoxide radical. The superscripts a-f in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

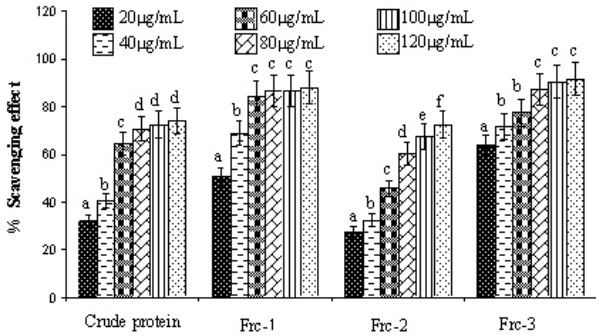

Figure 6. Scavenging effect of protein samples on hydroxyl radical. The superscripts a-f in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

effect was from 63.6-91.7%. Figure 7 shows the nitric oxide scavenging effects of CP, Frc-1, Frc-2, and Frc-3 at a concentration from 50-300 µg/mL. The results were found to be statistically significant ($P < 0.05$).

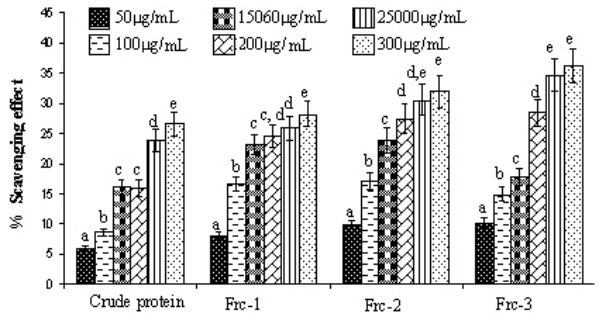

Figure 7. Scavenging effect of protein samples on nitric oxide radical. The superscripts a-e in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

4. Discussion

Separation of native *Chrysaora quinquecirrha* venom proteins was achieved by size-exclusion chromatography across the molecular mass separation range, 10-200 kDa. Reducing SDS-PAGE analysis of the crude venom and purified fractions revealed that all the three major fractions are significantly purified and the apparent molecular weight is same as calculated by size exclusion chromatography.

It is well known that the radical system used for antioxidant evaluation may influence the experimental results, and two or more radical systems are required to investigate the radical-scavenging capacities of a selected antioxidant (*32*). The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity (*33,34*). DPPH is one kind of the compound that has a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (*35,36*). All the protein samples had strong DPPH scavenging activities. Frc-3 showed maximum scavenging effect on DPPH radical.

Superoxide anion is also an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using four electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anion. It plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems (*37*). Therefore, studying the scavenging effects of sea nettle proteins on superoxide radicals is one of the most important ways of clarifying the mechanism of antioxidant activity.

The superoxide anion radicals scavenging activity of protein samples followed the order Frc-2 > CP > Frc-1 > Frc-3. IC_{50} of Frc-3 (53.8 µg/mL) is low when compared with other protein samples. Generally, the quenching of free radical has been attributed to the donation of hydrogen. Further, some amino acids such as His, Leu, Tyr, Met enhance the scavenging activities of peptides (*38*). These results indicated that *Chrysaora quinquecirrha* nematocyst proteins has a notable effect on scavenging of superoxide radicals.

Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions, such as copper or iron. Hydroxyl radicals react readily with lipid, polypeptides, proteins, and DNA, especially thiamine and guanosine. When a hydroxyl radical reacts with aromatic compounds, it can add across a double bond, resulting in hydroxycyclo-hexadienyl radical. The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxyl radical, or decompose to phenoxyl-type radicals by water elimination (*39*). Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases. The scavenging effect against hydroxyl radicals was investigated by using the 2-deoxyribose oxidation method. Of the four samples, Frc-1 and Frc-3 showed maximum scavenging effects. With this finding it can be concluded that *Chrysaora* proteins can be used as a good hydroxyl radical scavenger.

The NO• scavenging activity of a compound is of potential health interest as it has been proposed that NO• plays an important role in the progression of many diseases and pathological conditions such as septic shock, atherosclerosis, ischemia reperfusion, neurodegenerative disorders like Alzheimer's and Parkinson's diseases, cancer and diabetes (*40-43*). No significant nitric oxide scavenging effect was found at a concentration 20-40 µg/mL. The scavenging effect of protein samples followed the sequence CP > Frc-1 > Frc-2 > Frc-3.

The different protein fractions obtained by gel filtration exhibited different antioxidant and free radicals scavenging activities. Results revealed that Fra-3 has the highest antioxidant and free radicals scavenging activities. Frc-3 had the strongest radical scavenging activity for not only the small radicals (hydroxyl and superoxide) but also the relatively large species (DPPH), suggesting a nondiscriminating nature of the peptide antioxidants. Based on these findings, we believe that smaller peptides have a higher level of radical scavenging activity than larger proteins, a finding consistent with that of Moosman and Behl (*44*), where they have reported the antioxidant nature of smaller peptides. These findings are in agreement with observations from other studies and support the fact that functional properties of antioxidative peptides are highly influenced by properties such as molecular mass (*45,46*). The smaller fractions possibly contained some groups, which were electron donors and could react with free radicals to convert them to more stable

products and terminate the radical chain reaction. Neither the structure-activity relationship nor the antioxidant mechanism of peptides is fully understood (*47*).

The results of the present study showed that the purified proteins from *Chrysaora quinquecirrha* can be used as an easily accessible source of natural antioxidants. Furthermore, the observed radical scavenging activity can at least partially justify the therapeutic use of *Chrysaora quinquecirrha* proteins. Nevertheless, its potential toxicity should be addressed before any possible application on a practical scale.

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