8.0 GENERAL DISCUSSION

Assessment of white spot syndrome virus in broodstock and post-larvae of *Penaeus monodon* and *Penaeus indicus* in India shows that, the prevalence of diseases due to WSSV, MBV and HPV is a problem for shrimp hatchery and farm operations in India. Prevalence of viral infections due to WSSV, MBV and HPV have been reported from various countries (Fegan *et al.*, 1991; Lightner *et al.*, 1992; Lo and Kou, 1998; Ramasamy *et al.*, 1995; Lu *et al.*, 1993; Magbanua *et al.*, 2000; Umesha *et al.*, 2003; Corsin *et al.*, 2003; Withyachumnarnkul *et al.*, 2003). Among the various diagnostic methods, PCR is considered to be the most suitable method for accurate and rapid detection of shrimp viral pathogens. The PCR assays for the detection of shrimp viruses have been reported by various researchers (Sukhumsirichart *et al.*, 1999; Pantoja and Lightner, 2000; Phromjai *et al.*, 2002). It has been reported that, WSSV infection cause heavy mortalities in shrimp and subsequent production losses in shrimp aquaculture (Chang *et al.*, 1996; Chou *et al.*, 1998). The prevalence of WSSV in post larvae (11.8%) reported in this study suggested, the possibility of vertical transmission of the virus. Uma *et al.*, (2002) reported that, the prevalence of WSSV observed in this study is lower when compared to our earlier observation (13%) during the period from March 2000 to May 2002 in the South East coast of
India. Comparatively, the prevalence (75%) of WSSV is reported to be maximum from the West coast of India (Otta et al., 2003).

MBV is considered to be a potentially serious pathogen in the larval stages of shrimp (Baticados et al., 1991; Natividad and Lightner, 1992). A mortality of up to 90% has been recorded in India in the postlarvae of *P. monodon* due to MBV infection (Ramasamy et al., 1995). The MBV prevalence in post larvae has been reported in India by various researchers (Ramasamy et al., 1995; Karunasagar et al., 1998; Otta et al., 2003). An MBV prevalence of 81% by wet mount method and 54% by PCR has been recorded in the hatcheries located in the South East coast (Ramasamy et al., 1995) and West coast of India respectively (Otta et al., 2003). The MBV prevalence in *Penaeus monodon* broodstocks was reported in many countries including India (Fegan et al., 1991; Lightner et al., 1992; Ramasamy et al., 2000). The MBV prevalence reported in this study by wet mount method and PCR were 6.7% and 13.1% respectively. The variations in the sampling method and the number of samples screened may contribute for the difference in the prevalence rate. Transmission of MBV may occur from broodstocks to larvae by faecal contamination of the spawned eggs (Chen et al., 1990).
Use of infected broodstock would be the major source of MBV infection in the post larvae. Moreover, avoiding faecal contamination of spawned eggs and larvae by thoroughly washing naupliii or eggs with formalin, iodophore and clean seawater would help to prevent MBV infection (Chen et al., 1990). The lower prevalence of MBV observed in this study might be due to such preventive measures was followed in the chosen hatcheries by the present study. Keeping the economic loss due to WSSV prevalence, the present study also made an attempt to findout the alternative method to control the WSSV infection through the possible application of natural products from plant sources. For that, the present study has chosen 4 medicinal plants of which one marine medicinal plant.

Of the 4 medicinal plants tested, Sphaeranthus indicus exhibited potent antiviral activity against WSSV and they have also been reported to possess antiviral activity. The extract of S. indicus showed inhibitory activity against mouse corona virus and herpes simplex virus at a concentration as low as 0.4 µg. ml⁻¹ (Vimalanathan et al., 2009) and also exhibited antiviral activity against vaccinia and ranikhet viruses (Dhar et al., 1968). It was already reported that, the M. charantia is most effective herb against fish and shrimp pathogenic bacteria (Direkbusarakom et al., 1998a). The strong activity of M. charantia against HSV-1 was reported by Beloin et al., (2005). The antiviral activity of M. charantia is one of the most potent among a number of West
African plants that have been screened (Anani et al., 2000; Hudson et al., 2000).

Many species of *Phyllanthus* have also been widely used as herbal medicines (Unander et al., 1990). It was reported that, *P. amarus* had only partial activity against WSSV and found very effective against the fish viruses such as INHV and OMV and shrimp virus YHV (Direkbusarakom, 2004). Notka et al., (2004) also found that, the water and alcohol extracts from *P. amarus* blocked HIV-1. Zuckerman (1987) has reported that, *P. amarus* was effective against DHBV infection of Pekin ducks. The aqueous extract of the *P. amarus* has the ability to inhibit the reaction between HbsAg and antiHbsAg (Venkateswaran et al., 1987). Yeh et al., (1993) reported that, the aqueous fraction of *P. amarus* selectively inhibited the expression of the HBV HbsAg gene in infected liver cells.

The methanol extract of *A. marmelos* showed little antiviral activity against WSSV. Although, Aiyannathan and Narayanasamy (1998) reported that it had strong antiviral activity against herpes simplex virus-1 (HSV). Medicinal plants are good sources of natural antimicrobial agents. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of
activity can thus only be proven by using large doses. On the other hand, if the active principle is present in high quantities, there could be other constituents exerting antagonistic effects of the bioactive compounds. It is not surprising that there are differences in the antimicrobial activities of plant groups, due to the phytochemical differences between species. The present study was conducted to develop newer lead for better and safer hemotherapeutic agents from medicinal plants.


The antiviral effect of sulfated polysaccharides extracted from marine algae against mammalian viruses *in vitro* is well known (Witvrouw and De Clercq, 1997). Both *Spirulina platensis* (Hayashi and Hayashi, 1995) and *S. maxima* (Hernández-Corona et al., 2002) have antiviral activity. Sulfated fucans (fucoidan) can be isolated from 43 species of brown algae. Fucoidans from brown algae are extremely complex and heterogenous in structure (Berteau and Mulloy, 2003). In a study, fucoidan extracted from marine algae
*Sargassum polycystum* was incorporated in a diet. The diet was fed to *P. monodon* juveniles for four days before and after challenge with WSSV. Shrimp receiving the highest amount of fucoidan in the diet (100, 200 and 400 mg.kg\(^{-1}\) body weight of shrimp) showed increased survival rate (Chotigeat et al., 2004).

Seed extract of *Pongamia pinnata* plant has antiviral activity against herpes simplex virus (Elanchezhiyan et al., 1993). An ethanolic extract bis (2-methylheptyl) phthalate from *P. pinnata* leaves was incorporated in a diet and tested for antiviral activity against WSSV infection in *P. monodon*. *P. monodon* were fed for 4 days before and 15 days after WSSV challenge (200 and 300 µg.g\(^{-1}\) body weight of shrimp. day\(^{-1}\)). Increased survival (40 to 80%) was found with the diet containing the highest concentration of the extract (Ramesthangam and Ramasamy, 2006). Increased survival of *P. monodon* was also observed after administration of a mixture of WSSV and extracts of *Aegle marmelos, Cynodon dactylon, Lantana camara, Mimosa charanita* and *Phyllanthus amarus* plants (Balasubramanian et al., 2007). The mechanisms of the antiviral activity of these plant extracts against WSSV are not known.

Petroleum ether, alcohol and water extracts (10, 30 and 100 mg.kg\(^{-1}\) p.o.) from the flowers of *S. indicus* were evaluated for anxiolytic activity, using elevated plus maze, open field test and foot-shock induced aggression.
test. Petroleum ether extract (10 mg.kg\(^{-1}\)), alcoholic extract (10 mg.kg\(^{-1}\)) and water extract (30 mg.kg\(^{-1}\)) of *S. indicus* flowers produced prominent anxiolytic activity in mice. The study showed an increase in the time spent, percent entries and total entries in the open arm of the elevated plus maze; increased ambulation, activity at center and total locomotion in the open field test and decreased fighting bouts in the foot-shock induced aggression test suggesting anxiolytic activity (Ambavade *et al.*, 2006). Another study also reported the anxiolytic activity of hydroalcoholic extract of whole herb of *S. indicus* (100 mg.kg\(^{-1}\), p.o.) in the elevated plus maze test and open field test (Gelani, 2010)

Neuroleptic activity of petroleum ether, alcohol and water extracts of flowers of *S. indicus* (30, 100 and 300 mg.kg\(^{-1}\), i.p.) were evaluated using apomorphine induced cage climbing and catalepsy in mice model. Only the petroleum ether extract (300 mg.kg\(^{-1}\), i.p.) reduced total time spent in apomorphine induced cage climbing. Aqueous (300 mg.kg\(^{-1}\), i.p.) and alcoholic (300 mg.kg\(^{-1}\), i.p.) extracts showed catalepsy while petroleum ether extract was devoid of it (Mietre, 2006). Neuroleptic activity of hydroalcoholic extract of whole plant of *S. indicus* has also been reported. Hydroalcoholic extract of whole herb of *S. indicus* (100, 200 and 500 mg.kg\(^{-1}\), p.o.) produced catalepsy, potentiated haloperidol-induced catalepsy and antagonized apomorphine-induced stereotypy. The sedative potential of hydroalcoholic
extract of whole herb of *S. indicus* (100, 200 and 500 mg.kg\(^{-1}\), p.o.) has been reported using experiments in which it reduced locomotor activity of mice, exploratory activity and potentiated pentobarbital induced sleep in mice (Patel, 2009).

The immunomodulatory activity of *S. indicus* was explored by evaluating its effect on antibody titre, delayed type hypersensitivity response, phagocytic function and cyclophosphamide-induced myelosuppression in mice. Administration of methanol extract and its fractions (100 and 200 mg.kg\(^{-1}\), p.o.) showed immunostimulating activity. Methanol extract, petroleum ether, chloroform and remaining methanol fractions of flower heads of *S. indicus* Linn. were found to be effective in increasing the phagocytic activity, haemagglutination antibody titre and delayed type hypersensitivity, whereas only remaining methanol fraction was found active in normalizing total WBC levels in the case of cyclophosphamide-induced myelosuppression in mice (Mishra, 2004). Eudesmanolide type of sesquiterpene from *S. indicus* was reported to have immunostimulating activity (Shekhani *et al*., 1990).

In an *in vitro* study, ethanolic extract of *S. indicus* (1000 g.ml\(^{-1}\)) showed maximum scavenging of the radical 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 1,1-diphenyl, 2-picryl hydrazyl (DPPH), superoxide and
nitric oxide radical. The extract also showed moderate scavenging activity of iron chelation (Shirwaikar et al., 2006) In an in vivo study, methanolic extract of S. indicus exhibited a significant antioxidant effect showing increasing levels of superoxide dismutase, catalase and glutathione peroxides by reducing malondialdehyde levels in rats (Khosa, 2009).

The S.indicus showed anti-inflammatory activity by suppressing the capacity of Propionibacterium acnes induced reactive oxygen species and pro-inflammatory cytokines, the two important inflammatory mediators in acne pathogenesis. To prove the anti-inflammatory effects of S. indicus, polymorphonuclear leukocytes and monocytes were treated with culture supernatant of P. acnes in the presence or absence of the herb S. indicus (5 and 50 µg.ml⁻¹). This caused a smaller, still significant, suppression of reactive oxygen species. The aqueous extract obtained from the root of S. indicus was found to be moderately active in down-regulating P. acnes induced TNF-α and IL-8 production (Basal, 2003). Another study has also reported its anti-inflammatory activity (Heinrich et al., 1998)

Petroleum ether, benzene, chloroform, ethanol and triple distilled water extracts of whole plant of S. indicus, obtained by successive solvent extraction, were screened for analgesic and antipyretic activity (200 and 400 mg.kg⁻¹, p.o.) using Eddy's hot plate, tail immersion and brewer's yeast
induced pyrexia methods, respectively. The petroleum ether, chloroform and ethanol extracts showed significant analgesic activity at both the doses from 1st hour onward as compared to the standard drug diclofenac sodium. The chloroform and ethanol extracts showed potential significant antipyretic activity from 1st hour onward, whereas aqueous extracts exhibited this activity from 2nd hours onward as compared to the standard drug paracetamol amongst various extracts (Nanda et al., 2009). Ethanol extract (150 and 300 mg.kg⁻¹) and ethyl acetate extract (100, 150 and 300 mg.kg⁻¹) of S. indicus showed better protective action of mast cell degranulation in sheep serum induced allergy test and compound 48/80 induced allergy (Mathew et al., 2009).

The 50% ethanolic extract of plant was reported to have hypoglycemic activity (Dhar et al., 1968) Antihyperglycemic effect of alcoholic extract of S. indicus was evaluated in the nicotinamide (120 mg.kg⁻¹, i.p.) and streptozotocin (60 mg.kg⁻¹, i.p.) induced diabetes in rats. Fasting plasma glucose levels, serum insulin levels, serum lipid profiles, magnesium levels, glycosylated hemoglobin, changes in body weight and liver glycogen levels were evaluated in normal and diabetic rats. Fasting normal rats treated with the alcoholic extract of S. indicus showed significant improvement in oral glucose tolerance test. Oral administration of S. indicus for 15 days resulted
in a significant decrease in blood glucose levels and increase in hepatic
glycogen and plasma insulin levels (Prabhu et al., 2008).

The hepatoprotective effect of aqueous and methanolic extracts of
flower heads of *S. indicus* on acetaminophen-induced heptotoxicity was
studied in rats. A significant decrease in liver function markers such as
Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate
Pyruvate Transaminase (SGPT), Acid Phosphatase (ACP) and Alkaline
Phosphatase (ALP), bilirubin and total protein was observed while using
methanolic extract of *S. indicus* (300 mg.kg\(^{-1}\), p.o.) in comparison with the
same dose of aqueous extract. This fact was also confirmed by studying the
liver histopathology of treated animals (Khosa, 2009). Moreover, the
methanolic extract of *S. indicus* enhanced the activities of antioxidant
enzymes such as superoxide dismutase, catalase and glutathione peroxidase
and diminished the amount of lipid peroxides against acetaminophen-
induced hepatotoxicity in these animals (Nayak et al., 2007).

Ethanolic extract of aerial part of *S. indicus* Linn. was evaluated for
wound healing activity in guinea pigs. The cream containing the extract was
applied *in vivo* on the paravertebral area of six excised wounded models
once a day for 15 days. The cream significantly enhanced the rate of wound
contraction and the period of epithelialization and this effect was comparable
to neomycin (Sadaf et al., 2006). Various ointments of ethanolic extract of flower head of *S. indicus* in various proportions were screened for the assessment of wound healing activity in albino rats. Based on the comparison made by the wound healing activity of various formulations, the formulation comprising 2% (w/w) alcoholic extract of flower head of *S. indicus* was found to be superior to that of control and standard formulation. Hydroxyproline content was also found greater in healed wounds as compared to control and standard formulation (Jha et al., 2009).

A bicyclic sesquiterpene lactone isolated from the petroleum ether extract of the aerial part of the *S. indicus* was reported to have antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Fusarium* sp., *Helminthosporium* sp. and other microorganisms. Antimicrobial activity of alkaloidal and non-alkaloidal fractions of alcoholic extract of flowers has also been reported (Shaikh et al., 1986).

Alcohol and water extracts of *S. indicus* were reported to have antibacterial activity against *Alternaria solani*, *Fusarium oxysporum* and *Penicillium pinophilum* (Dubay et al., 2000). Ethanol extract of *S. indicus* has antibacterial activity against enteropathogens (Anandhan, 1997). Aerial parts of *S. indicus* showed antibacterial activity against *Bacillus cereus var. mycoides*, *Bacillus pumilus*, *Bacillus subtilis*, *Bordetella bronchiseptica*, *Micrococcus luteus*, *...*
S. aureus, Staphylococcus epidermidis, Klebsiella pneumoniae and Streptococcus faecalis. Essential oil from the leaves of S. indicus has antibacterial activity against Salmonella paratyphi A, Salmonella paratyphi B, Salmonella paratyphi C, Schigella Flexneri, Salmonella Enteritidis, Salmonella typhimurium, Shigella sonnei and Vibrio cholera (Kasera, 1983). The fruits of S. indicus exhibited excellent antibacterial activity against gram positive as well as gram negative bacteria (Naqvi et al., 1998). Petroleum ether, acetone, methanol (90%) and water extracts of flowers were tested for antibacterial and antifungal activities by diffusion method in bacterial and fungal test cultures (Kasera, 1982). All the extracts showed considerable antibacterial and strong antifungal activities (Lalla et al., 2005). In another study, n-hexane, benzene, chloroform, ethylacetate and acetone extracts of aerial parts and flowers of S. indicus were tested for antibacterial and antifungal activities using in vitro disk diffusion method at concentrations of 5, 2.5 and 1.25 mg.disk⁻¹. The n-hexane extract of flowers showed significant activity against S. aureus and Candida albicans (Duraipandiyan et al., 2009). Methanol extract of S. indicus showed inhibitory activity against mouse corona virus and herpes simplex virus at a concentration as low as 0.4 µg. ml⁻¹ (Vimalanathan et al., 2009). The plant extract also exhibited antiviral activity against vaccinia and ranikhet viruses (Dhar et al., 1968).
Acetone extracts of root and leaves of the plant (at concentrations of 750 and 1000 ppm) were shown to cause more than 50% mortality in a predominant Indian mosquito species which acts as a vector of filarial worm. Larvicidal activity was found to be higher in root extract than leaves extract (Shah, 2003). Purified fraction of acetone extract of *S. indicus* showed mosquito larvicidal effect. Methanolic extract of *S. indicus* showed repellent and feeding deterrent activities against *Tribolium castaneum* at 1% concentration. Complete feeding deterrent activity was observed at 5 ml dose, whereas repellent activity was noticed at 4 ml dose (Saxena, 2003).

The methanolic extract of *S. indicus* (1–10 mg.ml\(^{-1}\)) was screened for *in vitro* macrofilaricidal activity by worm motility assay against adult *Setaria digitata*, the cattle filarial worm. It showed macrofilaricidal activity at concentrations below 4 mg.ml\(^{-1}\) and an incubation period of 100 minutes (Nisha *et al.*, 2007). It produced toxic effects on the second and fourth instar larvae of *Cuex quinquefasciatus* mosquito at 100-500 ppm concentration. The fourth instar larvae were more susceptible than the second instar larvae (Saxena, 1994). Methanolic extract of dried fruit of the plant is reported to have nematocidal activity (Ali *et al.*, 1991).
The methanolic extract of whole plant of *S. indicus* Linn. and its various fractions (87 and 174 mg.kg⁻¹, p.o.) were tested for their bronchodilatory effect against histamine-induced acute bronchospasm in guinea pigs. The methanolic extract and its fractions *viz.*, petroleum ether, benzene, chloroform and ethyl acetate exhibited significant protective action against bronchospasm induced by histamine in guinea pigs (Sarpate *et al.*, 2009).

Antihyperlipidemic activity of alcoholic extract of *S. indicus* Linn. flower heads in atherogenic diet induced hyperlipidemia was studied in rats. *S. indicus* extract (500 mg.kg.day⁻¹, p.o. for 8 days) caused a marked decrease in body weight, total cholesterol, triglyceride and low density lipoprotein and very low density lipoprotein. A significant increase in the level of high-density lipoprotein was observed after treatment with *S. indicus* extract (Dubey, 2009).

The ethanolic extract of *S. indicus* was evaluated for nephroprotective screening in gentamicin-induced acute renal injury in rats. Gentamicin-induced renal injury resulted in elevated biochemical markers namely, blood urea and serum creatinine followed by a decrease in total protein and serum albumin. The histopathologic feature was that of acute tubular necrosis. The ethanolic extract of *S. indicus* at a dose level 300 mg.kg⁻¹ was found to
normalize the above mentioned biochemical markers and bring about near to normal recovery in the kidneys as evidenced microscopically (Srinivasan et al., 2008).

Extract of *S. indicus* has been reported to inhibit hyaluronidase (Nanba et al., 2006). The alcoholic extract of flowers of *S. indicus* is reported to have hypotensive, peripheral vasodilatory and cathartic activities (Srivastav et al., 1971). The plant is also reported to have anticancer activity and antiprotozoal activity against *Entamoeba histolytica* (Dhar et al., 1968).