SUMMARY

With the increased incidence of bacterial diseases, particularly of vibriosis in cultured shrimp coupled with the growing awareness of the problem associated with the use of antibiotics, the present study was aimed to develop potent novel antimicrobials from the marine secondary metabolites (MSMs).

Considering this, the thesis was initiated with the following three major objectives:

1. Residual kinetics and leaching rate of antibiotics in the shrimp tissue and in the environment.
2. Development of potent biologically active novel secondary metabolites from marine organisms using chosen bioassay systems.
3. Isolation, experimental transmission studies of shrimp bacterial pathogens and their eco-friendly management using marine bioactive secondary metabolites.

The first chapter of the thesis describes the findings of residual kinetics and leaching rate of antibiotics in the shrimp tissue and in the environment. During the first two hours, the rate of leaching of antibiotics was higher. At the end of 2 h of post immersion, 51.56% of OTC and 37.57% CAP respectively leached from the sprayed medicated feeds.

The level of residual antibiotic in the first day of post-treatment was 3.37 µg/g OTC and 7.1 µg/g CAP respectively. A substantial quantity of residue was retained on the observation days of 5,10 and 15. The residue of OTC was reduced to 0.42 µg/g or below the detectable limit on the 20th day. However, in the case of CAP, no residue was observed in the 20th day of post-dosing. These findings suggest that the medicated shrimps should be harvested at least after 25 days of post-treatment with antibiotics.

The second chapter describes the findings of isolation and bioactivity screening of MSMs along with the collection details of MSMs source organisms. Seventeen species of seaweeds and seven species of sponges were collected in addition to a holothurian (*Holothuria scabra*) from the southeast and southwest coast of India. Eco-friendly collection of sponges as by-catch was made and the method was presented. After drying under shade,

the seaweed extracts were prepared using Soxhlet apparatus. The highly active *Ulva fasciata* extract was scaled-up in the bulk extraction process. The MSMs from sponges and holothurian were extracted under reduced pressure in a rotary vacuum evaporator using methanol as the solvent.

The extracts thus prepared were screened initially for antibacterial activity using the clown fish isolate. Based on the results of the primary antibacterial activity profile, 2 species of seaweed, 3 species of sponges and one holothurian were taken-up for further experiments. The antibiogram indicated *U. fasciata* and *D. nigra* as broad-spectrum antibacterials, which inhibited the growth of all the eleven species of bacteria tested. *U. fasciata* exhibited the highest activity against *Bacillus cereaus* to the extent of 132.66 mm^2 inhibition area at 20°C. However at 30° C, the activity range was reduced to 78.5 mm^2. The shrimp isolates were moderately sensitive to *U. fasciata* whereas the clown fish isolate (CF–1) was nearly sensitive. In the case of *D. nigra*, it effectively inhibited the growth of *Micrococcus luteus* to the extent of 132.66 mm^2 and 283.3 mm^2 at 30 and 20°C respectively. High activity was also recorded against one of the shrimp pathogens, *V. fischeri* to the extent of 104.62 mm^2 at 20 °C. Based on the above results of antibacterial profile, MSMs of *U. fasciata* and *D. nigra* were identified to evaluate the 'in captivity control' of shrimp bacterial diseases.

In the *Artemia* lethality experiments, the MSM of *Clathria gorgonoides* were highly lethal to *Artemia* nauplii followed by *Axinella donnani*. The medium lethal dose of *D. nigra* was 0.28% while for *U. fasciata* it was 2.0 mg/ml. The data obtained from the *Artemia* lethality assay was used to extrapolate the cytotoxicity profile of MSMs and discussed. The results of micro-algal lethality assay indicated that though *Hypnea musciformis* extract had induced the growth of *C. salina* at 1.0 and 2.0 mg/ml, it inhibited the growth of *I. galbana* and *Nanochloropsis* sp. at all the concentrations tested. The secondary metabolites of *U. fasciata*, sponges and *Holothuria* were inhibitory to the algal growth at various concentration ranges.

Experiments on the larvicidal profile of MSMs revealed that *H. scabra* was highly active followed by *C. gorgonoides*. Extracts of *U. fasciata*, *D. nigra*, *H. musciformis* and *A. donnani* also exhibited similar profiles of activity. The secondary metabolites of *A. donnani*,

D. nigra and *U. fasciata* were found to be potent anticoagulants in the decreasing order of activity.

A rapid 'mollusc foot adherence assay' was developed and perfected to study the antifouling properties of MSMs. Using this assay, it was detected that the extract of *H. scabra* successfully prevented the foot adherence (fouling) of *Patella vulgata* at 4.0 % level. In the ichthyotoxicity experiments, the extract of *H. scabra* was toxic to the youngones of *O. mossambicus*, in which the lethal time was 2 h and 40 seconds in 1.0 and 4.0 % concentration respectively. Extracts of *U. fasciata* was less toxic as it was lethal to 60% of fingerlings at the concentration of 4.0 mg/ml.

The median lethal dose of *U. fasciata* extract was 1020 mg/kg in fish (*O. mossambicus*) whereas it was 1380 mg/kg in the case of Swiss albino mice. The LD_{50} values indicated that it was a 'slightly toxic compound' as per the standard toxicological code. However, *U. fasciata* did not exert significant influence in the pentabarbitone-induced sleeping time in mice. Sleeping time (narcosis) index of pentabarbitone administered was used to extrapolate the interaction of *Ulva* on liver metabolism of pentabarbitone (xenobiotics). As the increase or decrease of sleeping time is dependent on the interactive factor (*Ulva*), which suppresses or facilitates the hepatic microsomal enzyme release (which catalyses the oxidation of pentabarbitone), it is concluded that the MSM of *Ulva* was a safe compound.

The third chapter of the thesis highlights the isolation, experimental transmission, characterisation and 'in captivity control' of shrimp pathogenic bacteria such as: *Vibrio alginolyticus* and *Vibrio harveyi* in the farm-reared shrimp *Penaeus monodon*. The secondary pathogenic bacterium *V. alginolyticus* was isolated from the farm-reared and White Spot Syndrome Virus (WSSV) infected shrimps. The opportunistic pathogenic bacterial isolate was obtained from 'shell disease' affected *P. monodon* from a shrimp farm. Based on the morphological, biochemical and physiological characteristics, the secondary pathogen was characterised as *Vibrio alginolyticus* while the opportunistic pathogen was characterised as *Vibrio harveyi*. The antibiogram revealed that both pathogenic shrimp bacteria were highly susceptible to chloramphenicol. The pathogenicity was confirmed by

experimental transmission studies in healthy *P. monodon* in the laboratory. The median lethal dose of *V. alginolyticus* was $5x10^6$ cfu/shrimp with an average body weight (abw) of 6.5 g whereas for *V. harveyi*, it was $6x10^6$ cfu/shrimp (abw of 8 g).

Prior to the 'in captivity control' experiments, the LD_{50} values of the chosen MSMs such as *Ulva* and *Dendrilla* were determined in shrimp. The LD_{50} of *Ulva* was 'moderately toxic' (1380 mg/kg) whereas *Dendrilla* was 'nearly toxic' (420 mg/kg). Efficacy of medication in chosen routes of administration (oral, parenteral and immersion) was carried out in the primary experiments. From the exploratory experiments, 1000 mg/kg of *Ulva* and 500 mg/kg *Dendrilla* were selected for further experiments. At these feeding rates, *Ulva* gave higher survival (80%) against the infection caused by the potentiated pathogen (*V. harveyi*, 5×10^3 + *V. alginolyticus*, $5 \times 10^3 = 10^7$ cfu/shrimp) when compared to *Dendrilla* that exhibited 75.0% survival of which 25.0% was infected. Both the treatments gave complete protection against the pathogenic shrimp bacterial isolates of *V. alginolyticus* and *V. harveyi*. The PRP range and profile of survival against the bacterial infection in the treated and control group were discussed.

The mechanism of action of MSM in the shrimp body was studied in a separate experimental set up. Enhanced total haemocyte count (THC), agglutination titre, bacterial clearance, bactericidal activity and phagocytic rates were observed in the *Ulva* treated shrimp. However *Dendrilla* exhibited lower or no response over the control group of shrimps. Based on the present findings, it could be inferred that *Ulva* medication @ 1000 mg/kg shrimp could be one of the potent novel shrimp therapeutants for sustainable shrimp farming.