THE EFFICACY OF HERBAL PLANTS EXTRACT AGAINST INFECTION OF AEROMONAS HYDROPHILA, STAPHYLOCOCCUS XYLOSUS AND STREPTOCOCCUS AGALACTIAE IN SNAKEHEAD (CHANNA STRIATA) FISH

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by

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KEBERKESANAN EKSTRAK TUMBUH-TUMBUHAN HERBA TERHADAP PENYAKIT AEROMONAS HYDROPHILA, STAPHYLOCOCCUS XYLOSUS DAN STREPTOCOCCUS AGALACTIAE PADA IKAN HARUAN (CHANNA STRIATA)

oleh

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Tesis yang diserahkan untuk memenuhi keperluan bagi Ijazah Sarjana Sains

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LIST OF ABBREVIATIONS

ppm	Part per million
ppt	Part per thousand
rpm	Rotation per minutes
BLAST	Basic Local Alignment Search Tool
BSE	Bovine Spongiform Encephalopathy
CPK	Creatine phosphokinase
CTF II	CArdiotoxic factor II
CFU	Colony forming unit
CNS	Central Nerve System
DDS	Department Delivery System (DDS)
DNA	Deoxyribonucleic acid
DOF	Department of fisheries
DHA	Decosahexaenoic acid
EtBr	Ethdium Bromide
EPA	Eicosapentaenoic acid
FRIM	Forest Research Institute Malaysia
GC-MS	Gass Chromatography and Mass Spectrum
HMDS	Hexamethyldisilazane
IHNV	Infectious Haematopoietic Necrosis Virus
LC_{50}	Lethal Concentration that kill half of sample population
MIC	Minimum Inhibitory Concentration
MG	Mycotic Granulomatosis
MOA	Ministry of Agriculture and Agro-based industry

PCR	Polymerase chain reaction
RBC	Red Blood Cell
SFSE	Shol fish skin extract
SEM	Scanning electron microscope
SVCV	Spring Viremia Carp Virus
TEM	Transmission electronmicroscope
TAE	Tris-Acetate-EDTA
VHSV	Viral Haemorrhagi Septicaemia Virus
WBC	White Blood Cell
ZIA	Aquaculture Zone Industry

KEBERKESANAN EKSTRAK TUMBUH-TUMBUHAN HERBA TERHADAP PENYAKIT *Aeromonas hydrophila, Staphylococcus xylosus* DAN *Streptococcus agalactiae* PADA IKAN HARUAN (*Channa striata*)

ABSTRAK

Ujian saringan telah dilakukan untuk 13 spesis tumbuhan tergolong dalam 9 jenis famili seperti *Senna spectabilis, Jacaranda filicifolia, Samanae saman, Tamarindus indica, Carica papaya, Andira inermis, Morinda elliptica, Coleus aromaticus benth, Citrus hystrix, Milletia pinnata, Cymbopogon nardus, Cymbopogon citratus* dan *Polyalthia longifolia* untuk mencari aktiviti antibakteria terhadap tiga patogen ikan seperti; *Aeromonas hydrophila, Staphylococcus xylosus* dan *Streptococcus agalactiae*. Kepekatan perencatan minimum (MIC) menunjukkan, *S. xylosus* direncat oleh *S. saman* dengan kepekatan terendah iaitu 0.625 mg/ml berbanding dengan *T. indica* dan *J. filicifolia*; 2.5 mg/ml. *Tamarindus indica* dan *J. filicifolia* menunjukkan aktiviti terhadap semua patogen yang diuji. Ujian ketoksikan dengan udang air masin telah dijalankan ke atas tiga ekstrak tumbuhan dengan menggunakan nauplii *Artemia salina* sebagai sampel ujian dan keputusan menunjukkan , *T. indica* dan *J. filicifolia* adalah sangat toksik dan *S. saman* menunjukkan ketosikan yang terendah (> 1.0 mg/ml) ; dengan nilai LC_{50}, 2.22 mg/ml. Mikroskop imbasan elektron (SEM) digunakan ke atas dua sel patogen; *S. xylosus* dan *S. agalactiae* yang di rawat dengan ekstrak daun *S. saman* dan menunjukkan kerosakan struktur pada permukaan sel. Di samping itu, analisis fitokimia dilakukan untuk melihat komponen kimia yang hadir dalam ekstrak daun *S. saman*; menunjukkan kewujudan Alkaloid, Flavonoid, Tanin dan Sebatian Polifenolik. Kromatografi Gas dan Spektrum Jisim (GC-MS) dijalankan ke atas

sampel ekstrak *S. saman* dan menunjukkan kehadiran aktif kompaun hidrazin, 1-etil-1-(1-methylpropyl). Potensi *S. xylosus* dan *S. agalactiae* untuk menyebabkan jangkitan penyakit pada *C. striata* disiasat melalui ujian patogen secara *in-vivo*. Suntikan intra-muskular dilakukan ke atas sirip ikan dengan kepekatan 10^9, 10^7 dan 10^5/CFU larutan anggaran bakteria. Ikan disuntik dengan kepekatan 10^9 *S. xylosus* menunjukkan bisul seperti luka dengan erosi dan juga bisul seperti luka dengan pembentukan ulser, pada hari ke-3 selepas suntikan. Manakala, ikan yang disuntik dengan kepekatan 10^9 *S. agalactiae* menunjukkan sedikit bengkak pada hari pertama dan sembuh dengan lebih cepat. Keberkesanan ekstrak daun *S. saman* terhadap penyakit yang disebabkan oleh patogen *S. xylosus* diuji secara in-vivo dalam ikan haruan. Ikan dibahagikan kepada tiga kumpulan, Kumpulan 1 (dua kali rawatan), Kumpulan 2 (empat kali rawatan) dan Kumpulan 3 (kawalan tanpa rawatan). Daripada keputusan yang diperolehi, Kumpulan 2 (empat kali rawatan) menunjukkan bilangan kematian dan peratusan tahap keterukan iaitu 1 dan 5%, berbanding kawalan, 4 dan 25%.

THE EFFICACY OF HERBAL PLANTS EXTRACT AGAINST INFECTION OF *Aeromonas hydrophila, Staphylococcus xylosus* AND *Streptococcus agalactiae* IN SNAKEHEAD (*Channa striata*) FISH

ABSTRACT

Preliminary screening was done for 13 plants species belonging to member of nine families such as *Senna spectabilis, Jacaranda filicifolia, Samanae saman, Tamarindus indica*, *Carica papaya, Andira inermis, Morinda elliptica*, *Coleus aromaticus benth, Citrus hystrix, Milletia pinnata, Cymbopogon nardus, Cymbopogon citratus* and *Polyalthia longifolia* were screened to find an antibacterial activity against three common fish pathogens namely *Aeromonas hydrophila*, *Staphylococcus xylosus* and *Streptococcus agalactiae*. Minimum Inhibitory Concentration (MIC) showed, *S. xylosus* was inhibited by *S. saman* with lowest concentration at 0.625 mg/ml compared to *T. indica* and *J. filicifolia*; 2.5 mg/ml respectively. *Tamarindus indica* and *J. filicifolia* showed activity against all pathogens tested. Toxicity test of brine shrimp was conducted on three selected plants extract by using *Artemia salina* nauplii as a test sample and from the results, *T. indica* and *J. filicifolia* were highly toxic; and *S. saman* shows lower toxicity (>1.0 mg/ml); with LC_{50} value of 2.22 mg/ml. Scanning electron microscope (SEM) were used to observe on two pathogens; *S. xylosus* and *S. agalactiae* treated with *S. saman* leaf extracts and showed structural damage in the treated cells. In addition, phytochemical analysis was conducted to investigate the chemical constitution in *S. saman* leaf extract; and found presence of alkaloid, flavonoid, tannins and polyphenolic compounds. Gas chromatography and mass spectrum (GC-MS) on *S. saman* extract samples showed presence of active compounds Hydrazine, 1-ethyl-1-(1-methylpropyl). The potential of the *S. xylosus* and *S. agalactiae* to induce disease

infection in *C. striata* were investigated through *in-vivo* pathogenicity test. The intramuscular injection is done into dorsal fin of the fishes with concentration of 10^9, 10^7 and 10^5/CFU bacteria suspension. Fish injected with 10^9 of *S. xylosus* suspension showed furuncle like lesion with erosion and also furuncle like lesion with ulcerated core, in day 3 post injections. Fish injected with 10^9 of *S. agalactiae* suspension showed slight swelling in day 1 and healed faster. The efficacy of *S. saman* leaf extract against disease caused by *S. xylosus* pathogens was tested in-vivo in the to Snakehead fish. The experiment of fish were divided into three groups; Group 1 (two times treatment), Group 2 (four times treatment) and Group 3 (control without treatment). From the results obtained, Group 2 (four times treatment) showed lower number in mortality and percentage of severity which is 1 and 5 % compared to the control of 4 and 25 %.

CHAPTER 1

INTRODUCTION

Aquaculture provides big opportunities for the production of wide variety of aquatic foods including fish and shell fish. It provides important sources of protein and also source of food. Consumption of food fish is increasing had risen from 40 million tonnes in 1970 to 86 million tonnes in 1998 and reached 110 million tonnes in 2010 (Sihag and Sharma, 2012). A review on world fishery production showed that, the capture aquaculture production has maintained a level at 90 million tonnes for more than a decade, with fisheries showing an increasement from 34.6 million tonnes in 2001 to 55.7 million tonnes in 2009 (Pridgeon and Klesius,2012).

Aquatic diseases are the biggest constraint in aquaculture farmed production. Major pathogens been reported in aquaculture sector include: bacteria, fungi, viruses and parasites. In addition, as reported by Shariff and Subasinghe (1993) identified a number of parasitic, bacterial, viral and fungal pathogens common in the Malaysian aquaculture industry. Losses through diseases are considered significant, although hard data are lacking. Bacterial infection is commonly reported in aquaculture production especially in eggs, intestines, skin surface and gills (Cahill, 1990). This is because bacteria can survive in aquatic environment independently of their hosts and become major impediment to aquaculture. In Malaysia, the estimated losses in finfish cultured in floating cages in Peninsular Malaysia due to pathogenic bacteria was reported to have amounted to RM 20 million (Najiah *et al.,* 2011). Recent disease outbreaks reported in Kedah are thought to have caused the industry up to $10 million (Shariff 1995). The pathogenic bacteria such as *Aeromonas hydrophila,* *Staphylococcus xylosus and Streptococcus agalactiae* are among the common fish pathogens which have infected fish farms and caused mortality (Schaperclaus *et al.*, 1992).

The presence of antibiotic residues in fish farms is increasing due to their extensive used such as in animal feeds, bath treatment, in order to treat animal diseases and promote growth (Pericas *et al.*, 2010). Generally, chemical residues in aquaculture farms can accumulate in fish and could cause chronic health effects to consumers. Among the health problems caused by the chemical residues are cancer, nerve problems and immunological problems. In year 2001, antibiotic residue especially chloramphenicol and nitrofurans again became important issues due to their detection in shrimp farms in China, Vietnam and Thailand. Similarly, in Malaysia, chemical hazards including pesticides and antibiotic residues have been reported occur in freshwater aquaculture fish (Abu bakar *et al*., 2010).

1.1 Problem Statement

Uses of antibiotics and chemotherapeutic agents for treatment and prophylaxis in aquaculture production have been criticized due to it negative side effect (Cristea *et al.*, 2012). An urgent research on plant-derived based for treatment is needed; which have been reported by various workers; as an antistress, antimicrobial, immunostimulant agents, growth promoter and appetite enhancement (Citarasu *et al.*, 2001; Sivaram *et al*., 2004; Magdelin,2005). In addition, there is a lack of study done on water-based herbal plants extracts. Although, not many volatile active compounds have been found in these water based extracts, but, it is eco-friendly, safer to use, and also cheaper sources for farmers, which can prepare their own water extracts with minimal training. Furthermore, this also can help to reduce management cost by not purchasing any chemotherapeutic chemicals.

1.2 Research objectives

The aim of the study is:

i. To determine the effects of the plants extracts such *as Samanae saman, Jacaranda filicifolia, Tamarindus indica* and others on the activity of three common pathogenic bacteria namely; *Aeromonas hydrophila, Staphylococcus xylosus* and *Streptococcus agalactiae*.

ii. To gain a better understanding about herbal plants extracts and to find the efficacy in treating bacterial fish disease *in-vitro* and also *in-vivo.*

iii. To explore the possibility of using herbal plants extracts as an alternative treatment for Snakehead fish farmers in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture

Asia and the pacific region is the world's largest producer of fish. In 2008, the amount from capture fisheries reached 48.3 million tonnes, which was an increase of 2.9 per cent compared to 2006 (Lymer *et al*., 2010). Fish production growth in Southeast Asia has also been very high in the past four decades with marine capture production increasing almost linearly throughout this period (Table 2.1) (Lymer *et al*., 2010). The total capture production in 2006 was 15.4 million tonnes, of which marine capture was 88% while in 2008 was 16.1 million tonnes, which consisted mainly of pelagic marine fish. The increase in marine production in 2004 could be attributed to the increases in production from Myanmar, Indonesia, Vietnam and the Philippines, but slight drop in production occurred in Thailand and Malaysia, 2 and 3% respectively (Lymer *et al*., 2008). In 2008, aquaculture production in this region recorded an increase, of which ninety-four different species were reported (Lymer *et al.*, 2010).

Table 2.1: Fish capture production in Asia and the Pacific region (by sub region) (x1000 tones)

	China	Southeast Asia	South Asia	Other Asia	Oceania
Marine fishes	2103	4868	991	495	60
Marine molluscs	646	80	12	2	4
Marine crustaceans	0	9	81	17	2
Freshwater fishes	1615	1588	1361	13	8
Freshwater molluscs	268	63	0	2	2
Freshwater crustaceans	0	4	80	0	0
Total	4633	6613	2524	529	77
Total capture production	**15141**	**16133**	**6591**	**6467**	**1096**
Total contribution (%)	**31**	**41**	**38**	**8**	**7**

Source: Lymer *et al. (*2010)

2.1.1 Aquaculture in Malaysia

Aquaculture is one of the important branches in agriculture. In 1994, the total aquaculture production in Malaysia amounted to 114,114 metric ton of food fish valued at USD 145.8 million and USD 227.8 million aquarium fish valued at USD 17.5 million. Malaysian Department of Fisheries (DOF) has predicted that the aquaculture sector will generate almost USD 400 million (RM 1.39 billion) of revenue per year for the nation. Cage culture is one of the aquaculture systems in Malaysia that have a lot of potentials and can be further developed in the future. Cage culture has been producing more than 22,000 tons of fish per year and the value is about USD 106 millon (RM 371 million) per year (Ahmad Faiz *et al*., 2010).

In 2010, the total fishery sector showed an increase of 3.77 % in production compared to the year before. According to Department of Statistics Malaysia, the value of gross output generated by the fisheries sub sector was RM 689.6 million in 2008. Out of this, the states of Sabah, Perak and Pahang accounted for more than half of the total values of gross output (54.6%). Department of Fisheries stated that, deep sea fisheries showed an increase in value in a total of RM 1.271 million compared to RM 1.164 million in 2009. On the other hand, revenue from coastal fisheries was RM 5.305 million compared to RM 4.907 million in 2009 (Inside Malaysia, 2012)

The Ministry of Agriculture and Agro-based industry (MOA) through the Department of Fisheries (DOF) has developed an aquaculture industry zone (ZIA) program throughout Malaysia (Ahmad Faiz *et al.*, 2010). The aims of the program are:

(a) To create permanent areas for Aquaculture Industry Zone
(b) To increase the production of fish in line with the goal of balance of trade (BoT) plan.
(c) To increase the net income of aquaculturist to at least USD 850 per month
(d) To ensure the production of fish and fish products which have high quality and safe for consumption
(e) To increase private sector participation through the provision of ZIA areas, infrastructure and Department Delivery System (DDS) and
(f) To create a chain of efficient aquaculture fish production areas.

However, fish disease is considered a serious issue in aquaculture fish farming. Diseases in Malaysia aquaculture have been reported since the 1980s (Anderson, 1988). The common pathogens found in the Malaysia aquaculture industry are parasitic, bacterial, viral and fungal pathogens (Shariff and Subasinghe, 1993).

2.2 Snakehead fish

2.2.1 Taxonomy and Synonymy of Snakehead fish

Snakeheads (family Channidae) have two genera, *Channa* which is native to Asia, Malaysia and Indonesia; while *Parachanna* is endemic to a tropical Africa (Courtenay and Williams, 2004). Currently, Channidae are recognized for 26 species of *Channa* and 3 of *Parachanna* (Table 2.2). According to Nelson (2006), the snakeheads are classified into:

Class	:	Actinopterygii
Subclass	:	Neopterygii
Order	:	Perciformers
Suborder	:	Channoidae
Family	:	Channidae

Table 2.2: Species recognized for the family Channidae

No	Species	Name	References
1*	*Channa amphibeus*	Chel snakehead	McClelland,1845
2	*Channa argus*	Northern snakehead	Cantor,1842
3	*Channa asiatica*	Chinese snakehead	Linnaeus,1758
4	*Channa aurantimaculata*	Orangespotted snakehead	Musikasinthorn,2000
5	*Channa bankanensis*	Bangka snakehead	Bleeker,1852
6	*Channa baramensis*	Baram snakehead	Steindachner,1901
7	*Channa barca*	Barca snakehead	Hamilton,1822
8	*Channa bleheri*	Rainbow snakehead	Vierke,1991
9	*Channa burmanica*	Burmese snakehead	Chaudhuri,1919
10	*Channa cyanospilos*	Bluespotted snakehead	Bleeker,1853
11	***Channa gachua***	**Dwarf snakehead**	**Hamilton,1822**
12	*Channa harcourtbutleri*	Inle snakehead	Annandale,1918
13	***Channa lucius***	**Splendid snakehead**	**Cuvier,1831**
14	*Channa maculate*	Blotched snakehead	Lacepede,1802
15*	*Channa maurulius*	Bullseye snakehead	Hamilton,1822
16	***Channa marulioides***	**Emperor snakehead**	**Bleeker,1851**
17	*Channa melanoptera*	Blackfinned snakehead	Bleeker,1855
18	***Channa melasoma***	**Black snakehead**	**Bleeker,1851**
19*	***Channa micropeltus***	**Giant snakehead**[3]	**Cuvier,1831**
20	*Channa nox*	Night snakehead[1]	Zhang et al., 2002
21	***Channa orientalis***	**Ceylon snakehead**	**Schneider,1801**
22	*Channa panaw*	Panaw snakehead	Musikasinthorn,1998
23	***Channa pleurophthalma***	**Ocellated snakehead**	**Bleeker,1851**
24*	*Channa punctata*	Spotted snakehead	Bloch,1793
25	*Channa stewartii*	Golden snakehead	Playfair,1867
26*	***Channa striata***	**Chevron snakehead**	**Bloch,1797**
27	*Parachanna africana*	Niger snakehead	Steindachner,1879
28	*Parachanna insignis*	Congo snakehead	Sauvage,1884
29	*Parachanna obscura*	African snakehead	Gunther,1861

Source: Courtenay and Williams (2004) Note **(*):** Purpose as Food fish (Haniffa *et al.*, 2013) Highlighted *Channa* spp indicated occurred in Malaysia

2.2.2 Snakehead fish (*Channa striata*)

Channa striata is well-known as Haruan in Malaysia and is a native freshwater fish of tropical Africa and Asia (Ng and Lim, 1990). It belongs to the family Channidae and is also known as Murrels or Serpent-Headed fish. Some species of snakeheads are highly valued as food fishes, particularly in India, South eastern Asia, and China and to a lesser extent in Africa. Because of its popularity as a food in southern China and adjacent south-eastern Asia, the chevron snakehead (*C. striata*) has been reported as widely introduced into islands from the western Indian Ocean eastward to Hawaii. The northern snakehead (*C. argus*) has been a market leader and cultured in China and Korea. Other snakeheads utilized as food fishes include the Chinese snakehead (*C. asiatica*), Blotched snakehead (*C. maculata*), and Spotted snakehead *(C. punctata).*

2.2.2. (a) Taxonomy and distribution

Channa striata is a tropical, freshwater, carnivorous, air-breathing fish species, indigenous to Malaysia and widely distributed within the country. A study by Kajima *et al*., (1994) and Kumar (1995) on the genetic variability of Snakehead based on fish mitochondrial DNA revealed that this species has been present in Malaysia for more than 600,000 years providing evidence that the fishes are truly Malaysia indigenous species.

2.2.2. (b) Biology of *Channa striata*

Channa striata is not a good swimmer but with a fast flip action, is quite an efficient predator. It is also known as air-breathing fish that can survive in the environments with low dissolved oxygen and high ammonia contents (Marimuthu and Haniffa, 2007) and stay alive without water as long as its gills remain moist. However, *C. striata* have mostly been found in waters up to 12 meters deep and 4 to 80 meters wide. In addition, *C. striata* is usually found in rivers with salinity about 10 ppt and temperature around 20.7 to 26.4 °C with pH range of 4.3 to 7.9. Although, *C. striata* is known as a hardy fish and able to tolerate to some extend any deterioration of the water quality, it is very sensitive to contamination of its habitat and has developed a unique physiological adaptation namely the ability to move from pond to pond by crossing on land in order to find suitable and clean water (Mat Jais, 1991). It is carnivorous and feed on frogs, fish, insects, tadpole and earthworms (Muntaziana *et al.*, 2013).

In addition, *C. striata* is not a good swimmer and prefers stagnant, slow running and shallow water not more than two metre deep with dead log and aquatic plants so that it can easily hide and hunt for food (Mat Jais, 2007). The fish has a unique habit of settling itself into bottom mud of ponds during drought season and going deep and deeper into mud during the dry period and only comes out when the situation is better (Rahman *et al.,* 2012).

2.2.2. (c) Morphology of *Channa striata*

Channa striata possesses an angular head without patches of scales and with large mouth as shown in Plate 2.1. The pectoral fin length is about half the length of the head. The dorsal fin contains 37-46 fin rays; the anal fin has 23-29 fin rays; the pectoral fins bear about 15-17 rays and the pelvic fins with 6 rays, with rounded caudal fin. The colours of snakehead fish can be highly variable or complex but most often the dorsum appears to be dark brown to black (Courtenay and Williams, 2004).

Plate 2.1: Snakehead fish, *Channa striata*

2.2.2. (d) Importance Medicinal properties of *Channa striata*

The medicinal effects of *C. striata* are attributed to two major components, the amino acids and the fatty acids as described in Table 2.3.

Table 2.3: Important amino acids and fatty acids in different sources of *C. striata* extract

	Fillet	Roe	Mucus
Amino acids	Glycine Glutamine acid Arginine Aspartic acid	No published data	No published data
Fatty acids	Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA) Palmitic acid Oleic acid Stearic acid Arachidonic acid	Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA) Arachidonic acid Hexadeconoic acid Oleic Linoleic	Oleic acid Linoleic acid

Source: Mohd Shafri and Abdul Manan (2012)

A. Amino acids

Previously, Mat Jais *et al.,* (1994) have conduct the study on amino acid profile in the fillet extract of *C. striata* which found the extract to be rich in glycine, a non-essential amino acid. In addition, amino acid such as glutamic acids, arginine, aspartic acid and glycine have been found in *C. striata*. These amino acids are important in influencing the sense of pain and in healing wounds.

B. Fatty acids

The first study on lipid profiles on *C. striata* was reported by Endinkeau and Kiew (1993) which showed a high level of fat (11- 17%) wet weight and a high ratio unsaturated: saturated (1.2-2.3) and low omega-3 in general. The ability of *C. striata* to produce unsaturated fatty acids such as Eicosapentaenoic acid (EPA) and Decosahexaenoic acid (DHA) (Jaya Ram *et al.*, 2011) in high amounts (Abdul Rahman *et al*., 1995) showed the efficiency of the fish fatty acids as a wound healing agent. In addition, some of the most abundant fatty acids in *C. striata* are C16 (Palmitic acid), C22:6 (DHA), C18:1 (Oleic acid) and C18:0 (Stearic acid) (Zakaria *et al*., 2007; Dahlan-Daud *et al.,* 2010).

2.2.2. (e) Traditional health treatment

Channa striata is commonly consumed as a food fish as, freshwater fish consumption in Malaysia provides an important source of protein constituting up to 70% of total protein requirements (Osman *et al.*, 2001) and is also recognised as a source of omega-3 fatty acids (Ng, 2006). In addition, *C. striata* is also highly valued for its medicinal properties. Among many types of fishes in Malaysia, only the Malaysian Channidae (including *C. micropeltes, C. striata* and *C. gachua*), the mudskipper, *Periophtalmus* spp., and the freshwater eel, *Monopterus albus* (Abdullah *et al*., 2010) are known to be used in traditional Malay medicine. Other Southeast Asian countries such as Thailand, Vietnamese and Cambodians as well as the Chinese also use *C. striata* in the treatment of diseases (Wee, 1982). The popularity of *C. striata* as a therapeutic agent is related to folk belief in its efficacy in treating wounds, relieving pain and boosting energy in the sick and elderly. Mat Jais (1997) reported that *C. striata* is consumed to fasten healing especially for mothers who underwent caesarean operations and become supplementary among illnesses like diabetic gangrene and cancer.

2.2.2. (f) Pharmacological properties of *Channa striata*

The snakehead meat has good taste, high nutrient and also has high pharmaceutical medicinal values (Khanna, 1978). In addition, many of its health benefits have been studied and described in detail

A. Post-partum and energy booster meal

There has been a dearth of studies looking at current attitude of post-partum mothers towards *C. striata* as a medicinal food fish. Furthermore, among the Malays, *C. striata* is cooked in the form of curried, spiced, fried or roasted fish, playing the role of functional foods which provide health benefit beyond basic nutrition. Other forms of product such as broth or a tonic of *C. striata* extracts are quite popular. The energy-restoring properties of *C. striata* are also recognised in the Malay society where it is consume for recovery process from minor to major illnesses as well as a diet supplement for elderly people.

B. Wound healing

The uses of *C. striata* as a wound healing agent is thought to be influenced by high level of specific amino acids (e.g. glycine) and fatty acids (arachidonic acid), believed to be involved in the promotion of wound healing by the initiation of a series of reactions involving remodelling of collagen, re-epithelialisation of wound and induction of wound contraction. *Channa striata* extracts also has the ability to cause proliferation of mesenchymal cells and maintain sufficient cell viability for use as a biochemical agent and promoter of healing (Abdul Wahid *et al.*, 2009) which is not limited to dermal wounds but possibly involving other types of organs as well.

C. Anti-pain

In addition, the anti-nociceptive property of *C. striata* is thought to be due to its glycine and arachidonic acid constituents which are known to be involved in the anti-nociceptive pathway (Kapoor *et al*., 2006). The extracts of *C. striata* have better antinociceptive properties compared to extracts from other Channidae (Mohd

Hasan, 2005) and work in a concentration dependent manner (Zakaria, 2005) in a wide range of temperatures and pH (Dambisya *et al*., 1999).

D. Anti-inflammatory and anti-pyretic

The anti-inflammatory effect of *C. striata* extracts towards acute and chronic inflammation appears better than other Channidae (Somchit *et al*., 2004; Mohd Hasan, 2005). It is also used in treating diseases such as osteoarthritis (Michelle *et al*., 2004). In addition, *C. striata* may have a role in the treatment of joint diseases with a clearer inflammatory component such as rheumatoid arthritis. The anti-inflammatory property may also be the reason behind the observable antipyretic activity of the aqueous extract (Zakaria *et al*., 2008).

E. Anti-oxidants

Among freshwater fishes, *C. striata* appears to have a medium level of anti-oxidant activities (Lokman, 2006) possibly contributed by some of the major amino acids and fatty acids which it contains.

F. Anti-fungal and anti-bacterial

The skin and intestinal mucus extract of *C. striata* showed antibacterial activity against *Aeromonas hydrophila* and *Pseudomonas aeruginosa* (Dhanaraj *et al.*, 2009). As reported by Mat Jais *et al*. (2008), antifungal activities of *C. striata* ethanolic extract showed activity on *Neurospora crassa*, *Aleurisma keratinophilum* and *Cordyceps militaris*.

G. Cardiological effect

Fish oil supplementation is widely regarded as an effective preventative measure against cardiovascular problems. Calo *et al*. (2005) concluded that, fish oil supplementation could be useful in preventing post-operative atrial. The skin extract of *C. striata* known as Shol fish skin extract (SFSE) which, has been found to contain potent active compound, cardiotoxic factor II (CTF-II) (Karmakar *et al*., 2002), with hypotensive effect and cardiotoxic property that influence the increase in cardiac marker enzyme creatine phosphokinase (CPK) and creatine phosphokinase-MB (CPK-MB) values (Karmakar *et al.*, 2004).

H. Haematological treatments

The cardiotoxic factor II (CTF-II factor) found in SFSE also has blood-modulating properties. This factor could induce a decrease in haemoglobin, total Red Blood Cells (RBC), White Blood Cell (WBC), and platelet count (Karmakar *et al.*, 2004).

I. Neurology and neurophysiology

The skin extract of *C. striata* could initiate apnoea and irreversible blockade of nerve-muscle preparation (Karmakar *et al.*, 2002) and also influence the serotonergic receptor system which possible role as anti-depressant (Saleem *et al.*, 2011).

J. Skin disease

Channa striata is very useful for treating skin disease problems such as acne, pimples, allergy, psoriasis, sclerosis, infection and other related skin problems. Basically, it is due to the content of fatty acid, Docosahexaenoic acid (DHA) in *C.*

striata which was recognized as a nutraceutical with clinical value in skin problem treatment (Mat Jais *et al*. 1998; Mori *et al*., 1999).

K. Platelet-aggregation

Aggregation of platelet is one of the steps in blood clotting and wound healing. *Channa striata* extract produced positive results in diabetic patients whom undergoing drug treatments. This is beneficial to those suffering diabetes mellitus and it will also contribute as an alternative treatment for dengue haemorrhagic patients (Mat Jais 2007).

L. Antinociceptive properties

Hydromethanolic fraction of Haruan fillets extracts produces a dose-dependent anti-nociceptive property, which also plays a role in the healing process (Mat Jais *et al*., 1998).

M. Other Uses

Due to high demand for a special diet which increases awareness on the benefits of food from beef products and public concern over bovine spongiform encephalopathy (BSE), the demand for fish meat has increased dramatically, which in turn has led to higher amounts of unwanted fish parts being disposed such as fish skin. Thus, in order to avoid wasteful disposal of fish skin, researchers looked at alternative ways to utilize this valuable food source (Babji *et al*., 2011). For instance, the production of gelatin has been shown to be comparable to commercial gelatins made from cold-water fishes and bovine skin (See *et al*., 2010).

2.3 Fish diseases

Aquatic diseases are one of the major problem in aquaculture production. Adding to that, water is a very suitable medium for disease transmission in aquatic animals (Sihag and Sharma, 2012). According to Kinne (1980), a disease may be caused by genetic disorders, physical injury, nutritional imbalance and abiotic factors such as pollution and pathogens. The presence of the disease outbreak are varied due to the complex interaction between the host and the disease-causing situation. The interrelationships between the host organisms, the pathogens and environmental factors are shown in Figure 2.3. The natural balances between these three factors are correlated with unpolluted environment with the normal fluctuations in ambient conditions. However, a reduction in the quality of environment (E), may lead to the frequency and severity of disease (D). In addition, an increase in the host population density may cause an increase in the risk of disease outbreaks and the increase in virulence of the pathogens (P).

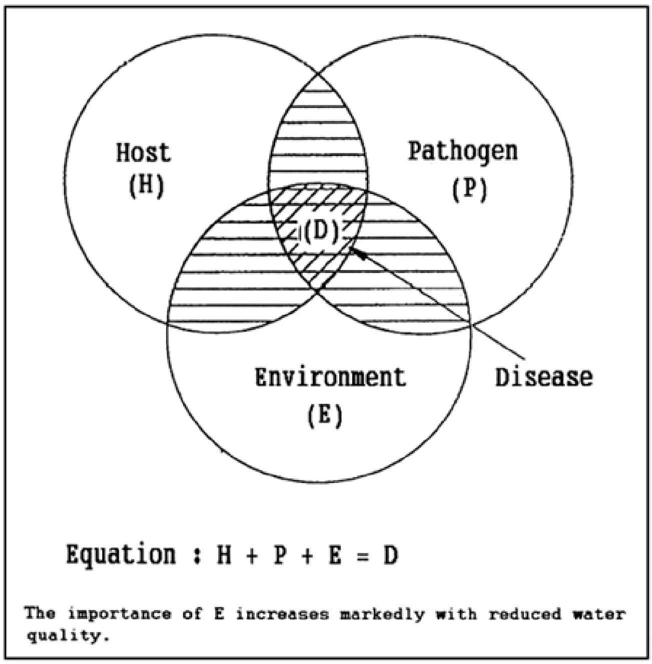

Figure 2.1: Interaction between host, pathogen, environment and the outbreak of diseases Source: Bohl (1989)

2.3.1 Types of fish diseases

2.3.1. (a) Microbial Diseases

The causative agents of diseases are divided into four categories; Viral, Bacteria, Fungi and Protozoa

2.3.1. (a). 1 Viral

Viral diseases in fishes are very serious due to the fact that the diagnosis is difficult and in a significant number of cases they are acute or sub-acute (SANO, 1995). Viral Haemorrhagic Septicaemia Virus (VHSV) and Infectious Haematopoietic Necrosis Virus (IHNV) are caused by viruses of the genus *Novirhabdovirus*, family Rhabdoviridae (Brudesth *et al.*, 2002). According to William *et al.,* (1999), this virus genus can infect Salmonid fish of all ages and cause 80% to 100% mortality. Disease outbreaks are found mainly in fingerlings less than 6 months of age. Infectious pancreatic necrosis virus (IPNV) belongs to family

Birnaviridae, and genus *Aquabirnavirus* (Roberts, 2001). The IPNV and IHNV are both important disease agents and can lead to disease outbreaks with huge losses in fish farming worldwide among wild and cultured salmonid fish (La Patra *et al.*, 2001). Spring Viremia of carp virus (SVCV) belongs to genus *Vesiculovirus*, family Rhabdoviridae which causes severe disease in wild and cultured in the common carp (*Cyprinus carpio*) with very high mortality (Oreshkova *et al*., 1999).

2.3.1. (a). 2 Bacteria

Bacterial pathogen diseases are responsible for heavy mortalities in both cultured and wild shell fish species all over the world (Jakhar *et al*., 2010a). In addition, certain bacteria have been reported as pathogenic to fish and shell fish. Three gram positive cocci (*Micrococcus*, *Streptococcus* and *Staphylococcus*) and six gram negative rods (*Aeromonas, Proteus, Citrobacter*, *Pseudomonas, Flavobacterium* and *Chromobacterium*) genera of bacteria have been categories as potential pathogens to *Aristichthys nobilis* and *Ctenopharyngdon idella* fish fingerlings (Welker *et al.*, 2005; Verma *et al*., 2006). Dahiya and Sihag (2009) also stated that, three bacteria (viz *Vibrio anguillarum, Vibrio alginolyticus* and *Aeromonas hydrophila*) have been reported to cause pathogenicity in the Indian magur (*Clarias batrachus* L.). In addition, bacterial necrosis in freshwater prawn, *Macrobrachium rosenbergii* was caused by *Aeromonas hydrophila*, *Pseudomonas flourescens* and *Enterobacter aerogenes* (Jakhar *et al*., 2010b) while, *Micrococcus luteus, Micrococcus varians, Cellabiosococcus scuri*, *Streptococcus* group Q1 and *Staphylococcus aureus* were found to cause tail rot disease (Jakhar *et al.*, 2010c). Table 2.4 below is a list of all the known pathogenic bacteria species found in fresh water and marine water fish farms around the world.

Table 2.4: Occurrence of bacterial pathogens in Fresh water and marine water

Taxonomy	**Presence in fresh water**	**Presence in Marine water**
Acinetobacter calcoaceticus	+	+
Aeromonas hydrophila	+	
Agrobacterium spp.	+	
Alcaligenes denitrificans	+	
Alcaligenes Faecalis	+	
Alcaligenes Piechaudii	+	
Alteromonas haloplanktis		+
Arthrobacter spp.	+	
Asticaccaulis spp.		+
Bacillus cereus	+	+
Bordetella bronchiseptica	+	
Caulobacter spp.		+
Coryneforms	+	+
Cytophaga spp.	+	+
Cytophaga fermentans	+	+
Cytophaga hutchinsonii	+	
Cytophaga salmonicolor	+	
Enterobacter aerogenes	+	+
Erwinia herbicola	+	+
Erwinia stewartii	+	
Escherichia coli	+	+
Flavobacterium spp.	+	+
Flexibacter spp.	+	+
Hafnia alvei	+	
Hyphomicrobium vulgare		+
Hyphomonas polymorpha		+
Janthinobacterium lividum		+
Klebsiella spp.	+	
Listeria spp.	+	
Lucibacterium harveyi		+
Micrococcus spp.	+	+
Micrococcus roseus	+	
Moraxella spp.	+	
Photobacterium angustum		+
Prosthecomicrobium spp.		+
Pseudomonas fluorescens	+	+
Serratia spp.	+	+
Serratia liquefaciens		+
Serratia marinorubra		+
Staphylococcus spp.	+	+
Vibrio alginolyticus		+
Vibrio parahaemolyticus		+
Yersinia spp.	+	

Source: Austin and Austin (1987)

2.3.1. (a). 3 Fungi

Fungi, which cause fungal disease, are present in both marine and freshwater fishes. Fungi are commonly known as 'Fish-molds' which attack eggs, fry, fingerlings and adult fishes and usually in the initial stage of infection, the fungal infections starts when the host has been injured. Fungal diseases are easily recognized by relatively superficial, colony of fluffy growth on the skin and gill of fishes. Various workers have reported the mycotic infection in fishes which occurred in India (Srivastava and Srivastava, 1978). Myazaki and Egusa (1973) observed an invasive component in histological sections of ulcerative disease affected as mycotic granulomatosis. *Achyla, Saprolegnia* and *Aphanomyces* were commonly identified from the lesion surface of affected fish (Roberts *et al*., 1993). Below in Table 2.5 is the list of common fungi species that can cause infection in fish:

Table 2.5: Occurrence of fungi on fishes

Fungus	***Scientific name of Fishes***
Achlya sp.	*Labeo calbasu*
	Channa gachua
	Notopterus chitala
	Puntius conchonius
	Labeo bata
	Puntius ticto
	Labeo calbasu
Dictyuchys sterile	*Anabas testudineus*
	Channa punctatus
	Puntius conchonius
Saprolegnia sp.	*Colisa fasciatus*
	Channa punctatus
	Cirrhinus mrigala
	Labeo calbasu
	Channa punctatus
	Channa striata
	Anabas testudineus

Source: Srivastava and Srivastava (1978)

2.3.1. (a). 4 Protozoa

Fishes are hosts for many protozoan parasites. In fish farm, parasitic protozoa may lead to epidemics and mortalities resulting in economic losses (Khalil and Polling, 1997). A study by Neave (1906) reported the presence of *Trypanosomes* in the blood of *Synodontis shall, Bagarus bajad* and *Mugil* species. Studies done by Al Wasila (1976) reported the presence of parasites in Sudan which infect *Oreochromis niloticus* fish. *Trypanosoma alhusaini* and *Myxobolus* sp. are the protozoa that live in freshwater fishes such as *Polypterues sengalus*, *Clarias lazera*, *Synodontis shall* and *Serrtatus* (Idris, 1986). Below in Table 2.6 shows a list of parasitic protozoa species which can infect the blood and internal organs of both *Oreochromis niloticus* and *Clarias gariepinus.*

Table 2.6: Protozoa detected in infected blood and organs of *Oreochromis niloticus* and *Clarias gariepinus*.

Fish species	Infected blood/organs	Detected protozoan
Oreochromis niloticus	Liver, kidneys and ovaries	*Myxobolus* sp.
	Liver	*Cryptobia* sp.
Clarias gariepienus	Blood and livers	*Trypanosoma* sp.
	Livers, kidneys and ovaries	*Myxobolus* sp.
	Blood	*Haemogregarine* sp.

Source: Adam *et al*. (2009)

2.4 Bath treatments for sick fish

2.4.1 Types of bath treatment

The aim of bath treatment is to eliminate the external infections which occur on skin, gills and fins of the fish that may be caused by bacterial, parasite or fungal diseases (Francis-Floyd, 1996). There are three basic of the bath treatments, which are listed below:

2.4.1. (a) Dip bath

The fishes are dipped into concentrated chemical or solution for a short time period which is often less than one minute. The prolonged exposure of fishes in the chemicals at a high concentration would make stressful condition and cause fatality to the fishes.

2.4.1. (b) Short bath

The fishes are subjected into moderate concentration of chemical or solution for time period between 30 minutes to several hours. The duration of exposure to the chemical is determined by the types of chemical used, the concentration used and the facility in which the fish are housed. This is an excellent method for administering many medications to fish which are placed in aquaria, tanks or raceways.

2.4.1. (c) Prolonged bath

Usually, this bath treatment is applied to pond fishes. The small concentration of chemicals used and left in the water without changing or flow through where the chemicals will eventually disappear or break down.