Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

BIOLOGICAL ACTIVITIES OF NEMATOCYSTS EXTRACT OF JELLYFISH *CHRYSAORA QUINQUECIRRHA* (DESOR1848) FROM VELLAR ESTUARY, SOUTHEAST COAST OF INDIA

K. SUGANTHI* AND P. PERUMAL

Department of Biotechnology, Periyar University, Salem 636011, Tamil Nadu, India. Email: krishsuganthi@gmail.com

Received: 24 July 2012, Revised and Accepted: 06 Sep 2012

ABSTRACT

An investigation was made on the quantitave and qualitative characterization and biological activity of nematocyst extracts of 50 different samples of jellyfish, *Chrysaora quinquecirrha* collected from Vellar estuary, Southeast coast of India. The extracts of the jellyfish tentacles were characterized with FT-IR and their biological activities were determined through antioxidant and hemolytic analyses. This preliminary study suggests that the extracted molecule could be secondary amides (amino- acids) originating of protein with 15- 96% of scavenging activity and with significant hemolytic activity.

Keywords: Jellyfish, *Chrysaora quinquecirrha*, Nematocyst, Antioxidant, Biological activity and hemolysis.

INTRODUCTION

The Jellyfish, *Chrysaora quinquecirrha* (Desor, 1848) belongs to the phylum, Coelenterata, (Class-Scyphozoa) is found to occur abundantly in Indian coastal waters [1].The jellyfish secretions from nematocysts, during feeding and defence are frequently responsible for skin irritations in swimmers. The nematocyst contains a mixture of proteins, polypeptides and enzymes, which react with amazing speed [2]. Anderluh[3] and Menestrina (2002) reported the medical uses, inducement and immunological responses, formation of pore forming proteins of jellyfish and Sea anemone[4-5]. As there is only a very little information on the venom of the *C. quinquecirrha,* the present study was aimed to estimate the biological activity of nematocyst extract of *C.quinquecirrha* collected from Vellar estuary, Southeast coast of India.

MATERIALS AND METHODS

Specimen collection and protein purification

The samples of jellyfish were collected from Vellar estuary, Parangipettai, along the Southeast coast of India during the summer season (April & May, 2011). The collected live specimens were kept in the glass bowl along with ice for 15 minutes. Due to stress condition, the tentacles released the nematocysts, which were filtered using 0.5 mm mesh sieve and filtered by Whatman No.1 filter paper. The nematocysts were centrifuged at 5000 rpm for 15 min. The supernatant was collected in separate cleaned beakers for lyophilization and stored at 4° C until further use[6]. 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° C in labeled 25-mL vials kept in containers until their analysis. The nematocyst extract yield and aperture were estimated by counting at 0.2 mL under a light microscope. In order to qualitatively characterize the nematocyst extract, proteins, amino acids, antioxidant, hemolytic and FTIR sweep (615.40 to 3385 nm) were done in Lachrom D- 7000 HPLC System.

The presence of protein was confirmed qualitatively in the released nematocyst extract by the standard method of Rayment [7]. Afterwards, the biological activity of the extract was tested, by evaluating the free radical scavenging assays, a DPPH assay[8] (Diphenyl Picrylhydrazyl,in triplicate). For this, 1 mL of the extract was added to 2 mL of DPPH solution (10 mg DPPH (99%) in 1 L of methanol, DPPH 10 mg.L-1), that was later incubated in a thermo regulated bath for 30 min. Its absorbance was then read at 517nm by using a methanol as blank. The extract's antioxidant activity was calculated as follows: A=100. (1-Abs sample/ Abs reference[9]. This activity was compared to BHT (Hydroxytoluene Beutylated), a commercial antioxidant, frequently used in the food industry.

The hemolytic activity of crude and fractionated venom of *C. quinquecirrha* was tested on chicken, goat, cow and human erythrocytes following the procedure of Paniprasad and Venkateshwaran[10]. The chicken, goat and cow blood were obtained from the nearby slaughterhouse in Parangipettai and the clinically healthy human blood samples were obtained from Parangipettai hospital using 2.7% ethylenediaminetetraacetic (EDTA) solution as an anticoagulant. The blood was centrifuged at 3000 rpm for 10 minutes with normal saline (pH 7.2). The supernatant was discarded and the RBC pack was suspended again in normal saline. This process was repeated thrice and finally concentrated RBC was obtained. The final RBC was used to prepare 1% RBC suspension.

Hemolytic assay by microtitre plate

Hemolytic assay was performed in 'V' shaped sterile Laxbro microtitre plate. Serial two fold dilutions of the venom extracts (100 µl; 1mg jellyfish extract in 1 ml PBS) were made in PBS (pH 7.2) starting from 1: 2. An equal volume of 1% human RBC was added to each well. The plates were shaken for mixing the RBC and venom extracts. Then the plates were incubated at room temperature for 2 hours before reading the results. Appropriate control was followed in the tests. Erythrocytes suspension to which distilled water and saline was added (100 µl) served as positive and negative control respectively. Formation of the knob in the wells was taken as negative. Reciprocal of the highest dilution of the venom extracted showing the haemolysis was taken as one hemolytic unit.

Determination of LD_{50} value

The LD_{50} value was determined in mice following the method of Miller and Tainter [11] Male Swiss albino mice, each of 20 ± 2g body weight were used for the study. The mice were grouped in to four and each group comprising of 10 mice were injected *i.p* with *C. quinquecirrha* venom in logarithmic concentration. Toxic symptoms and behavioural changes of mice were observed. After 24 hours, the percentage of death in each group was calculated and converted in to probit values. The 50% lethal dose (LD_{50}) of the venom was then obtained by plotting the logarithmic dose against the probit value.

Amino acid compositions of the proteins in *C. quinquecirrha* were determined with an Amino acid analyzer (Lachrom D- 7000 HPLC System).

RESULTS AND DISCUSSION

The jellyfish, *Chrysaora quinquecirrha* is a Scyphozoan species, identified based on morphological features of its nematocysts. The occurrence, histological and cytological studies of *Discomedusa lobata* medusa recorded in Egyptian coastal water [12]. The only study in India was that of Ghosh et al [13] with the jelly fish *Acrmitus rabanchatu* a venomous Scyphozoan jelly fish, which is quite

abundant along the coastal areas of Bay of Bengal. In India, so far, studies regarding nematocysts structure, pharmacological properties of jelly fish *C. quinquicirrha* toxins are very meager. Hence, the present attempt on the isolation of nematocysts and characterization of biologically active substances present in *C. quinquecirrha*.

All the jellyfish samples were alive and mature. Their bell diameters fluctuated between 130 and 250 mm. The nematocyst extract yield per specimen ranged between 70 and 80%, whereas the released nematocyst found in each specimen ranged between 40 and 50%. Jellyfish extract was found to be lethal in sea shore crab, *Ocypoda macrocera* and it has been lethal and very sensitive in mice at highest concentration (Institutional Ethical committee Reg. No: (1085/ac07/PU-IAEC/Feb2012/08). However, *C. quinquecirrha* extract, the molecular component tested in the nematocyst show low, almost mild hemolytic activity.

The SDS-PAGE analysis revealed the presence of medium sized proteins in the crude and fractionated venom of jellyfish (Fig.1). The bands indicated proteins of 17, 35, 50 and 70 kDa in the crude and 105, 65 and 9 kDa in fractionated samples against the molecular marker. Proteins with molecular weight of 29.0 kDa have been recorded from *Heteractis magnifica* and *Stichodactyla haddoni*[12]. Similar results have also been reported by Anderluha and Macek[13].

The presently obtained toxic proteins from the *C. quinquecirrha* could be categorized as (i) Medium sized Cytolytic Actinoporins (~ 20kDa), (ii) Cardio stimulatory proteins (~ 28kDa) and (iii) Cytolysin with or without Phospholipase in both crude and fractionated proteins (~ 40kDa). However, further purification and characterization of compounds are required to confirm the type of toxins in the jellyfish, *C. quinquecirrha*.

The FTIR spectrum of *C.quinquecirrha* venom shows an increase in Amides I, II and III (Fig.2). Similar [14] results have used pressure tuning FTIR Spectra to find out the structural differences between the connective epithelial and malignant epithelial cervical tissues. The IR sweeps of the extracts revealed signals of the most concentrated bands of the extract on group 1(615.40 to 3385 nm) (Figs.3&4).

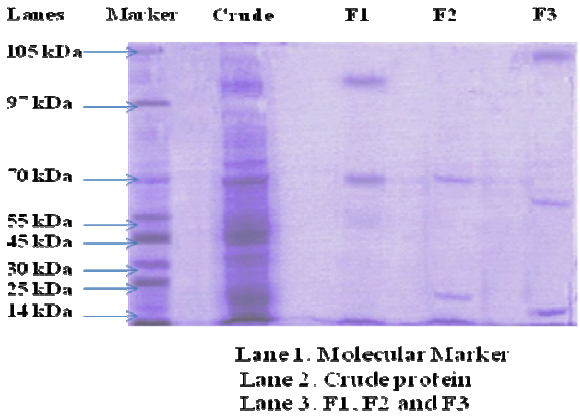

Fig. 1: SDS-PAGE analysis of the crude and fractionated proteins of *C. quinquecirrha* venom

The percentages of free radical scavenging effect are given in Table 1. Antioxidant study revealed that the preliminary actions of the active protein isolated from *C. quinquecirrha*. All the venom samples had strong DPPH radical, superoxide anion radical, hydroxyl radical and nitric oxide scavenging activities (15 to 96 %) at a range of concentrations of 20-120 µg/mL in both crude and fractionated venoms. The scavenging properties of *C. quinquecirrha* may be due to the presence of amino acids such as Histidine, Lysine, Tryphtophane and Metheonine which enhance the scavenging activities of peptides. These results indicated that *C. quinquecirrha* nematocyst proteins have a notable effect on scavenging of superoxide radicals. On the other hand, the nematocyst extract showed mild to moderate hemolytic activity in chicken, goat, cow and human erythrocytes (Table 2).

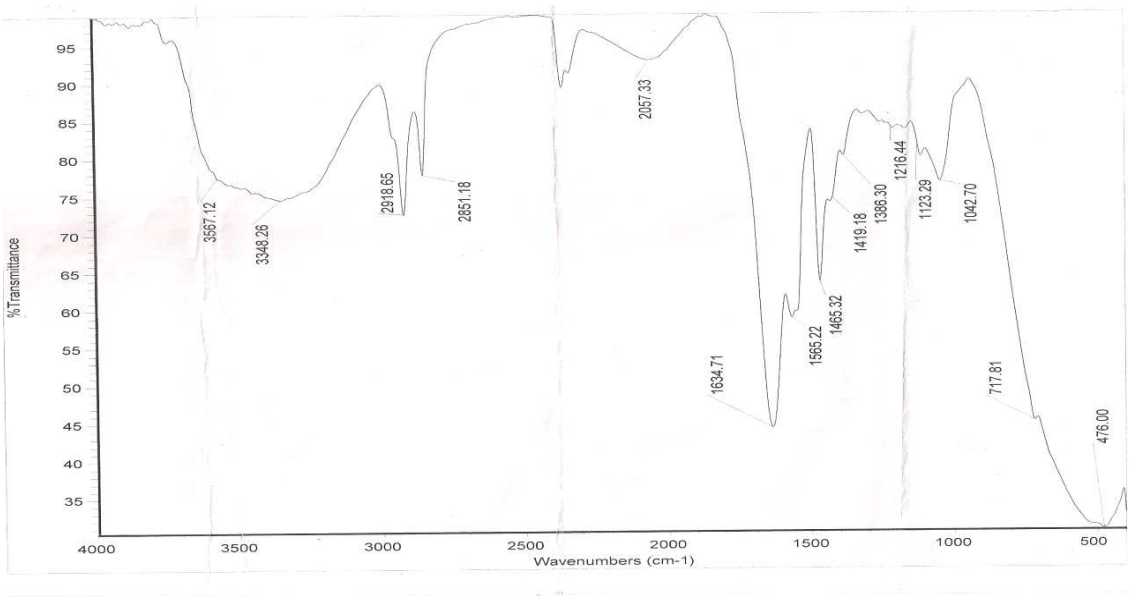

Fig. 2: FT-IR analysis of the crude and fractionated proteins of *C. quinquecirrha* venom.

Table 1: Scavenging effect of *C. quinquecirrha* nematocyst venom

Assays	Crude Protein	Frc-1	Frc-2	Frc-3
DPPH Radical scavenging	78.2 ± 1.60	82.0 ± 1.48	73.0 ± 1.69	92.0 ± 0.75
Superoxide Radical scavenging	76.04 ± 2.40	85.0 ± 1.64	74.0 ± 1.10	96.01± 0.51
Hydroxyl Radical scavenging	73.1 ± 0.65	87.0 ± 0.58	72.0 ± 1.55	95.5 ± 0.84
Nitric oxide radical scavenging	28.2 ± 49.1	30.1 ± 18.46	35.0 ± 8.62	40.5 ± 0.85

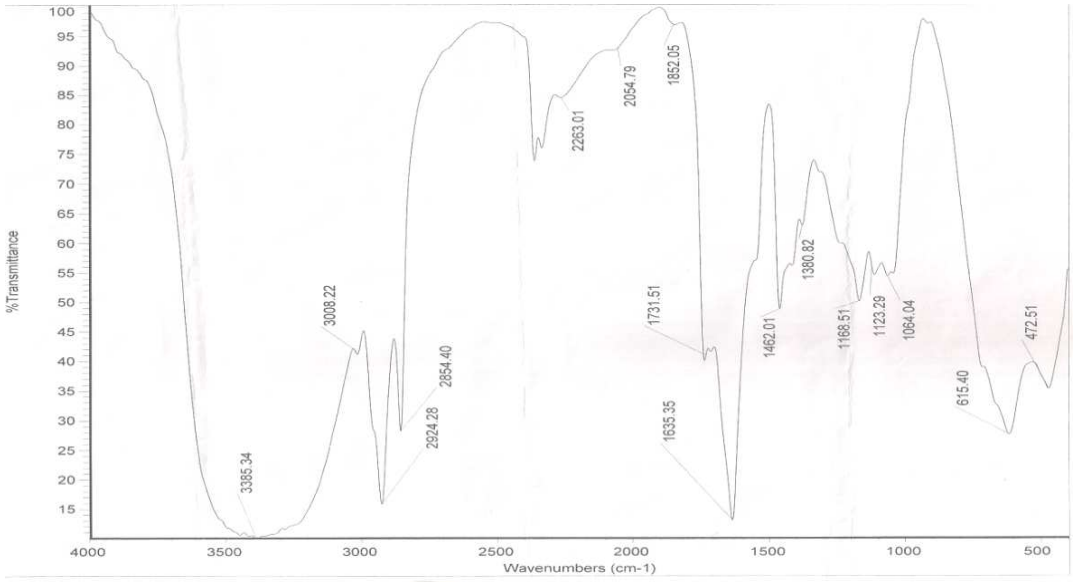

Fig. 3: FT-IR analysis of the fractionated proteins of *C. quinquecirrha* venom.

Table 2: Haemolytic activity of crude and partially purified fractions *C. quinquecirrha* nematocyst venom

S. No	Crude/ Fraction	Haemolytic unit (HU)				
		Chicken blood	Goat blood	Cow blood	Human blood	
					'B'	'O'
1	Crude	1024	128	32	64	32
2	Frac-1	8	8	4	4	8
3	Frac-2	2	32	8	2	2
4	Frac-3	256	512	32	32	128
5	Frac-4	256	8	8	4	16
6	Frac-5	16	16	8	16	16

In the acute toxicity determination, the various concentrations of *C. quinquicirrha* venom extract revealed that the percentage of mortality was directly proportional to the concentration of the venom (Table 3). The LD_{50} dose of the *C. quinquicirrha* venom extract was found to be 200µg/kg as calculated graphically by inter plotting against probit value (in between 4.75 and 5.25), minimum and maximum lethal dose was 160µg/kg and 230 µg/kg respectively (3.04 to 6.96). The signs shown by the mice in gross behavioral changes were writhing, tremor, convulsion, hind limb paralysis, mortality followed by respiratory failure and cardiac arrest (Table 4).

Table 3: LD_{50} results of *C. quinquecirrha* extract on mice.

Group	Dose(µl/kg) *i.p.*	Log dose	Dead/Total	Dead %	Corrected %	Probit
1	160	2.638	0/10	0	2.5	3.04
2	180	2.648	2/10	20	20	4.10
3	200	2.658	4/10	40	40	4.75
4	220	2.667	6/10	60	60	5.25
5	220	2.677	8/10	80	80	5.84
6	230	2.686	10/10	100	97.5	6.96

Table 4: Results of *C. quinquicirrha* extract in gross behavioral studies on mice.

Observation	Effects								
Gross activity	Upto 3 Hrs	3 ½ hrs	4 hrs	4 ½ hrs	5 hrs	5 ½ Hrs	6 hrs	12 Hrs	24 hrs
Respiration	↓	↓	↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓
Writhing	+	+	+	-	-	-	-	-	-
Tremor	+	+	+	-	-	-	-	-	-
Convulsions	+	-	-	-	-	-	-	-	-
Hindlimb paralysis	+	+	+	+	+	+	+	+	+
Sense of touch and sound	↑	↑	↑	-	-	-	-	-	-
Salivation	↑	↑	↑	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	x	x	X
Urination	+	+	+	+	+	+	+	-	-
Skin and Fur	+/-	+/-	+/-	-	-	-	-	-	-
Eyes	+/-	+/-	+/-	-	-	-	-	-	-
Mucous membrane	-	-	-	-	-	-	-	-	-

+Mild effect; +/- either normal or abnormal; ↓Mild depression; ↑ Mild stimulation; - (No Effect); ↓↓ Strong depression; x Death

Some reports are available on the hemolytic activity of the venoms from jellyfish such as *Chrysaora achlyos Carybdea alata, Rhopilema nomadica* and *Carybdea marsupialis*, respectively [15-19]. Chung *et al* [20] have isolated and characterized a novel hemolytic protein from the venom of *Carybdea alata* which indicated the presence of a potent hemolytic protein from sheep red blood cells. Previous study, some jelly fish *Crambionella stuhalmanni, Chrysaora quinquecirrha* was screened for biological activity by Suganthi *et al* [27].

Amino acid (21 amino acids) compositions of the proteins in *C. quinquecirrha* are presented in Tables 5&6. The concentration of essential amino acids Methionine, Histidine, Tryptophane and Lysine and non essential amino acids, Aspartic acid, Glutamic Acid, Taurine, Asparginine and Proline were high. Threonine was found to be minimum. The present results are consistent with previous reports that nematocysts of various cnidarians contain large quantities of polypeptides rich in glutamic and aspartic acids[21-22] including poly-γ-glutamic acid[23]. Our findings are in agreement with the observations from other studies and support the fact that functional properties of antioxidative peptides are highly influenced by properties such as molecular mass[24].

All the jellyfish extracts had strong DPPH radical, superoxide anion radical, hydroxyl radical and nitric oxide scavenging activities (15 to 96%) at a range of concentrations of 20-120 µg/mL in both crude and fractionated venoms. The observed scavenging properties of *C. quinquecirrha* may be due to the presence of amino acids such as Histidine, Lysine, Tryptophane and Methionine which enhance the scavenging activities of peptides.

Table 5: Essential Amino acid composition in *C. quinquecirrha* venom.

Amino acid	Std (%)	Venom content %
Threonine	3.575	2.045
Valine	10.970	1.787
Arginine	12.780	2.116
Methionine	0.991	**1.978**
Isoleucin	7.890	1.214
Leucine	16.77	2.565
Lysine	1.398	**1.564**
Phenyl Alanine	4.890	2.114
Histidine	**0.445**	**1.565**
Tryptophane	0.699	**0.897**

Table 6: Non- essential Amino acid composition in *C. quinquecirrha* venom.

Amino acid	Std (%)	Venom content %
Aspartic acid	0.124	**0.908**
Glutamic acid	0.0325	**0.434**
Cysteine	7.44	0.897
Tyrosine	5.37	1.656
Taurine	0.567	**0.675**
Alanine	7.210	0.511
Asparginine	0.023	**0.967**
Glycine	14.55	0.715
Proline	0.645	**0.897**
Serine	2.020	1.056
Glutamine	1.610	0.876

CONCLUSION

Biomedical prospecting of the marine environment in relation to marine invertebrates is still in its infant stage. Several marine organisms are of considerable current interest as a new promising source of metabolites and enzymes with unsuspected application potential. The present investigation showed that the venom of *C. quinquecirrha* contains many biologically active substances, which could be useful tools for probing biological, pharmacological activities. Molecular studies in order to determine the structure of proteins of nematocyst and pharmacological efficacy of the extract are in progress.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of the Periyar University for providing facilities and to the UGC-New Delhi, for financial support to the first author through Dr.D.S. Kothari Post Doctoral Fellowship Scheme.

REFERENCES

1. Fernando SA, Fernando J. A field guide to the common invertebrates of the east coast of India. CAS in Marine Biology, Annamalai University, 2002; 63pp.
2. Burnett FE, Scott LV, Weaver MG, Medbak SH, Dinan TG. The effect of naloxone on adrenocorticotropin and cortisol release and evidence for a reduced response Weber, J., 1990. Poly (γ-glutamic acid) are the major constituents of nematocysts in *Hydra* (Hydrozoa), Cnidaria. J Biol Chem 1999; 265: 9664 - 9669.
3. Anderluh G, Menestrina G. Pore forming proteins from sea anemones and the construction of immunotoxins for selective killing of harmful cells. In: M. Fingerman an N. Rachakonda, (Eds.), Bio-organic Compounds: Chemistry and Biomedical Applications. Science Publishers 2001; 131 -148.
4. Vera CK, Kolbach M, Zegpi MS, Vera F, Lonza JR. Medusas en Chile: a proposito de un caso. Rev Chil Dermatol 2005; 21(2): 96-101.
5. Cairnes SD, Calder DR, Brinckmann A, Clovis CB, Pugh PR, Cutress CF et al. Common and scientific names of aquatic invertebrates from the United States and Canada: Cnidaria and Ctenophora. American Fisheries Society Special Publication 1991; 22pp.
6. Yanagihara A, Kuroiwa J, Oliver L, Chung J, Kunkel DD. Ultra structure of a novel eurytele nematocyst of *Carybdea alata* (Cubozoa: Cnidaria). Cell Tissue Res 2002; 308: 307-318.
7. Rayment I. Small-scale batch crystallization of proteins revisited: an underutilized way to grow large protein crystals. Structure 2002; 147-151.
8. Abuin E, Lissi E, Ortiz P Henriquez C. Uric acid reaction with DPPH radicals at the micellar interface. Bol Soc Chil Quim 2002; 47(2): 145-149.
9. Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. J Agric Food Chem 2001; 49(6): 2774-2779.

10. Pani Prasad K, Venkateshwaran K. Microhaemolytic assay, International Training Manual on Advance Techniques in Marine Biotoxinology, CIFE, India,1997; 41pp.
11. Miller C, Tainter ML. Estimation of ED_{50} and its errors by means of logarithmic probit graph paper. Proc Soc Exp Bio Med 1944; 57: 261 - 264.
12. Mariottini GL, Pane L. Mediterranean jellyfish venoms: A review on Scyphomedusae. Mar Drugs 2010; 8(4): 1122 -1152.
13. Ghosh TK, Gomes A, Chaudhuri AK. Isolation of a toxin from jellyfish *Acromitus rabanchatu* and its effect on skeletal muscle. Toxicon 1993; 31(7): 825-928.
14. Thangaraj S, Bragadeeswaran S, Suganthi K, Sri Kumaran N. Antimicrobial properties of sea-anemones *Stichodactyla mertensii* and *Stichodactyla gigantea* from Mandapam coast of India. Asian Pac J Trop Biomed 2011; 43-46.
15. Anderluh G, Macek P. Cytolytic peptide and protein toxins from sea anemones (Anthozoa: Actiniaria) Toxicon 2002; 40: 111-124.
16. Wang YS, Huang MX, Zhang QS. Release of tagged jellyfish. J Zhejiang Coll Fish 1993; 13(3): 201-204.
17. Rottini GL, Gusmani EP, Avian M, Patriarca P. Purification and properties of a cytolytic toxin in venom of the jellyfish *Carybdea marsupialis.* Toxicon 1990; 33: 315-326.
18. Gusmani LM, Avian B, Galil P, Rottini G. Biologically active polypeptides in the venom of the jellyfish *Rhopilema nomadica*. Toxicon 1997; 35(5): 637-648.
19. Nagai H, Takuwa K, Nakao M, Sakamoto B, Crow GL, Nakajima T. Isolation and characterization of a novel protein toxin from the Hawaiian box jellyfish *Carybdea alata.* Biochem Biophys Res Commun 2000; 275(2): 589-594.
20. Radwan FF, Burnett JW, Bloom DA, Coliano T, Eldefrawi ME, Erdely H et al. Comparison of the toxinological characteristics of two *Cassiopea* and *Aurelia* species. Toxicon 2001; 39: 245-257.
21. Chung JJ, Fernandez K, Ratnapala LA, Cooke IM, Yanagihara AA. Partial purification and characterization of Hawaiian box jelly fish *Carybdea alata* venom. Pacific Science 2000; 90-97.
22. Moosman B, Behl C. Secretory peptide hormones are biochemical antioxidants: structure-activity relationship. Mol Pharmacol 2002; 61: 260-268.
23. Blanquet RS. Ionic effects on discharge of the isolated and in situ nematocysts of the sea anemone *Aiptasia pallida*: A possible role of calcium. Comp Biochem Physiol 1970; 35: 451-461.
24. Mariscal RN. Scanning electron microscopy of the sensory surface of the tentacles of sea anemones and corals. Z Zellforsch Mikrosk Anat 1974; 147: 149 - 156.
25. Weber J. Poly (γ-glutamic acid) are the major constituents of nematocysts in *Hydra* (Hydrozoa), Cnidaria. J Biol Chem 1990; 265: 9664-9669.
26. Kim IY, Seo SJ, Moon HS, Yoo MK, Park IY, Kim B et al. Chitosan and its derivatives for tissue engineering applications. Biotechnol Adv 2008; 26: 1-21.
27. Suganthi K, Bragadeeswaran S, Sri Kumaran N, Thangaraj S, Balasubramanian T. Biological and pharmacological activities of jelly fish *Crambionella stuhalmanni* (Chun, 1896) and *Chrysaora quinquecirrha* (Desor, 1848). Inter J Pharm Pharmaceut Sci 2011; 3(2): 230-236.