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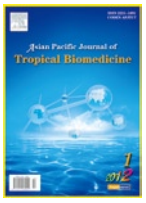

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In vitro antioxidant activities of jelly fish *Chrysaora quinquecirrha* venom from southeast coast of India

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ABSTRACT

Objective: To extract the venom from *Chrysaora quinquecirrha* (*C. quinquecirrha*) and tested for antioxidant potential of both crude and fractions. **Methods:** These venom proteins were purified through DEAE- cellulose column chromatography. The protein content from crude and fractionated samples (Frc 1 to Frc 3) showed 398, 180, 203 and 286 μg/mL respectively. The molecular weight of purified protein were determined by SDS- PAGE and we found five distinct bands with molecular weight of 20, 35, 45, 70, 74 and 100 kDa in the crude 70 and 100 kDa in Frc-1, 21 and 68 kDa in Frc-2 and 14, 65 and 104 kDa in Frc-3. The *in vitro* antioxidant activity of crude and purified samples were evaluated in different assay systems *Viz*. radical scavenging activity by DPPH reaction, Superoxide radical scavenging activity in PMS/NADH-NBT system, hydroxyl radical by Fe^{3+}-Ascorbate- EDTA, H2O2 system and Nitric oxide (NO) activity in sodium nitroprusside/Greiss Reagent system. **Results:** Fre-3 displayed the maximal antioxidant activity from all the assay system tested. Inhibitory activities in DPPH radicals 15%-92% Superoxide radicals 21%-96% hydroxyl radicals 30%-95% and Nitric oxide activity also showed significant activities. **Conclusions:** These results indicate that the jelly fish *C. quinquecirrha* venom have remarkable antioxidant activities. Further studies will fulfill for purification and structural elucidation.

1. Introduction

The jellyfish, *Chrysaora quinquecirrha (C. quinquecirrha)*, a Cnidarian of the class Scyphozoa, the order, *Semaeostomeae* and the family *Pelagiidae* is distributed widely in the Bay of Bengal, India. Toxicity is a common feature of Cnidarians and a large number of toxins have been identified in Cnidarian nematocysts. A variety of toxins are produced and used for prey capture and defense by the cnidarians, which include Anthozoa, Scyphozoa, Cubozoa and Hydrozoa. Toxins derived from some of the jelly fishes as a model for the development of new drug which also has promising applications in cardiovascular medicine and target medicine of nerve molecular biology [1].

Regarding jellyfish derived peptides, to the best of our knowledge there are only a few reports dealing with their antioxidant properties [2]. Antioxidants have an important role to protect the human body against damage by the free radicals. An antioxidant is a molecule capable of inhibiting other molecules oxidation. 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. The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases [3]. Various antioxidant compounds are identified in many natural sources including some protein compounds. Proteins of jellyfish and protein hydrolysates from different sources, such as milk protein, maize zein, egg-yolk, porcine proteins, yellow stripe trevally, yellow fin sole frame, mackerel, have been found to possess antioxidant activity[2]. Thousands of marine toxic organisms may be regarded as a precious treasure for man. A number of compounds from marine organisms possessing anti-bacterial, anti-coagulant, anti-diabetic, anti-inflammatory, antifungal, antimalarial, anti tuberculosis and anti-viral properties have recently

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been reported. The aim of this study is to investigate In vitro antioxidant activities of jelly fish *Chrysaora quinquecirrha* venom.

2. Materials and methods

2. 1. Specimen collection and protein purification

During the summer season (April and May, 2009) the live specimens of *C. quinquecirrha* were collected from Vellar estuary, Parangipettai coast. Collected animals were identified by the standard literature of Puertas *et al.*, [4]. The live animals were kept inside the glass bowl along with some amount of distilled water with ice in container for 15 min. During stress condition, the nematocysts were released from the tentacles. The same procedure was repeated thrice. The collected nematocysts containing toxins were collected with 0.5 mm mesh sieve and filtered by Whatman No.1 filter paper. In order to remove the debris from the extracted crude toxin, residues were centrifuged at 5 000 rpm for 15 min. The supernatant was collected in separate cleaned beakers for lyophilization and stored at 4 ℃. The crude extract was filtered and dialyzed by using Sigma (USA) dialysis membrane-500 (average flat width: 24.26 mm; average diameter: 14.3 mm; approximate capacity: 1.61 mL/cm) against D-glucose to remove excess water. Then, the supernatant obtained was lyophilized (Free Zone® Freeze Dry Systems, Labconco, USA) and stored at 4 ℃ in a refrigerator. The crude extract was purified by DEAE- cellulose column. Both crude and fractionated extracts were evaluated for further studies.

2. 2. Protein estimation

Protein estimation was carried by the method of Bragadeeswaran *et al.* [5] using bovine serum albumin as standard.

2. 3. Molecular weight determination SDS PAGE

The proteinaceous nature of *C. quinquicirrha* crude and fractions were subjected to electrophoresis followed the method of Bragadeeswaran, [5] in 12% polyacrylamide slab gels. This is the most convenient way for determining the molecular weight of proteins. In this technique, SDS detergent was used to make uniform charge all over the protein samples and β-Merceptoethanol was used to break the disulphide linkage, which makes all proteins in the same shape, so migration of proteins in the gel was only according to their molecular weight. The CPMI was used as the molecular marker [1].

2. 4. 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. A measurement of superoxide anion scavenging activity of proteins was performed based on the method followed by Panda *et al.* [6]. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compound (proteins) for hydroxyl radical generated by Fe3+-Ascorbate-EDTA-H_2O_2 system (Fenton reaction) according to the method described by Dubey and Batra, [7]. The nitric oxide radical inhibition activities of proteins were measured by the method followed by Balamurugan and Menon, [2]. All the tests were performed six times.

2.5. 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

3. 1. Specimen identification and protein purification

The jelly fish, *C. quinquicirrha* (248 g. in wet wt.) collected and the crude extract was fractionated by DEAE- cellulose column and 3 fractions were collected.

3. 2. Protein estimation

In the present study amount of protein content from crude and fractionated samples (Frc 1 to Frc 3) showed 398, 180, 203 and 286 μg/mL respectively.

Table 1.
Scavenging effect of C. quinquecirrha nematocyst protein samples.

Assays	Crude Protein	Frc-1	Frc-2	Frc-3
DPPH Radical scavenging	78.20±1.60	82.00±1.48	73.00±1.69	92.00±0.75
Superoxide Radical scavenging	76.04±2.40	85.00±1.64	74.00±1.100	96.01±0.51
Hydroxyl Radical scavenging	73.10±0.65	87.00±0.58	72.00±1.55	95.50±0.84
Nitric oxide Radicalscavenging	28.20±49.10	30.10±18.46	35.00±8.62	40.50±0.85

\The values are mean ± SD for six experiments in each group. P values < 0.05 were considered as level of significance.

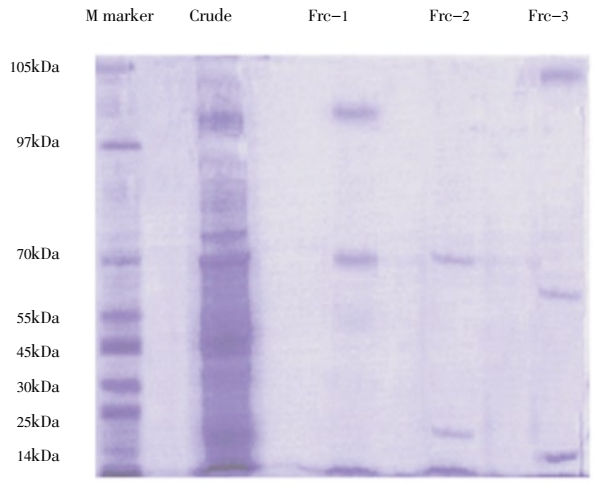

Figure 1. SDS-PAGE.
(12 % polyacrylamide gel stained with Commassie Blue) analysis of *C. quinquecirrha* with Molecular marker, Crude, Fraction 1,Fraction 2 and Fraction 3.

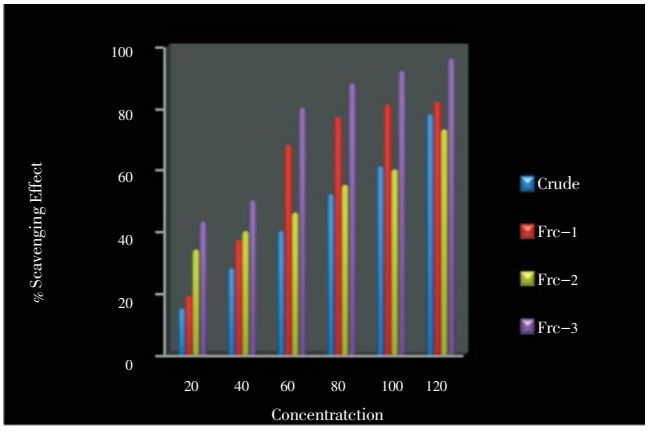

Figure 2. Scavenging effect on DPPH radical.
The superscripts a-f in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

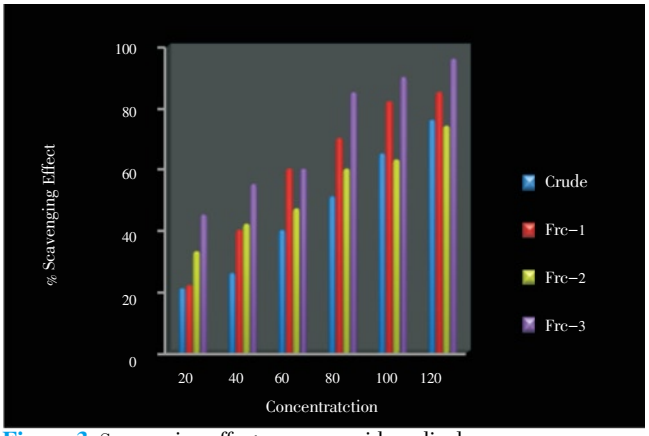

Figure 3. Scavenging effect on superoxide radical.
The superscripts a-f in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

3. 3. Molecular weight determination SDS PAGE

In SDS PAGE five distinct bands with molecular weight of 20, 35, 45, 70, 74 and 100 kDa in the crude 70 and 100 kDa in Frc-1, 21 and 68 kDa in Frc-2 and 14, 65 and 104 kDa in Frc-3 (Figure 1).

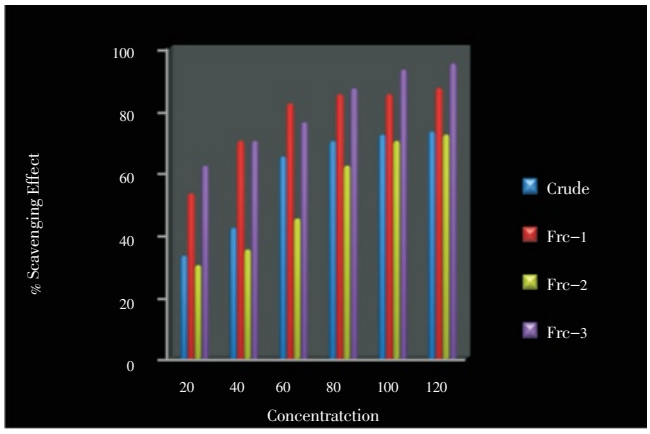

Figure 4. Scavenging effect on hydroxyl radical.
The superscripts a-f in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

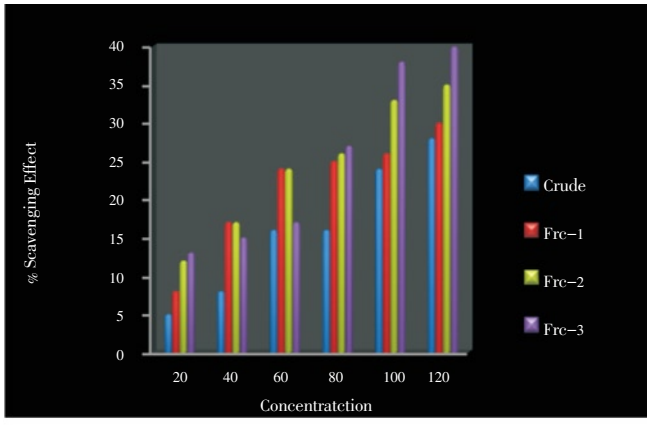

Figure 5. Scavenging effect on nitric oxide radical.
The superscripts a-e in the figure represents that the difference between the samples is less than 0.05 ($P < 0.05$) which is statistically significant.

3. 4. Radical scavenging assays

The scavenging effect of protein samples followed the sequence crude > Frc-1 > Frc-2 > Frc-3. Based on these findings, we believe that smaller peptides have a higher level of radical scavenging activity than larger proteins (Table 1). The DPPH assay results of *C. quinquicirrha* nematocyst proteins exhibited powerful DPPH radical scavenging activity. All fractions showed strong scavenging activities. Especially crude protein at a concentration from 20-120 μg/mL showed a scavenging effect on the DPPH radical from 15 to 92% (Figure 2). The Superoxide radical-scavenging activities of proteins are Frc-1, Frc-2, and Frc-3 were originated to be 82.0, 73.0 and 92.0 % (Figure 3) in dose dependent manner. In the hydroxyl radicals scavenging effect the crude, Frc-1 and Frc-3 showed maximum scavenging effects (Figure 4). Crude at a concentration from 20-120 μg/mL, the percentage scavenging effect was from 33-73 % and for Frc-1, Frc-2, and Frc-3 the % scavenging

effect was from 53-87%, 30-72 and 62-95%. The Nitric oxide scavenging effects of Crude, Frc-1, Frc-2, and Frc-3 at a concentration from 50-300 μg/mL, the scavenging effects were 5-28, 8-30, 12-35 and 13-40% (Figure 5).

4. Discussion

Jelly fish toxins have been extensively studied and chemically characterized as most proteinaceous. The sea nettle has a model for jelly fish toxinological research because of its predictable appearance and its venom also consists of proteins, peptides and enzymes, which react with amazing speed. Jelly fish toxins have been extensively studied and chemically characterised as most proteinaceous. In the present study amount of protein content from crude and fractionated samples (Frc 1 to Frc 3) showed 398, 180, 203 and 286 μgmL-1 respectively. These findings were correlated with the investigation made by Nagai et al [8, 9]. Separation of native *C. quinquecirrha* venom protein was achieved by Ion exchange chromatography across the molecular mass separation range, 14 - 105 kDa. Reducing SDS-PAGE analysis of the crude venom and purified fractions were significantly apparent being molecular weight almost same. In this, five distinct bands with molecular weight of 20, 35, 45, 70, 74 and 100 kDa in the crude 70 and 100 kDa in Frc-1, 21 and 68 kDa in Frc-2 and 14, 65 and 104 kDa in Frc-3 (Figure 1). Subsequent results were found from the cnidarian L. danae with molecular weight of 62.5 and 58 kDa [10]. Jelly fish venoms from different species vary in activity and composition. It was reported that a major component in *C. quinquecirrha* venom had a 19 kDa molecular weight, where as major protein component in Chrysaora achlyos venom was 55 kDa [11]. The venom of Chiropsalmus quadrigatus contained a hemolytic toxin of 44 kDa [9].

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. DPPH is a compound that has proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers [12]. It is well known that the radical system used for antioxidant evaluation may influence the experimental results; two or more radical systems are required to investigate the radical-scavenging capacities of a selected antioxidant [13]. The results obtained by DPPH assay are shown in Figure 2. Chrysaora nematocyst proteins exhibited powerful DPPH radical scavenging activity. Crude protein at a concentration from 20-120 μg/mL showed a scavenging effect on the DPPH radical from 15 to 92%. All the protein samples had strong DPPH scavenging activities. Frc-3 showed maximum scavenging effect on DPPH radical.

The superoxide radical-scavenging activities of proteins are Frc-1, Frc-2, and Frc-3 were originated to be 82.0, 73.0 and 92.0 % in dose dependent manner. The protein samples scavenge superoxide anion radical in a dose dependent manner. Crude, Frc-1, Frc-2, and Frc-3 at a concentration from 20-120 μg/mL showed a scavenging effect on the superoxide anion radicals from 21 to 96 %. Frc-2 is low when compared with other protein samples. Generally, the quenching of free radical has been attributed to the donation of hydrogen and supplementary some amino acids such as His, Leu, Tyr, and Met enhance the scavenging activities of peptides [14]. These results indicated that *C. quinquecirrha* nematocyst proteins have a notable effect on scavenging of superoxide radicals. Hydroxyl radical is the most reactive free radical which 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 scavenging effect against hydroxyl radicals was investigated by using the 2-deoxyribose oxidation method. Of the four samples, crude, 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. Figure 4 shows the scavenging effects % on hydroxyl radical of Crude, Frc-1, Frc-2, and Frc-3. Crude at a concentration from 20-120 μg/mL, the % scavenging effect was from 33-73 % and for Frc-1, Frc-2, and Frc-3 the % scavenging effect was from 53-87%, 30-72 and 62-95%. Figure 5 shows the nitric oxide scavenging effects of Crude, Frc-1, Frc-2, and Frc-3 at a concentration from 50-300 μg/mL, the scavenging effects were 5-28, 8-30, 12-35 and 13-40%. The results were found to be statistically significant ($P< 0.05$).

The nitric oxide ($NO^{\bullet}$) scavenging activity of a compound is of potential health interest as it has been proposed that $NO^{\bullet}$ 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 [15]. No significant nitric oxide scavenging effect was found at a concentration 20-40 μg/mL.

The different protein fractions exhibited different antioxidant and free radicals scavenging activities. Results revealed that Frc-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 non discriminating nature of the peptide antioxidants. 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. Antioxidant activity of *C. quinquecirrha* extract may be due to the presence of polypeptides, proteins, and some

amino acids such as His, Leu, Tyr, and Met. These results supported that *C. quinquecirrha* extract could be a potential therapeutic agent in oxidative stress-induced diseases and can be used as an accessible source of natural antioxidants. Nevertheless, its potential toxicity, possible application on a practical scale is in progress.

Conflict of interest statement

We declare that we have conflict of interest of University Grant Commission (UGC) Office Memorandum No: G4/7453/07 Dated 01.03.2010.

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