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Research Article

IN VITRO ANTIOXIDANT STUDY OF JELLY FISH RHOPILEMA HISPIDUM DERIVED PROTEIN

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ABSTRACT

The present investigation was to study the *in vitro* antioxidant activities of the protein from jelly fish from *Rhopilema hispidum*. The protein was isolated and purified through DEAE-cellulose ion exchange chromatography. *In vitro* antioxidant activity was determined by free radical scavenging assays from this study we observed the total antioxidant activity (88.47± 0.63), Reducing power assay (0.063± 0.07)- (0.786 ± 0.05), DPPH assays (79.82± 0.33%), ABTS assays (70.91± 0.57%), Superoxide anion assays (81.27± 0.66 %), Nitric oxide radical assay (49.74±0.63%) and Hydroxyl assays (77.49± 0.45%). Based on this result, we concluded the isolated protein of jellyfish found to be the best antioxidant source.

Keywords: Jelly fish, *Rhopilema hispidum,* Protein, DEAE-cellulose, Antioxidant activities

INTRODUCTION

Marine toxins are notoriously more potent substance as useful drugs. Among various constitutions of the coelenterates (Cnidarians) the cytolytic properties have been demonstrated (Halstead, 1965). The jelly fish venoms have exposed the presence of several components and various investigators fractionated the extracts of venom in order to isolate the compounds responsible for the biological activity (Blanquet, 1970; Toom *et al*., 1975). Protein isolated from jellyfish, with a unique structure, has many bioactivities such as enzymatic activities, hemolysis, hepatocyte toxicity, myotoxicity, cardiac toxicity and neuro-toxicity (Cao *et al*., 1998; Chung *et al*., 2001; Gusmani *et al*., 1997; Radwan *et al*., 2000).

Toxins derived from some of the jelly fishes as a model for the development for new drug and could have promising applications in cardiovascular medicine and target medicine of nerve molecular biology (Wang *et al*., 2002). Evidence from rat experiments show that jelly fish can be used to cure arthritis (Hsieh *et al*., 2001). While other investigators have reported that various venoms from different jilly fish species have biological funcation (Rottini *et al*., 1990; Gusmani *et al*., 1997; Li *et al*., 2005). Moreover, the active protein isolated from jelly fish Rhopilema esculentum possesses antioxidant and insecticidal activities (Yu *et al*., 2006).

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 (Balamurugan and Menon, 2009).

An antioxidant is molecule capable of inhibiting other molecules oxidation. Oxidation is a chemical reaction that transfers electrons from a substance to oxidizing agantsoxidation reactions can produce free radicals. In turn, these radicals can start a chain reaction that damages the cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1993). The main objective of the present study is to purify the protein from jelly fish venom from *R.hispidum* obtained from Parangipettai, south east coast, and India and analyse its antioxidant activity.

MATERIALS AND METHODS

Sample preparation:

The jelly fish *R. hispidum* was collected from the Parangipettai, south east coast of India during post monsoon season, during may 2014. The live animals were kept inside the glass bowl along with some amount

of distilled water in an ice container for 15 min. During stress condition the nematocysts were released from the tentacles. The same procedure was repeated for thrice. The collected nematocysts contain 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 5000 rpm for 15 min. The supernatant was collected in separate cleaned beakers for lyophilisation and stored at -20 ºC until further use (Yanagihara *et al*., 2002).

Partially purification of protein:

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 ºC in a refrigerator. The crude extract was purified by DEAE- cellulose column (Suganthi *et al*., 2012). The collected fractions were estimated for the protein content by Lowry *et al*. (1951). The protein positive fractions were pooled together lyophilized.

Free radical scavenging activity venom protein:

Total antioxidant activity of protein was determined according to the method of prieto *et al.* (1999). Reducing power of the protein was determined by the following method of Yamaguchi *et al.* (1998). The free radical scavenging activity of protein was measured by the 1-1-Diphenyl-2-picryl-hydrazyl (DPPH) following the method of Blois (1958). The ability of the extract to scavenge ABTS radical scavenging was determined by the method of Re *et al.* (1999). Measurement of superoxide anion scavenging activity of the protein was done based on the method of Nishimiki *et al*. (1972). 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 by Gulcin (2006). 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 (1990).

RESULTS

Free radical scavenging activity of protein in R. hispidum:

Total antioxidant capacity:

The concentration of 100µg/ml, the protein of *R. hispidum* exhibited higher antioxidant activity (88.47± 0.63) which was found significant when compared with the standard ascorbic acid (97.54± 0.21) shown in figure 1.

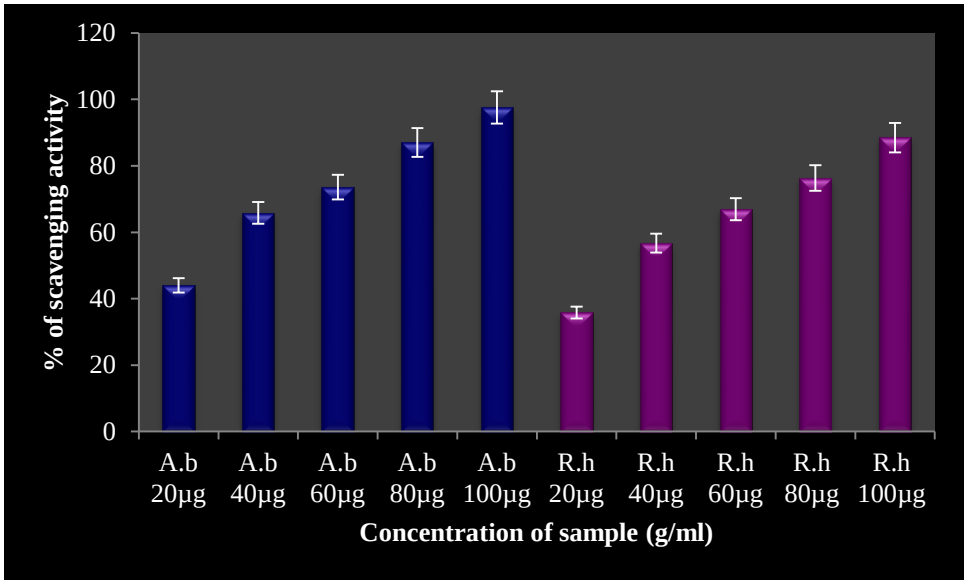

Figure 1: Total antioxidant activity of protein from *R. hispidum* compared with standard ascorbic acid (AC).

Reducing power:

The reducing power of protein of *R. hispidum* (0.063± 0.07) - (0.786 ± 0.05) was compared with the standard ascorbic acid (0.187± 0.01) - (1.021± 0.05) shown in figure 2.

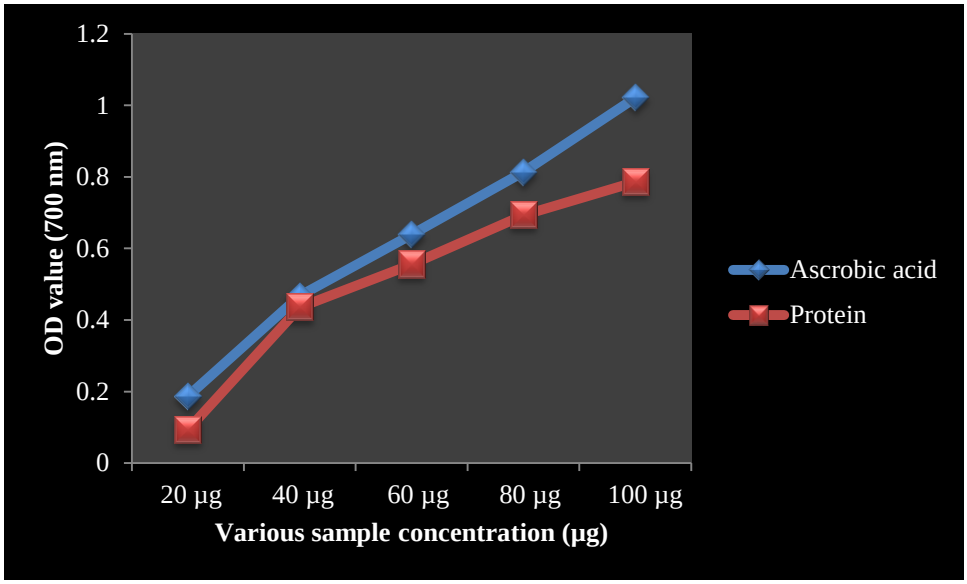

Figure 2: Reducing power of protein from *R. hispidum* compared with standard ascorbic acid (AC).

DPPH radical scavenging assay:

The protein of *R. hispidum* possessed (79.82± 0.33%) scavenging activity whereas Gallic acid possessed (94.65± 0.39%) shown in figure 3.

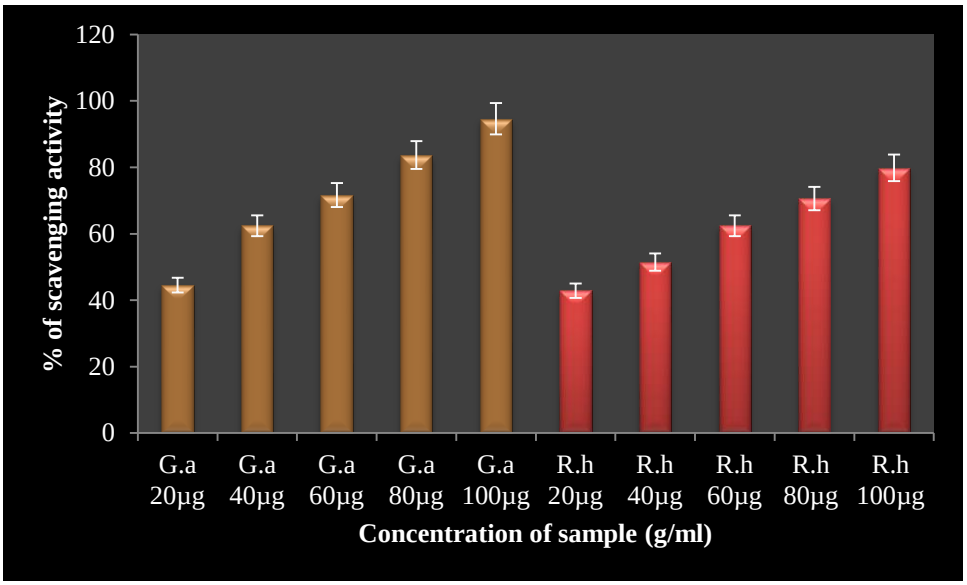

Figure 3: DPPH scavenging assay of protein from *R. hispidum* compared with standard gallic acid (GA).

ABTS inhibition assay

The protein of *R. hispidum* possessed (70.91± 0.57%) scavenging activity on ABTS whereas Gallic acid possessed (92.41± 0.52%) shown in figure 4.

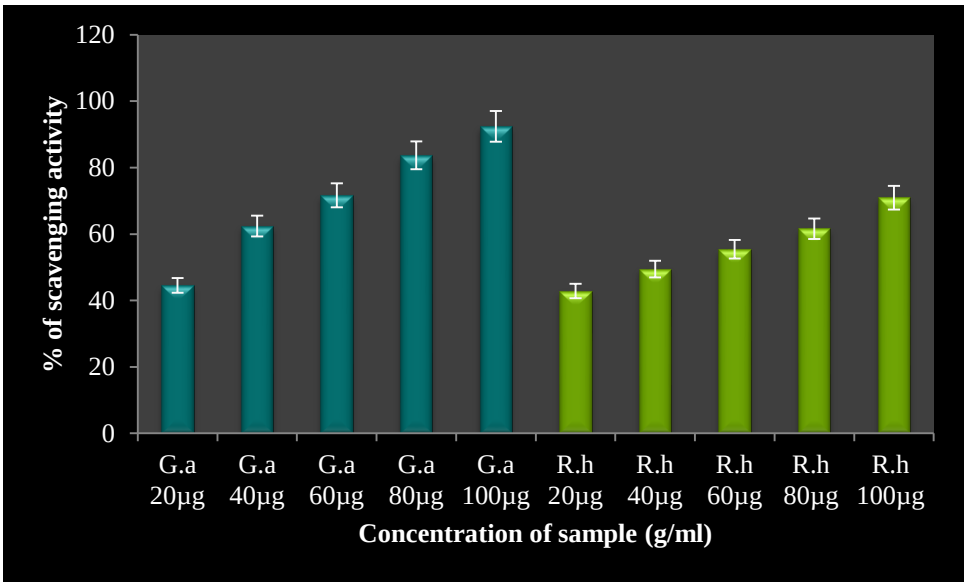

Figure 4: ABTS scavenging assay of protein from *R. hispidum* compared with standard gallic acid (GA).

Superoxide anion radical scavenging activity:

The protein of *R. hispidum* exhibited the maximum superoxide scavenging activity of (81.27± 0.66 %). The result of Superoxide anion radical scavenging activity is shown in figure 5.

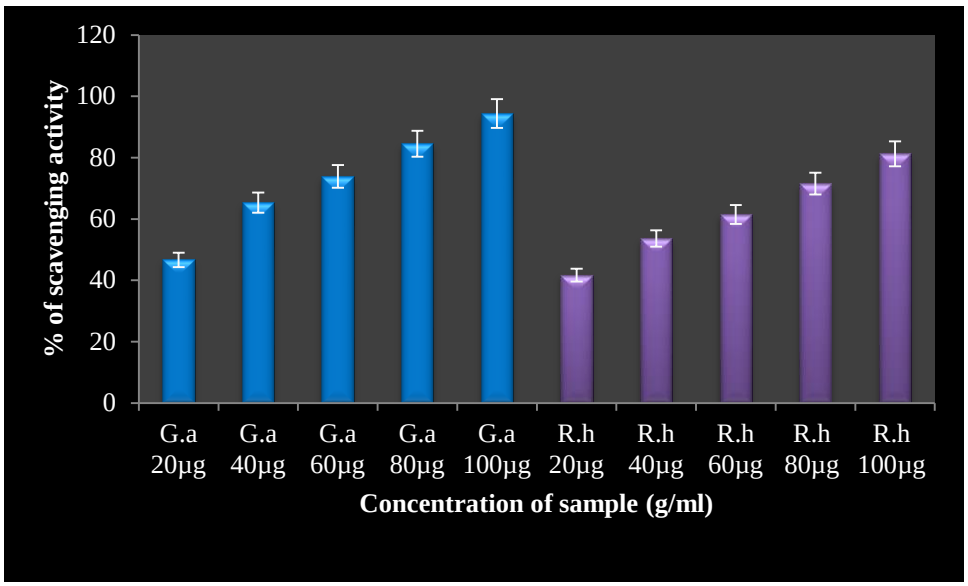

Figure 5: Superoxide scavenging assay of protein from *R. hispidum* compared with standard gallic acid (GA).

Nitric oxide scavenging activity:

The protein of *R. hispidum* exhibited the maximum nitric oxide scavenging activity of (49.74±0.63%) shown in figure 6.

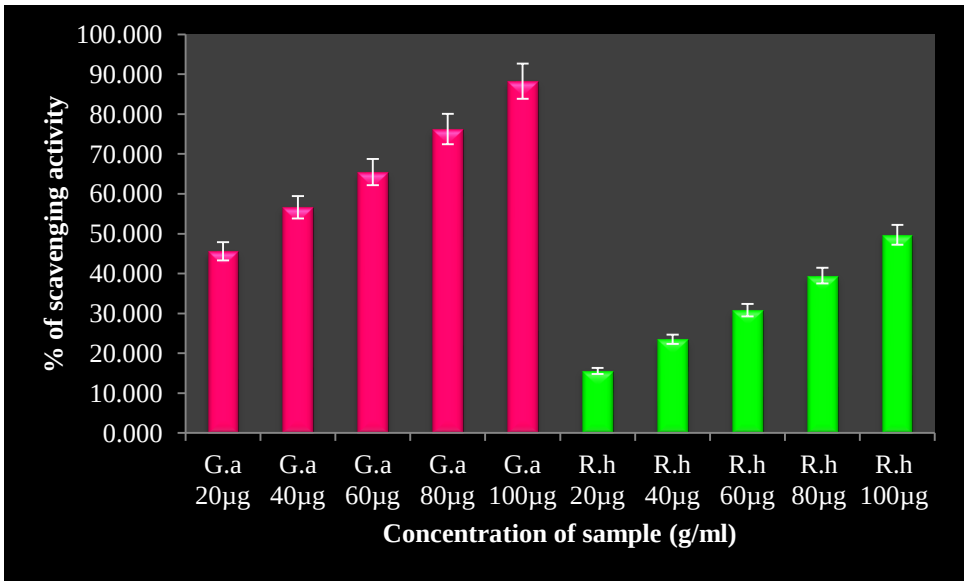

Figure 6: Nitric oxide scavenging assay of protein from *R. hispidum* compared with standard gallic acid (GA).

Hydroxyl scavenging activity:

The scavenging effect of hydroxyl was investigated using the fenton reaction and the protein of *R. hispidum* exhibited the inhibition of about (77.49± 0.45%) shown in figure 7.

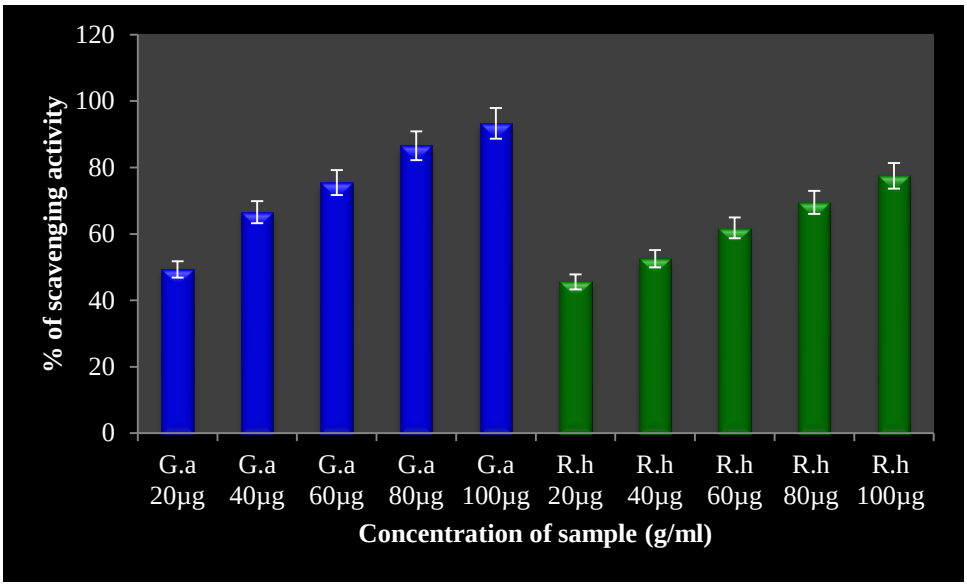

Figure 7: Hydroxyl scavenging assay of protein from *R. hispidum* compared with standard gallic acid (GA).

DISCUSSION

In the present study the protein from jelly fish *R. hispidum* was isolated and purified by DEAE-cellulose ion exchange chromatography. Suganthi *et al.* (2012) report that the jelly fish, *C. quinquicirrha* (248 g in wet wt) crude extract was fractionated by DEAE- cellulose column. In this study the total antioxidant capacity of protein from *R. hispidum* was found to be (88.47± 0.63). Reactive oxygen species can damage the protein. However, some antioxidants in organisms belong to protein and these proteins play an important role in the antioxidant defense system. A dietary deficiency of protein not only impairs the synthesis of antioxidant enzymes but also reduces tissue concentrations of antioxidants, thereby resulting in a compromised antioxidant status (Fang *et al*., 2002; Sies, 1999).

In the present study the reducing power of protein of *R. hispidum* (0.063± 0.07) to (0.786 ± 0.05). Yong *et al*. (2010) reported that the reducing power of three series of gelatin polypeptides increased with the use of increased concentration, suggesting that the reducing power of jellyfish gelatin peptides was dependent on the concentration.

Our studied express the DPPH and ABTS protein of *R. hispidum* was found to be (79.82± 0.33% and 70.91± 0.57%) scavenging activity. Similarly, Suganthi *et al*. (2012) report that the *Chrysaora quinquecirrha* 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. Elumalai and Venugopal (2009) observed that the *Chrysaora* nematyocyst proteins exhibited powerful DPPH radical scavenging activity. Cured protein at a concentration from 20-120 µg/mL showed a scavenging effect on the DPPH radical from 18.0-70.7%.

In the present study the superoxide anion radical scavenging activity from protein of *R. hispidum* was 81.27± 0.66 %. Similarly, Suganthi *et al.* (2012) report that the *C. quinquecirrha* nematocyst proteins exhibited 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.. Elumalai and Venugopal (2009) reported that the *C. quinquecirrha* nematocyst protein has a notable effect on scavenging of superoxide radicals.

In the present study the nitric oxide scavenging activity from protein of *R. hispidum* was found to be 49.74± 0.63%. Suganthi *et al*. (2012) report that the *C. quinquecirrha* nematocyst proteins exhibited the nitric oxide scavenging activity in crude protein (28.20± 49.10%), Frc-1, Frc-2, and Frc-3 were originated to be 30.10±18.46, 35.00±8.62 and 40.50±0.85 %. Elumalai and Venugopal (2009) reported that the nitric oxide scavenging effects of crude protein, Frc-1, Frc-2, and Frc-3 at a concentration from 50-300 µg/mL. The results were found to be statistically significant ($P < 0.05$).

From our observation the hydroxyl scavenging activity from protein of *R. hispidum* was found to be (77.49± 0.45%). scavenging effects. Suganthi *et al*. (2012) report that the *C.quinquecirrha* proteins can be used as a good hydroxyl radical scavenger. The protein of *R. hispidum* had potential antioxidant activity which clearly indicates. Further studies are needed to investigate the biological activities such as anti-cancer, antitumor and anti-inflammatory activities.

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