6. DISCUSSION

Marine organisms are known to have enormous resources for novel compounds and are considered as the largest treasury of known and unknown drugs (Devries and Beact, 1995). There are varieties of ecosystems that exist in the ocean, where the organisms have various adaptations to survive in these competitive environments and such environment requires the production of specific active molecules in the biological system for their existence and well being. Such specific active molecules consist of proteins which are otherwise known as bioactive compounds (Aneiros and Garateix, 2004).

The discovery of different endogenous peptides in the organisms helps to recognize the molecular mechanism of action on specific cellular targets. The peptides derived from marine biological resources afford new vistas for the pharmaceutical drug development (Miljanich, 1997). Discovery of peptides have enriched our knowledge about potent cytotoxic effects and their usages and these facts pave way for the biomedical research in marine peptides (Aneiros and Garateix, 2004).

The search for toxins especially from marine sources, which have important biological properties, is intensified in recent decades. The venom of an organism is used for its defense mechanism, but there is a lot of scope to analyze its various other functional specificities. In the present study, a fish *M. monodactylus* (scorpaenidae) was taken up to study the biomedical properties of venom present in its toxic spines.

In general, the epidermal toxins and venom in spines of poisonous fishes are produced by the protein elaborating cells (Al Hassan *et al.*, 1982). The venom of both in fish mucus and spines are composed of glycoprotein (Al Hassan *et al.*, 1985). To know the importance of venom, this study has been carried out to find out the lead molecules of peptides with pharmacological properties. In the present
study protein content of the crude and purified venom were estimated around 22 mg/ml and 6 mg/ml respectively. Likewise, a study carried out in *Arius maculates* (Manivasagam et al., 2009) showed around 12.6 mg/g and 9.34 mg/g of protein both in crude and purified fractions respectively in the venom of cat fish. The mucus of catfish, *Arius thalassinus* was reported to have contained over 60 proteins and a variety of other components, including sugars, lipids, nucleotides and nucleosides (Al Hassan et al., 1982, 1987). Annadurai et al. (2012) emphasized that the protein content of spine venom of *Plotosus canius* and *Plotosus limbatus* were found to be 0.95mg/ml and 0.75mg/ml respectively. The protein concentration of *Synanceja horrida* was estimated around 1 mg/ml (Chen et al., 1997). The level of protein estimated in the venom of *M. monodactylus* during the present study was found to be higher than the previous studies.

Several authors had reported that purification of peptide from complex mixtures especially venoms / toxins require one or more purification processes to find out the better pharmacological activity (Batista et al., 2010). In the present study, totally ten fractions were collected and each fraction (20 ml) was analyzed by DPPH and MTT assay. The active fractions were pooled and further the molecular weight of concentrated protein was determined on the SDS PAGE. The purity was checked by reverse phase HPLC using C18 column. A single peak with the retention time of 3 min was obtained. Likewise, Mendis et al. (2005) purified the peptide of *Dosidicus gigas* jumbo squid by sephadex G-25 and fractionated into three fractions, and found that fraction III was more active and it was further purified by RP-HPLC Capcell pack C18 UG120. Similar findings were noticed as in the case of present study that Ranathunga et al. (2006) had selected the fish *Conger myriaster* (conger eel) to purify the peptide by ion exchange chromatography on C-25 sephadex column and fractionated into eight fractions and found that fraction IV was more potent to scavenge free radicals and therefore, it was further purified by RP-HPLC on Capcell Pak C18 UG 120 column. Jun et al. (2004) purified the protein hydrolysates from the fish *Limanda aspera* by ion-exchange chromatography and fractionated into three fractions and found that fraction III was found with higher antioxidant activity and it was further purified
by RP-HPLC. Present consequences were emphasizing significance of multiple chromatographies which are necessary for obtaining active fractions of better pharmacologically active compounds.

In the present study, electrophoretic mobility pattern of crude and purified fish venom was assessed and the molecular weight of the purified peptide was found to be around 19 kDa. The single band was observed in both crude and partially purified samples. In addition, weakly stained bands were also observed. Whereas, the electrophoretic pattern of proteins in two catfishes *Heteropneustes fossilis* and *Clarias batrachus* was demonstrated that the presence of 15-20 kDa protein bands (three or five prominent bands) in the extract of poisonous spines Datta *et al.* (1982). The stonustoxin [SNTX] isolated from the venom of stonefish (*Synanceja horrida*) showed a single protein peak with a molecular weight of 148 kDa and the SDS PAGE was reported that the protein peak comprising of two subunits designated as α and β with molecular weights of 71 kDa and 79 kDa respectively (Poh *et al.*, 1991). Kreger (1991) reported that the purified cytolysin and trachynlysin of stone fish, (*Synanceja trachynis*) were found to have monomeric protein with a molecular weight of 158 kDa. Unlike the above studies, Shiomi *et al.* (1993) isolated and purified verrucotoxin from stone fish *Synanceja verrucosa* and described that monomeric lethal protein having a molecular weight of 98 kDa. Garnier *et al.* (1995) reported verrucotoxin, a tetrameric glycoprotein having a molecular weight of 322kDa comprising 2 α and 2β (3 subunits with MW 83 kDa and 78kDa) with lethal cytolytic and hypotensive activities in *Synanceja verrucosa*. The polypeptide, dracotoxin from greater weever fish, *T. draco* was found with the molecular weight of 105 kDa (Chhatwal and Dreyer, 1992b). Trachinine, a 324 kDa protein having four identical subunits was isolated from lesser weever fish, *T. vipera* (Perriere *et al.*, 1998). A 15 kDa PC protein was isolated from the Indian catfish, *Plotosus canius* (Auddy and Gomes, 1996). Nocitoxin, a single monomeric soluble protein from the venom of *Notesthes robusta* was found to have yielded a molecular weight of 170kDa (Hahn and Connor, 2000). The venom of Brazilian fish, *Thallassophryne nañfereri* was yielded with 47 kDa protein (Lopes-Ferreira *et al.*, 1998). The present investigation revealed that the molecular weight of active peptide from the venom of *M. monodactylus* was comparatively lesser which indicates that it is a smaller group of peptide.
The free radical scavenging activities of the extracts were evaluated based on the ability to scavenge the synthetic DPPH. This assay provides useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum as the electron becomes paired off in the presence of free radical scavenging. The absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption was representing the capacity of the test drugs to scavenge free radicals independently (Guno sindhu and Ghorpad. 2010). The change in absorbance of DPPH radical caused by the extracts was due to the reaction between the antioxidant molecules and the extracts, which resulted in the scavenging of the radical by hydrogen donation. It was visually noticeable as a discoloration, from purple to yellow (Villano et al., 2007). The venom of M. monodactylus showed promising free radical scavenging effect of DPPH in a concentration dependant manner. The activities are found to be more significant when compared with control (p ≤ 0.005). The result indicates that venom of M. monodactylus contains antioxidant potential when compared to commercial standard antioxidants BHT. Similar results were reported in tuna backbone 35.82 % (Je et al., 2007), big eye tuna muscle 40.1% (Je et al., 2007), loach 92.2% (You et al., 2009), Nile tilapia 72.04% (Ngo et al., 2010). Rajapakse et al. (2005) and also reported that trypsin derived peptides from giant squid muscle showed more antioxidant activity than pepsin and chymotrypsin. Therefore, the protein hydrolysates and peptides of M. monodactylus were found capable of quenching DPPH radicals by pairing the odd electrons of DPPH.

Superoxide anion radicals are produced endogenously by flavor enzymes like xanthine oxidase, which converts hypoxanthine and then to uric acid. Bora et al. (2010) indicated that the consumption of superoxide anion in the reaction mixture, exhibiting a dose dependent increase in superoxide scavenging activity. The present study reveals that the active fraction exhibited maximum scavenging of 28.61% at 100 μg/ml concentration and minimum scavenging of 17.12% at 50 μg/ml concentration. Scavenging of fish venom by superoxide anions found to be significant when compared with control (p ≤ 0.005). Rajapakse et al. (2005)
reported that marine blue mussel can scavenge superoxide radicals in higher concentration at 98%. The values were compared with hoki frame protein (Kim et al., 2007) and Tuna backbone antioxidant peptide (Je et al., 2007) and found that they were able to scavenge superoxide radical at 28.97% and 29.89% respectively. Superoxide radical was known to be very harmful to cellular components and acts as a precursor for more reactive in oxygen (Aurand et al., 1977). Hence, its great importance in scavenging superoxide anion radical that may cause damage to DNA, cell membrane and protein hydrolysates. Thus, fish protein hydrolysates are able to quench superoxide radicals to a greater extent as proved by the above cited literatures related to superoxide radical quenching abilities.

Hydroxyl radical scavenging assay showed that the purified active protein fraction extract was found with maximum scavenging of 47% at 100 µg/ml concentration and minimum scavenging of 24 % at 50 µg/ml concentrations. Hydroxyl radical scavenging of fish venom was found to be significant when compared with control (p ≤ 0.005). In contrast to the report of Je et al. (2007), the purified peptide isolated from Alaska pollack (Theragra chalcogramma) scavenged 35% on hydroxyl radical, but the current result showed higher activity. Whereas, hydroxyl scavenging ability of different hydrolysates and peptides produced from different sources like conger eel Conger myriaster (71.3%) by Ranathunga et al. (2006), tuna backbone (81.08%) by Je et al. (2007), shrimp biowaste (80.04%) as revealed by Sachindra and Bhaskar. (2008), bullfrog Rana catesbeiana (63.8%) as studied by Qian et al. (2008), Loach Misgurnus anguillicaudatus (55%) by You et al. (2009) and Nile tilapia Oreochromis niloticus (42.80%) as described by Ngo et al. (2010). The chemical activity of hydroxyl radical is the strongest among ROS and it reacts readily with other groups or substances in the body subjected to cell damage (Halliwell et al., 1989). Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of living body against various diseases in a living body. The peptides with antioxidant amino acids could play a vital role in quenching hydroxyl radicals. Active fractions of M. monodactylus showed maximum scavenging effects and these findings conclude that the fractions could be used as a good hydroxyl radical scavenger.
Nitric oxide (NO) is an essential bio regulatory molecule required for several physiological processes like neural signal transmission, immune response, control of vasodilatation and control of blood pressure (Bredt and Snyder, 1990; Gold et al., 1990). It plays many roles as an effective molecule in diverse biological systems. The increased of the NO is resulting in several pathological disorders including cancer (Nathan and Xie, 1994). The fish products may have the property to counteract the effect of NO formation and in turn can prevent the ill effects of excessive NO generation in vivo. In the present study, the active fraction was produced the strongest radical scavenging activity, not only in the small radicals (hydroxyl and superoxide) but also in large radicals (DPPH). This finding supports the functional properties of anti-oxidative peptides as observed by (Chang et al., 2007; Kim et al., 2007). The smaller fraction groups of electron donors react with free radicals to convert them into more stable products in radical chain reaction (Saito et al., 2003). Antioxidant activity of active fraction of M. monodactylus contains Tyrosine (15.45%) which was found to be the major amino acid followed by Tryptophan (13.5%), Lysine (11.4%), Phenyl alanine (9.9%), Glycine (8.11%), Proline (7.03%), Arginine (4.8%), Leucine (4.7%), Iso-leucine (4.6%), Valine (4.13%), Alanine (4.11%), Threonine (3.9%), Aspartic acid (1.3%) and Glutamic acid (0.8%). These results supported the present study that M. monodactylus could be a potential therapeutic agent in oxidative stress-induced diseases. Based on the above findings, it is inferred that smaller peptides have a higher level of radical scavenging activity than larger peptides, which is consistent with the report of Moosman and Behl, (2002).

Cancer is one of the major health hazards in both developing and developed countries. Currently one fourth of deaths in the United States are due to cancer (Jamel et al., 2004). In all the types of cancers, a genetic alteration gives rise to changes in expression, activation and localization of regulatory proteins in the cells. They affect the signaling pathways that alter their response to the regulatory stimuli allowing the unrestricted cell growth (Marks et al., 2000). Unlike cancer cells, normal cells have intact and programmed cell death mechanism and most treatments designed to kill cancer cells also affect normal cells, resulting in side
effects to organs including the gastrointestinal tract, bone marrow, etc. (Gerl and Vaux, 2005). The approach of testing venom as anti tumor agents dates back to the beginning of the last century, when Calmette et al. (1993) reported on the anti tumor activity of snake venom (Naja species venom) in adenoma carcinoma cells. It was also demonstrated that purified protein from cobra venom was selectively cytotoxic to cancer cells (Braganaca et al., 1976; Baldi et al., 1988). Particularly, Proteinacious venom from several animals like snake, scorpion, spider etc., was reported to have excellent cytotoxicity in cultured cancer cell lines and also reduces the tumor growth in mice (Abu sinna et al., 2003; Orsolic et al., 2003). Among all, snake venom had been studied extensively. In this context, several authors have reported the anti cancer potential of snake venom, but the exact mechanism remains unclear (Abu Sinna et al., 2003). Lipps (1994) had suggested that venoms act directly on the tumor cells and cause their lysis, but Marchland, (1986) had suggested that venoms act indirectly by destroying the micro environment produced by the tumor cells.

In the present study, the anticancer efficacy of the scorpion fish *M. monodactylus* venom was first evaluated in vitro on Mcf 7, HeLa and Vero cell line. The viability of Mcf 7, HeLa and Vero cell line was adversely affected on adding crude proteins. The symptoms of toxicity shown by the cancer cells (Mcf 7, HeLa and Vero cell) were cell rounding, lysis and detaching from the substratum. Where in active fraction analysis in MTT Assay, after 24 h incubation of *M. monodactylus* venom against Mcf 7 cell line showed cytotoxic activity, and the level of inhibition of the growth of HeLa cell line by the active fraction of *M. monodactylus* ranged between a maximum of 34.57% at the lowest concentration of 50 µg/ml and a minimum of 55.8% at the highest concentration of 100 µg/ml. In the case of the Vero cell line, the active fraction of *M. monodactylus* inhibited the growth at the lowest concentration of 14.7% at 50 µg/ml and at highest concentration by of 23.4% at 100 µg/ml. Similar findings were noticed on cytotoxicity in the fish venoms of *T. nattereri* on endothelial cell lines of capillary origin (Lopes - Ferreira et al., 2002) and *G. marmoratus, P. volitans* and *S. trachynis* in cultured murine cortical cells (Church et al., 2003). Studies have
shown that cells were distended after exposure to fish venoms of Scorpaeidae family, supporting the hypothesis that depolarization of the cell and ionic imbalances leading to an influx of fluid into the cytosol resulting in swelling of cells and eventually cell lysis (Church et al., 2003). Recently the HeLa cells incubated with S. argus venom of different concentrations for nearly 4 hours and was found the cells distended Sivan. (2009). The observed anti proliferative effect on cancer cells and significantly less cytotoxic effect on normal lymphocytes, motivated further search for the anti cancer potential in scorpion grey sting fish M. monodactylus venom using MTT assay model.

In order to understand the mechanism of antitumor effect of fish venom, the main apoptotic marker Caspase was estimated in the cancer cells. Caspases are the central executioners of the apoptotic pathway. The report was also made about the visible changes like cell shrinkage, blebbing of plasma membrane, nuclear condensation and cell death which characterize the apoptosis (Nancy and Luzebink, 1998). Caspase was found to be activated during apoptosis which cleaved substrates such as poly (ADP-ribose) polymerase, actin and lamin (Hengartner, 2000, Porter and Janicka, 1999). A Caspase activity with Mcf7 is significantly increased in the concentration of dose dependent manner. This suggested that, fish venom can destroy the existing tumor cells by activating the mitochondrial pathway at a specific concentration of 100 µg/ml.

Animal venoms are usually complex mixture of bioactive molecules recognized as potential source for pharmacological and physiological function. Enzymes are an important and common component of the venom of many animals including bees, snakes, spiders and scorpions, with several functions, probably involving in the toxic action (Nget Hong and Ponnudurai, 1992). In this respect, the venoms of fishes are also the same in having various enzymatic activities.

Studies have suggested that proteolytic enzymes in venom are the prime cause for lysis in blood cell membrane. They digest the integral proteins and thereby weakening certain membrane proteins, which in tum may lead to the increased membrane permeability, increased osmotic fragility and hemolysis
(Saminathan et al., 2006). In the present study, the presence of proteolytic enzyme observed in *M. monodactylus* crude venom was observed about 3.45 units/ml, whereas in the active fractions it was found as 0.355 units/ml, which was greater when compared with *S. argus* venom 0.416 units/ml. Similarly, in the present study the proteolytic enzyme was also found lesser when compared with other venomous fishes *Thalassophyme maculosa* (2.0 - 4.4 µl/mg) and stingrays, *Potamotrygon cf. scobina* and *Potamotrygon. gr. orbignyi* (Sosa Rosales et al., 2005; Magalhaes et al., 2006). Mild proteolytic activities had been observed in bullrout, *Noteesthes robusta* venom (Hahn and Connor, 2000). The presence of proteolytic activity in the venom of *M. monodactylus* showed that could be a possible role of these enzymes in increasing local tissue damage and necrosis.

Hyaluronidase is an enzyme, commonly found in the animal venoms (Tan and Ponnudurai, 1992). In general, venom was found to have the ability to hydrolyze the connective tissue by catalyzing the action of gelatinase (Birkedal-Hansen et al., 1993). In the present study, the crude enzyme activity of *M. monodactylus* was found to be 5.45 units/ml whereas in the active fraction, trace quantity of enzyme 2.35 units/ml was observed. Previous studies showed that hyaluronidase activity was detected in the sting grays of fresh water *P. motoro* and marine sting gray *P. falkneri* venom (Magalhaes, 2006; Haddad et al., 2004). Thus the hyaluronidase associated with the protease activity of the scorpion fish *M. monodactylus* could be one of the key factors playing crucial role in the toxic effects. Such proteases could contribute in the degradation of hyaluronidase enzyme present in the extracellular matrix, favouring injury (Barbaro et al., 2007).

Hyaluronidases are well-known spreading factor of venom. Additionally, these enzymes could be used in biological, biochemical researches and in pharmaceutical applications. Hyaluronidase enzyme was involved in the processes of mammalaian fertilization and recombinant molecules could be developed as tools for *in vitro* fertilization and contraceptive design (Kreil, 1995). Since hyaluronidase cause greater tissue permeability, it is used to increase the absorption of drugs. Eradication of HA by hyaluronidase administration reduced human breast
cancer Xenografts in SCID mice, showing the probability of this enzyme which could provide a new class of anticancer therapeutics (Smith et al., 2004). Thus, the fish venom of *M. monodactylus* could play a vital role in the medicinal field.

Venom is considered as a mixture of compounds which include proteins, peptides and metal ions organic compounds. Some toxins were found to exert toxic action through various enzymatic actions which includes serine, protease, hemorrhagin, fibrinolytic activators, metallo proteases, hemolysins, phospholipase etc. They mainly target haemostatic system, extracellular matrix components, cardiovascular and neuromuscular systems.

This study focuses on the effect of *M. monodactylus* venom on the components of blood. The crude venom exhibited hemolytic activity on washed erythrocytes of chick, goat, and human. These activity was found to be specific, showing high rate of activity in chick erythrocytes (5lysis-32 HT/mg) followed by goat (4lysis-16 HT/mg) and also in human erythrocytes (3lysis-6 HT/mg). These results were in good agreement in line with the earlier findings by several researchers, which revealed that the *Carybdea marsupialis* jelly fish venom exhibited unpredictable hemolytic activities in different species, such as sheep, human and rabbit (Rottini et al., 1990). Similarly, *Cassiopea xamachana* jelly fish venom showed a higher hemolytic activity in human RBCs than in sheep RBCs (Torres et al., 2001).

Whereas, the hemolytic activity was not observed in the active fraction of *M. monodactylus* venom with chick, goat and human washed erythrocytes. This result was similar with that of potamotrygon species, the fresh water stingray *P. falkneri* and the marine water stingray *D. guttata*, confirming data published by Vallard (1931, 1932). Studies on the mechanism of the hemolytic activity of stonefish venom had shown to produce hemolytic by forming hydrophilic pores in cell membranes, which then results in cell lysis (Chen et al., 1997). This result was previously observed in the crude venom of stonefish, *Synanceja horrida*, and toadfish, *T. nattereri* (Khoo et al., 1995; Lopes-Ferreira et al., 2002). Therefore, it is unlikely that the hemolysis could be an important factor in mediating the toxic action of the venom.
In the present study, low molecular weight venom peptide was confirmed from HPLC and FT-IR. The amino acid composition of *M. monodactylus* spine venom consists of different amounts of essential amino acids. In the present study, active fraction contains Tyrosine (15.45%) which was found to be the major amino acid followed by Tryptophan (13.5%), Lysine (11.4%), Phenylalanine (9.9%), Glycine (8.11%), Proline (7.03%), Arginine (4.8%), Leucine (4.7%), Isoleucine (4.6%), Valine (4.13%), Alanine (4.11%), Threonine (3.9%), Aspartic acid (1.3%) and Glutamic acid (0.8%), these consequences were confirmed based on the Rf value of standard. Previously, amino acid analyses by Al Hassan *et al.* (1985) in both crude and fraction proteins from the skin secretion of Arabian Gulf catfish *Arius thallasimus* yielded similar results. Further the toxin from the red sea flat fish *Pardacuieutis marmoratus* is a hydrophobic acidic protein (paradoxin) with a molecular weight of 17,000 Da, was composed of 162 amino acids Primor *et al.* (1978; 1980). The basic amino acids residues, such as lysine and arginine may play important role in blocking the acetylcholine receptor sites on the postsynaptic membrane. The spider venom consists of amino acids like glycine, serine, threonine, lysine, glutamine, alanine, arginine, asparagines, leucine and histidine (Fischer and Bohn, 1967; Gilbo and Coles, 1964; Bettini and Maroli, 1978). The high level of taurine and histidine in insect hemolymph increases the sensitivity and the role of these compounds act synergistically with other toxins and increasing their lethality (Kuhn-Nentwig *et al.* 1994). Among these, Glutamine and Glycine are important neurotransmitters (Danyysz and Parsons, 1998) and the presence of amino acids in them might play a role in envenomation. This study clearly indicated that the purified *M. monodactylus* can be used as potential source for amino acids.

Each compound has a characteristic set of absorption bands in its infrared spectrum. Characteristic bands found in the infrared spectra of protein and polypeptides include the amide I and amide II. These arise from the amide bonds that link the amino acids. The absorption associated with the amide I band leads to stretching vibrations of the C=O bond of the amide, absorption associated with the amide II band leads to primarily bending vibrations of the N-H bond. Because both
the C=O and the N-H bonds are involved in the hydrogen bonding that takes place between the different elements of secondary structure, the locations of both amide I and amide II bands are sensitive to the secondary structure content of a protein. Studies with proteins of known structure have been used to correlate systemically the shape of the amide I band to secondary structure content (Byler et al., 1985; Sureuicz and Mantsch, 1988).

In the present study the FT-IR spectra shows the amine bonds, I, II and III at 1651-1636 cm\(^{-1}\), 1560 cm\(^{-1}\) and 1418 cm\(^{-1}\) respectively. These regions were expected for proteins with secondary structures of largely random in nature. Which consist of C=O and N-H/C-N stretching vibration of the backbone peptide bonds in proteins and it has been used to estimate quantitatively the secondary structural features of proteins (Dong et al., 1990; Vedantham et al., 2000). Two intensive absorptions were arising from the amide group of proteins at 1653 and 1418 cm\(^{-1}\). The amide I mode is primarily C=O stretching band. The amide II and III mode is an out-of-phase combinations of largely NH in plane bending and C-N stretching. This region, which consist of C=O and N-H/C-N stretching vibration of the backbone peptide bonds in proteins (Vedantham et al., 2000). Further, the bands at 1398, 1340 and 1088 cm\(^{-1}\) in infrared spectrum are less intensive and belong to additional vibrations of amino groups and alkyl side chains. At the outset, the amide III band region 1418 cm\(^{-1}\) is very important peptides for secondary structure determination. Similarly, C. quinquecrrhge jelly fish venom showed, FT-IR spectra increased in Amide I, Amide II and Amide III regions (Suganthi et al., 2011). Wong et al. (1993) studied IR absorption spectra of extensive structural changes during carcinogenesis and noticed enormous increase in absorption intensities of Amide-I and Amine-II. In the present study FT-IR analysis also showed a varied range of peaks which consists of nitrocompounds, sulphates, phosphates and methylene. As such spin venom consists of heterocyclic amine NH stretch at 3433cm, 1048cm. In addition, it contains asymmetrical /symmetrical C-H stretch of methyl and methylene, methoxy, methylether, isothiocyanate of S-S stretching, aldehyde, alkanyl C=C stretch, asymmetrical / symmetrical aliphatic nitro compounds of XO\(_2\) stretch sulfonates, dialkyl /acyl sulfones and aliphatic nitro compounds of C-I stretch. More recently the FTIR analysis of the mucus of two marine fishes
Cynoglossus Arel and Arius Caelatus showed distinct spectral profile which confirms the presence of primary amine-group, aromatic-compound, halide-group, aliphatic alkyl-group and polysaccharides (Bragadeeswaran et al., 2011). The presence of free amino acids and peptides could also play vital role during envenomation by acting directly or indirectly in the victims. These non-enzymatic components could act synergistically with other components and it could enhance toxic effects of the venom.

The discovery of peptide in scorpaeniformes provides new clues for fundamental understanding of scorpaenidae family. The studies on the penaeid shrimp have largely contributed to this knowledge as they are the first antimicrobial peptide fully characterized in a crustacean (Chen et al., 2004; Chiou et al., 2005) and for which expression studies were performed. Several methods have been proposed for determining the binding affinity of peptides to cancer cells. Here the utilization of MALDI-TOF/TOF-MS was proposed as a reliable and efficient method for high throughput peptide screening. The major advantage of the technique consists of finding peptide that are selective and specific to a wide range of cancer cells, gram negative and gram positive bacteria, providing a simple reliable screening tool to determine the potential candidates for broad spectrum antimicrobial drugs (Mandal et al., 2012).

In the present study, the anticancer peptide extracted from the fish M. monodactylus was purified by RP-HPLC using C18 column and the first peak fraction which shows anticancer activity against Mcf 7, HeLa, Vero cell line. The obtained and purified peptide sample was subjected to Matrix-Assisted laser Desorption/Ionization (MALDI) Time-of-flight (TOF). The isolated peptide was identified using the tryptic-digested mixture by peptide mass finger printing mass spectra. The Peptide mass finger printing which is coupled with MASCOT search results predicted the peptide sequence to be 259 amino acid lengths, where serine consists of major share 40%.

The obtained amino acid sequences were matched for the existing peptide in the database by MASCOT search. The isolated peptide was found to have homology with the nuclear phosphoprotein, activating peptide in Tetraodon fluitatilis (puffer fish). The
identification of peptide of amino acid of the present study is almost similar with the following findings. Peng et al. (2010) reported the structural and functional characterization of two antimicrobial peptide (Scolopin 1 and 2) identified from centipede venoms of Scolopendra subspinipesmultilans by sephadex gel filtration and reverse-phase high performance liquid chromatography (RP-HPLC). The peptide amino acid sequence length is 21 FLPKMSTKLRVPYRRGTKDYH. The peptide obtain was in arginine rich of 14.3% among its constituent. Likewise, the present study showed the serine and arginine are rich peptide from the fish M. monocactylus with the amino acid sequence length of 259.

Similarly, Jirvanichpaisal, (2007) reported that the fourteen amino acids residues which carry a proline and arginine rich antibacterial peptide designated as astacidin 2 was purified and characterized from hemocytes of the fresh water crayfish, Pacifastacus leniusculus. In this, Astacidin 2 had a broad range of antibacterial activity and the primary sequence is RPRPNYRPRPYRP which was obtained by MALDI- TOF and amino acid sequence length is 15. In this peptide arginine and proline each consists of 34.5%. In the same way, Miyata et al. (1989) reported the Tachylepsin was an antimicrobial peptide found in the acid extract of hemocytes from the Japanese horse shoe crab Tachylepsus tridentatus. The amino acid sequence length is 17 RWCFRVCYRGICYRKCR and arginine consists of 29.4%.

Centipede (Polistes dominulus) venoms were found to have complex mixtures and two new antibacterial peptides were denominated with Dominulin A and B. The amino acid sequences of the two peptides, determined by Mass spectrometry, were INWKKIAEVGGKILSSL for Dominulin A, INWKKIAEIGKGVLSAL for Dominulin B and was confirmed using MALDI-TOF/TOF by means of micro - extraction and direct analyses on body parts. Both the peptides were found to be Isoleucine rich (Turillazzi et al., 2006). These findings were found similar with the present study but different in consisting of Isoleucine as a major source, while the anticancer peptide of M. monocactylus is serine and arginine are rich. The yellow catfish Pelteobagrus fulvidraco skin mucus has recently been recognized to be a potential source of antimicrobial peptides, which provides the first line of defense
against invading pathogens. MALDI-TOF/TOF MS was involved in the identification of the peptide. Pelteobagrin was leucine rich and consists of 20 amino acids in length (GKLNFLSRLILKLFVAGL) and showed no clear homology with any known bioactive peptides (So, 2011). This result has dissimilarity with the present study by consisting of Leucine as a source. The identification of the peptides was made as specified by Edman degradation and the peptide sequence length which was almost similar to the length obtained from *M. monodactylus* but they differ in consisting of glycine as a rich source such peptides are followed. The amino acid sequence of the peptide purified by RP-HPLC (C18 column) was deduced by Mass spectrometric *de novo* sequencing and conformed by Edman degradation is as follows

```
MMFTSFNAECDSRSSRCASPSDNYYYPSPAGSYSSMGPQSQDLTLTASSASFVPTVTA
ISTSPDLQWMVQPLVSSVAPSFILAAHQPICKIPSQMDSDFPVSVMSPVHAYLSTAAS
TQPQTAPTEATTVTSSHSTFTSTSNSIFGSNSDSLSTTVSDSVKMTDLESVLEESL
DLLAKTEVETVEPDVNLSSLYTAAQDEPELHATIGSSDFEPLCTPVCTPACTITI
FVFTFPEAETFPTCCVAHR
```

This work supports to the present study in the line of peptide sequence length and glycine rich peptide. The peptide amino acid sequence length is 15 Eumenitin is a novel antimicrobial peptide isolated from the venom of the solitary Eumenine wasp *Eumenes rubronotatus*. The sequence of Eumenitin, Leu- Asn-Leu-Lys-Gly-Ice-Phe-Lys-Lys-Val-Ala-Ser-Leu-Thr was mostly analyzed by Mass spectrometry together with Edman degradation and corroborated by solid phase synthesis (Sousa *et al.*, 2009). The amino acid sequence length was reported to be 15 and leucine rich (Konno *et al.*, 2006). The crab-eating frog, *Rana cancrivora*, is one of the amphibians and the antimicrobial peptide was obtained and named as cancin found with an amino acid sequence length of GSAQPYKQLHKVWNWDYG (19) and its glycine rich peptide (Lu *et al.*, 2008). The results of the above findings are similar to the resent study in the line of the sequence length of amino acid.
The MALDI-TOF/TOF analysis is used major in the identification of allergens, investigations of neuropeptide expression and sequence determination of toxin. The usage of MALDI-TOF/TOF for the identification of anticancer peptide is limited, although studies of anticancer peptide were increased especially in vertebrates. Thus, the peptide sequence from the *M. monodactylus* was identified and the serine rich homology search of sequence producing significant alignment confirms that the amino acid corresponding to the anticancer peptide is predicted to a nuclear phosphoprotein.

*Insilico* peptide analysis is a well-established technique for the assessment of allergenicity and immunological cross-reactivity (Binder *et al.*, 2001; Fluckiger *et al.*, 2002). Cycophilins constitute a family of proteins involved in many important cellular functions. They had also been identified as Pan-allergen family, able to elicit IgE-mediated hypersensitivity reactions (Cadot *et al.*, 2000 and Fujita *et al.*, 2001). Potential allergens are identified among the characterized proteins of *Periplaneta americana* using web-based and the commercially available allergen prediction tools (Ahmed *et al.*, 2010).

In the present study, the amino acid sequence is translated into protein. The structure predication and computed information were validated with the help of computational biology and bioinformatics tools. The amino acid sequence analysis was performed for the active fish peptide using Protein BLAST. The receptors were subjected for protein BLAST against PDB database. All the template sequence possesses similarity in alignment, which is greater than 95%, thereby indicating high similarity between target and template sequence. The receptors of NFkB anticancer peptide with active fish peptide of *M. monodactylus* were selected, based on the results of template sequence and then the structure was predicted by using Modeller 9.10.

Expasy’s Protparam computes the extension coefficient (EC) with a range of 18575 wave length. However, it is similarly correlated to EC of VEGF human proteins at 280 nm ranging from 7615 to 214235 M⁻¹ Cm⁻¹ with respect to concentration of cysteine, Trpsine and Tyrosine. Expasy’s Prot Param validated the
fish peptide as unstable and most of the VEGF human proteins are also unstable on the basis of instability index (II>40). The obtained result similarly correlated with Sivakumar et al. (2007) with chosen seventeen fish antifreeze proteins in studying their Physio-chemical properties, primary and secondary structures by using computational tools and servers and also primary structure analysis revealed on the AFPS were hydrophobic in nature. Expasy’s Prot param computes the extension coefficient on predicted active peptide was within a range of 276-289 nm wave length, and extension coefficient on AFPS at 280nm was ranging from 1280-42990 cm⁻¹. Insilico studies by (Ashokan and Pillai, 2008) on ten different silk fibrin proteins (SFS) retrieved from Swiss-port database, analyzed for their Physico-chemical characterization and structure analysis which exhibited, computed PI value as Q967G5, Q815B3, Q9BLL9, Q81750, Q9B1U3, Q4F623 and A81M76 (PI<7) thereby indicating that the SFS were acidic and the PI of A81M39, Q9BLL6 and Q9GUB4 (PI>7) were basic in character.

Amino acid sequence analysis provides important insight into the structure of proteins, which in turn greatly facilitates the understanding of its biochemical and cellular function (Nagano, 1973; Chou and Fasmon, 1974). Computational methods were used in predicting protein structure based only on sequence information. Regarding the secondary structure of protein, globular proteins are easily distinguishable because of their regular (periodic) character. Other types of secondary structures such as different turns, bends, bridges, and non-α helices are less frequent and more different to observe and classify. The non-α structure is often referred to as coiler loop. The majority of secondary structure prediction methods were aimed at predicting only these three classes of local structure: coil, helix and strand. In general, globular proteins have more coil structure of 50% than others: 30% α helix, 20% β- strands (Garnier et al., 1978). The secondary structure analysis of the fish *M. monodactylus* peptide showed highly random coiled which found to be a mix of secondary structural contents. The computed percentage of residues of α-helices was found to have extended the strands at 14.60%, β-strands as Nil and random coil at 61.3%. By using bioinformatics tools Akhilesh et al.
(2012) characterized viral envelope proteins such as P04290, PO6477, and PO4486, having a great importance in the keratitis disease caused by HSV and found the important proteins which were highly coiled in nature.

The Ramachandran plot (2D plot of the $\phi$-$\psi$ torsion angles of the protein backbone) provides a simple view of conformation of a protein. The $\phi$-$\psi$ angles cluster into distinct regions in the Ramachandran plot, where each region corresponds to a particular secondary structure (Ramachandran et al., 1963). The Ramachandran plot is used to find out the accuracy in the stereochemistry of active peptide / protein of fish. In the present study, the result of Ramachandran plot showed that the initial model had some disallowed regions. The loop refining was performed the final structure and validation were predicted which indicated the presence of amino acids as nil in the disallowed region of predicted active peptide. Docking analysis was performed for the active peptide with NFκB antiapoptotic binding protein which involves in the regulation of inflammation, cancer and bacterial resistance.

Peptides were evaluated using binding interactions. The algorithm for the entire rotational and translational space of the ligand with respect to the receptors was studied. The patch dock software resulted in identifying the best peptide that interacts with the receptor. The results were evaluated based on the binding compatibility i.e. Docked energy in kcal/mol (fitness). Active site of NFκB antiapoptotic protein offers different binding modes for the protein as they are strongly dependent on the attached substituent in disallowed region. NFκB binding protein bound ligand was docked deeply within the binding pocket region forming the interactions. The 259 amino acid residues from NFκB binding protein provide a cavity for the active peptide to interact with the receptor.

From the present study, the amino acid sequence of *M. monodactylus* was translated to the protein in order to know the primary structure, Physico-chemical properties, secondary structure and docking states. The peptide was identified as a membrane phosphor protein with $\alpha$- helices and it was hydrophobic in nature. The information derived from the computational tools was reliable and cost effective.