3. REVIEW OF LITERATURE

Though the studies on the biomedical and pharmacological studies on marine flora and invertebrates are wide and large, little focus has been given to such studies on marine fishes, particularly venomous. This is primarily because of the availability, difficulty in collection and getting a sizable quantity of toxins or venoms for such research.

There are at least 1200 species of venomous fish, and they include the Stonefish, Lionfish, Scorpion Fish, Stargazer, Toadfish, Surgeon fish and Sting rays. The puffer fishes are also found to be most toxic. Scorpaenidae, are a family of mostly marine fish that includes many of the world's most venomous species. As the name suggests, scorpionfish have a type of "sting" in the form of sharp spines coated with venomous mucus. The family is a large one, with hundreds of members (Eschmeyer, 1998). They are widespread in tropical and temperate seas, but mostly found in the Indo-Pacific. General characteristics of family members include a compressed body, ridges and/or spines on the head, one or two spines on the operculum, and and three to five spines on the preopercle. The dorsal fin has 11 to 17 spines, often long and separated from each other, and the pectoral fins are well-developed, with 11 to 25 rays. The spines of the dorsal, anal and pelvic fins all have venom glands at their bases (Taylor, 2000).

Of all the venomous fish known, the stonefish is one of the most common venomous fish, encountered by man. Studies on its venom started in the 1950s, but little work has been performed until then several groups revived interest in the 1980’s.
Bottard (1889) reported the existence of venom apparatus in the lionfish. He reported that the venom apparatus of *Pterois sp.* is identical with the Scorpionfish, *Scorpaena sp.* Duhig and Jones (1929) described the structure and function of the skin glands of *Synanceia horrida* with special reference to the poison glands of the dorsal spines of the fish. Hallstead *et al.* (1955) described in detail the complete anatomy of the venom apparatus of the lionfish, *Pterois volitans*. Gopalakrishnakone and Gwee (1993) studied the structure of the venom gland of stonefish *Synanceja horrida* by using light microscopy, transmission and scanning electron microscopy. They reported that the glands were covered with a fibrous capsule which divided the glandular tissue into many septa which carried numerous nerves and blood vessels. Transmission electron microscopy showed Type I cells with electron-dense material and tubular cisterns, Type II cells with dilated cisterns, sarcoplasmic reticulum and dense secretory granules. The secretory granules were globular and seen in monomer or polymer form. The secretory cells appear to be unique in comparison with the venom gland cells of snakes, scorpions or spiders.

Wright (2009) used histological preparations from over 100 catfish genera, basic biochemical and toxicological analyses of fin spine extracts from several species, and previous systematic studies of catfishes to examine the distribution of venom glands in this group. These results offer preliminary insights into the evolutionary history of venom glands in the Siluriformes.

Wiener (1959a) also observed that fresh stonefish *Synanceja trachynis* venom was opalescent, with a pH of about 6.0, and its protein nature was shown by its precipitation
by mineral acids, alcohol and picric acid, with no reducing sugars present on hydrolysis and the production of a copious precipitate on boiling. The venom was reported to be quite stable for 24 hr between pH 7.0 and 7.6 and at 4°C, but a 50% loss in toxicity occurred during 48 hr storage, while a complete loss of toxicity occurred after 4 or 5 days. Almost immediate destruction of the venom occurred at a pH below 4 and at pH 8.6, there was about 50% loss in toxicity in about 3 hr and almost complete loss after 24 hr. The loss of toxicity is enhanced by freezing and thawing, and pH effects on venom stability are temperature-dependent. However, venom that has been lyophilized and stored in a desiccator essentially retained its toxicity for 3 months. Weiner (1959c) reported the venom of stonefish, *Synanceja horrida* was non-dialyzable and a high molecular weight protein.

Poh *et al.* (1991) purified stonous toxin (SNTX) by two-step procedure on Sephacryl S-200 High Resolution gel permeation and DEAE Bio-gel Anion exchange chromatography. SNTX has a native molecular weight 148 kDa and an isoelectric point of 6.9. Two subunits designated α and β were revealed by SDS-PAGE with molecular weights of 71 and 79 kDa respectively. Amino acid composition of both subunit and the N-terminal sequence of the β-subunit were also determined. The toxin exhibited potent Lethal Dose LD$_{50}$, haemolytic activity *in vitro* and endema-inducing activity with a minimum edema dose of 0.15 µg in mouse paw. Ghadessy *et al.* (1996) studied lethal factions from stonustoxin with a MW of 71 and 79kda. Yuen *et al.* (1994) amplified a base pair fragment from stonefish genomic DNA by the polymerase chain reaction (PCR). It showed 695 amino acids including 11 cystiens. Garnier *et al.* (1996) identified the structure of cDNA clone encoding the β-subunit of VTX, the venom of the stonefish.
S. verrucosa. They reported the presence of 13 aminoacids. Ghadessy et al. (1996) reported the first complete sequence of SNTX of Synanceja horrida. Khoo et al. (1999) attempted to elucidate the crystals of SNTX. They obtained and diffractions of 3.4Å resolution. The crystals belonged to the tetragonal space group p 422, with unit cell constant a=b=109.0Å, c=245.7Å. A native SNTX molecule of Synanceja horrida was reported to have two subunits, designated as α and β respectively and there was one SNTX molecule per asymmetric unit. Khoo et al. (2006) isolated 148 kDa, lethal protein factor from the venom of stonefish Synanceja horrida. The freshwater stingray Potamotrygon falkneri venom extract presented a major band of approximately 12 kDa. Several other components distributed between 15 and 130 kDa were detected in the venom extract. Many components with molecular mass above 80 and 100 kDa have gelatinolytic and caseinolytic activities, respectively. Hyaluronidase activity was detected only in a component around 84 kDa in P. falkneri venom extract. Whittington et al. (2010) reported on the proteinaceous nature of stonustoxin, verrucotoxin and neoverucotoxin venom of stonefish Synanceja sp. Barbaro et al. (2007) characterized certain properties of tissue extracts obtained from the glandular tissues covering the stinger apparatus of Potamotrygon falkneri and Dasyatis guttata stingrays. The tissue extracts were reported to have similar bands above 80 kDa in SDS-PAGE.

Gall and Rageau (1956) reported that venom contents of a single dorsal sting of S. verrucosa, produced spastic paralysis and death of a frog in about 4 hr. When injected into rats, the venom from S. verrucosa caused defaecation, respiratory distress, hypothermia, muscle spasms, marked weakness in the hind limbs and death between 7 and 16 hr, with evidence of blood in the thorax at autopsy. Injection of the venom from
the glandular contents of five dorsal stings into a dog produced some typical signs within 45 sec, including vocalization, trismus, convulsions, relaxation of the sphincters, respiratory distress and loss of consciousness, with death occurring within 1 minute of injection, attributed to cardiovascular collapse and respiratory paralysis.

Saunders (1958, 1959c, 1960) reported the effect of venom of *S. horrida* and *S. verrucosa* in mice and rabbits. He found low doses to cause arterial hypotension and large doses caused electrocardiographic (ECG) alteration respiratory arrest and death. Wiener (1959) observed that *S. horrida* venom was heat-labile and boiling of which caused coagulation and destruction of the venom, and exposure of the venom at 52°C resulted in a loss of toxicity within 30 min. Albumin-like nature, of this venom acted as a good antigen and easily promoted the formation of neutralizing antibodies in rabbits. Saunders and Taylor (1959) tested the pharmacological effect of stone fish venom extracts on anesthetized rabbits and found it to produce a slight fall in blood pressure and respiratory rate. Large doses caused a more predefined drop in blood pressure, respiratory rate and evidence of myocardial injury.

Austin *et al.* (1961) reported that the venom of *S. horrida* possessed potent myotoxic effects on cardiac and involuntary muscles. Saunders *et al.* (1962) investigated the cardiovascular actions of the venom of *S. horrida*. They reported that small intravenous doses of the venom in rabbits produced hypotension as a consequence of peripheral vasodilation. According to Austin *et al.* (1965) there are four major components in stonefish venom *viz.*, hyaluronidase, a capillary permeability factor, a corridor vascular factor which produces a dose department hypotension and a pain
producing factor. They performed chemical studies on the venom of the *S. trachynis* and *S. verrucosa* and found similar elution profiles when chromatographed on speared A-75. In both cases, the lethal activity was only associated with first peak of increased absorbance at 280nm, representing a high molecular weight protein.

Carlson *et al.* (1971) reported mild *in-vitro* haemolytic activity. Cohen *et al.* (1989) examined fluid aspirated from blisters following a lionfish (*Pterois volitans*) sting. The results showed the pressure of prostaglandin F\textsubscript{2α}, E\textsubscript{2}, F\textsubscript{1α}, and thrombokinase platelet aggregation could also be seen. Cohen *et al.* (1989) reported that a soluble toxic extract derived from spine tissue of the lionfish, *Pterois volitans* decreased heart rate and force of contraction in isolated clam and frog hearts. These actions were due to the presence of micromolar concentrations of acetylcholine in the extract. Shiomi *et al.* (1989) extracted crude venoms from dorsal spines of the six species of venomous fishes. All venoms exhibited lethal activity against mice and hemolytic activity specific for rabbit erythrocytes. The lethal activity (or hemolytic activity) of each venom was reported to be very unstable to freezing, lyophilization and heating. Both lethal and hemolytic activities of *S. verrucosa* venom were remarkably neutralized by the stonefish *Synanceja trachynis* antivenom available commercially. Results of neutralization tests have suggest that venoms from the six species were comparable in terms of antigenecity.

Kreger (1991) reported a cytolytic toxin with a MW of 158kDa from the stonefish, *Synanceia trachynis*. The toxin is reported to be cytolytic *in vitro* for rabbit, dog, rat, pig and guinea pig erythrocytes, in that order, but is largely or completely inactive against sheep, cow, human, monkey, mouse, goat, horse, burro and cat.
erythrocytes. The results suggest that the toxin is haemolytic, lethal and has vascular permeability-increasing activities. Sugahara et al. (1992) reported stonustoxin having acetylcholine esterase to act against cancerous cell formation and also the hyaluronidase enzyme present acts on the ion channels between the kidney cells of rabbit. Khoo et al. (1992) confirmed the enzymatic properties of *Synanceja horrida* venom, It showed only the hyaluronidase activity and the other activities such as phospholipase A₂, acetylcholine esterase, protease and Ca²⁺/ Mg²⁺-activated triphosphatase activity were not present. Sugahara et al. (1992) identified the reaction products of the purified hyaluronidase from stonefish *Synanceja horrida* venom.

Kreger et al. (1993) characterized the neuromuscular toxicity of stonefish *Synanceja trachynis* venom by electrophysiological and electron microscopic examination in isolated murine and frog nerve-skeletal muscle preparations exposed to various concentrations of venom. Low concentrations of venom (2.5-10 µg/ml) acted presynaptically by causing release and depletion of neurotransmitter from the nerve terminal. Higher concentrations of venom (100-300 µg/ml) acted postsynaptically and presynaptically. They caused irreversible depolarization of muscle cells and microscopically observable muscle and nerve damage.

Vasuthevan et al. (1993) have reported stonefish hyaluronidase appears to be a potent and safe agent for murine *in vitro* fertilization research. Chen et al. (1997) examined the pore forming property of Stonustoxin by an osmotic protection assay and CD spectrum analysis; however it was shown that SNTX induces the potent haemolytic
activity through the formation of pores in the cell membrane. Low et al. (1993) discovered that SNTX caused vasorelaxation of precontracted rats’ aorta.

Low et al. (1994) observed that the SNTX produces a contracture followed by a decrease in electrically-evoked twitches in both the mouse hemi-diaphragm and the chick biventercervicis muscle preparations. Garnier et al. (1995) reported the crude venom of the stonefish Synanceia verrucosa to contain hyaluronidase, eight esterases and ten aminopeptidases. Verrucotoxin, as the crude venom is lethal for mice, haemolysed rabbit erythrocytes and induced a fall in arterial pressure in anaesthetized rats.

Abe et al. (1996) examined Cardioleputin, a cardioactive toxin, purified from stonefish venom using column chromatography. The purified toxin was found to be an unstable protein that was susceptible to heat and freeze-thawing. This protein showed to have a molecular size of 46 kDa and its amino acid composition was rich in serine and glycine, but low in basic amino acids. The crude venom induced a sudden drop in blood pressure and heart rate of rats right after administration. Both the blood pressure and heart rate returned to their original values as time elapsed, and thereafter continued to show a gradual decrease. In addition, crude venom is reported to actively affect the contractile response and suppressed the heart rate of guinea pig atria. The purified toxin caused irreversible inotropic and chronotropic increases in guinea pig atria. The action of the toxin on the atria was completely different from that of lysolecithin. Hopkins et al. (1996) reported that stonefish venom contains a component capable of stimulating the release of endogenous tachykinins. The venom also is reported to stimulate sodium channels on sensory nerves. Lethal, dermonecrotic and myotoxic
activities were reported in *P. falkneri* tissue extract. Edematogenic activity was similar and reported to be dose dependent in both tissue extracts. Nociceptive activity is reported in both tissue extracts, but *P. falkneri* is reported to present a two-fold higher activity than *D. guttata* tissue extract (Abe *et al.*, 1996).

Breton *et al.* (1999) reported that the crude venom of *S. verrucosa* had severe neurotoxic effects when the venom was administered *i.c.v.* to rats. However, they have reported that Verrucotoxin, the lethal toxin isolated from *S. verrucosa* venom, was unable to produce the same severe neurotoxic effects produced by crude *S. verrucosa* venom when administered *i.c.v.* Sauviat *et al.* (2000) isolated Trachynilysin (TLY), a protein toxin from stonefish *S. trachynis* venom and tested its effects on the electrical and mechanical activities of frog atrial fibres. TLY induced an increase of basal Ca\(^{2+}\) influx in atrial cells, enhances Ca\(^{2+}\) influx in atrial cholinergic nerve terminals favouring the release of ACh (acetylcholine). Church and Hodgson (2000) noted that the *S. trachynis* venom produced concentration-dependent biphasic vasoconstriction/vasodilation response. Ouanounou *et al.* (2000) isolated neurotoxin from the venom of the stone fish *Synanceia trachynis* and reported it to affect neuromuscular junctions.

Roger *et al.* (2004) reported antitumor activity of Tetradoxins (TTX) extracted from the Masked Puffer fish and some dramatic changes in the electrophysiological properties of three breast cancer cell lines exposed to different concentrations of TTX. Carrijo *et al.* (2005), described some biological properties and a partial biochemical characterization of the *Scorpanea plumieri* crude venom. The fresh venom is reported to induce a decrease in blood pressure, cardiac and respiratory frequency, and exhibited
hemorrhagic, hemolytic and proteolytic activities. The LD\textsubscript{50} (i.v) to mouse was 0.28 mg/kg. The pharmacological activities were found to be very unstable and this fact is reported to be associated with proteolytic activity. Enzymes which hydrolyze casein and gelatin were reported to be found in this venom. A gelatinolytic protease (Sp-GP was purified to homogeneity from \textit{S. plumieri} venom through a combination of three chromatographic steps: gel filtration on Sephacryl S-200; ion exchange on DEAE-cellulose and reverse-phase/HPLC on a Vydac C4 column. The molecular mass of the Sp-GP scorpionfish gelatinase estimated by SDS-PAGE was around 72-80 kDa. Verrucotoxin has been reported to be isolated by DEAE and hydroxyapatite chromatography, followed by FPLC gel filtration on Superdex 200 HR 10/30. It was found to be a glycoprotein with a MW of 322 ± 2 kDa, comprising subunits, 2α (83) and 2β (78).

The results showed that at a concentration of 30 μM which fully blocks sodium ions input to the cells reduces the proliferation, migration and invasive properties of the cell line by about 30%. No direct hemolysis, phospholipase A\textsubscript{2} and coagulant activities have been reported. The extracts have been reported to possess gelatinolytic, caseinolytic and fibrinogenolytic activities (Barbaro \textit{et al.}, 2007).

Sivan \textit{et al.} (2007) reported biological properties of the weever fish \textit{Scatophagus argus} venom. \textit{S. argus} venom showed relatively low LD\textsubscript{50} of 9.8 mg/kg \textit{via i.p}. Haemolytic activity in human erythrocytes was recorded. Platelet lysis expressed as LDH activity of lysed cells was dose dependent. \textit{S. argus} venom failed to induce any clot in human plasma. No PLA\textsubscript{2} activity was found in \textit{S. argus} venom. Mild proteolytic activity
was observed. The injection of venom in mice produced lesions and nociception, which were not inhibited by antihistamine pheniramine maleate, suggesting that histamine was not involved in the inflammatory process. The increase in serum creatine kinase activity is reported to indicate myotoxicity. Cytotoxicity on HeLa cells was observed.

Magalhaes et al. (2007) reported on the biological and biochemical properties of the Brazilian venomous fishes *Potamotrygon scobina* and *P. orbignyi*. Both stingray venoms induced significant edematogenic and nociceptive responses in mice. Edematogenic and nociceptive responses reduced when the venom was incubated at 37 or 56°C. The results have shown striking augments of leukocytes rolling and adherent cells to the endothelium induced by both venoms in mice. It is presented that injection of both venoms in mice induced necrosis of tissues, low level of proteolytic activity, without inducing haemorrhage. It is reported that when the venoms of both stingray species were injected together with their mucus secretion, the necrotizing activity was more vigorous. Enzor et al. (2011) reported that the spine venom of the Atlantic stingray *Dasyatis sabina* effected the resting metabolism of mice. The LC$_{50}$ for the fish *Cyprinodon variegates* was reported to be 0.181 mg protein g fish$^{-1}$. The ballistic bomb calorimetry revealed an average spine caloric density of 0.238 kcal per gram of spine.

Yamamoto et al. (2009) reports that a chef encountered severe pain in his finger while cutting a stonefish. The finger became reddish, swollen and tender. The pain is reported to have disappeared after 18 hrs after immersing in hot water. Garcia et al. (1965) recorded and discussed eighty one cases of stings by stonefish (genus *Synanceja*) encountered over in the polar Hospitals, Singapore. Ngo et al. (2009) reported that
stonefish envenomation causes extreme pain, swelling and erythema. Chan et al. (2010) reported most common fish stings were of catfish and stonefish.

Haddad et al. (2004) studied the clinical aspects of 84 patients injured by freshwater stingrays. Intense pain was the most conspicuous symptom. Skin necrosis was observed in a high percentage of the victims, mostly fishermen and bathers. The initial therapeutic procedures, like immersion of the affected member in hot water were effective in the initial phases of the envenoming, especially in the control of the acute pain; however, they did not prevent skin necrosis.

Dehghani et al. (2009) studied the clinical aspects of injuries induced in three patients bitten by the stingray Himantura gerrardi. Intense pain was noticed in all human cases. Redness was observed in two cases, and spasm and seizure were each recorded in only one case.