



In vivo screening of mangrove plants for anti WSSV activity in *Penaeus monodon*, and evaluation of *Ceriops tagal* as a potential source of antiviral molecules

N.S. Sudheer^a, Rosamma Philip^b, I.S. Bright Singh^{a,*}

^a National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Cochin 682016, India

^b Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Cochin 682016, India

ARTICLE INFO

Article history:

Received 8 September 2010

Received in revised form 5 November 2010

Accepted 15 November 2010

Available online 23 November 2010

Keywords:

Mangrove

Ceriops tagal

White spot syndrome virus

Shrimps

Penaeus monodon

Antiviral

ABSTRACT

The objective of the study was to find out a natural way to fight white spot syndrome virus (WSSV) in cultured shrimps, as the present scenario necessitated an organic remedy for the devastating pathogen in crustaceans. Under this research programme seven mangrove plants were collected, identified and aqueous extracts screened for their protective effect on the giant tiger shrimp *Penaeus monodon* against WSSV. The experimental design consisted two modes of application, such as exposure of the virus to the extract and injection challenge, and oral administration of the extract coated feed followed by oral challenge. All experimental animals were monitored through a nested diagnostic PCR analysis. Of the seven mangrove extracts screened aqueous extract from *Ceriops tagal* imparted total protection to shrimp from WSSV when challenged by both methods. Shrimps administered with the aqueous extract from *C. tagal* were devoid of virions. The HPLC fingerprint of the aqueous extracts from *C. tagal* showed more than 25 peaks and 7 of them were larger and well separated. Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, polyphenolics, cardiac glycosides, saponins and sterols. The study indicated suitability of the aqueous extract of *C. tagal* as a possible prophylaxis for WSSV infection in shrimp. This is the first report on the anti WSSV property of the mangrove plant *C. tagal*.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

White spot syndrome virus (WSSV), an enveloped non occluded DNA (300 kb) virus of the family Nimaviridae under the new genus *Wispovirus* (Mayo, 2002), is the most devastating shrimp pathogen ever isolated and studied; it causes total mortality to a rearing stock within 3–7 days of infection in a culture system (Lightner, 1996). The virus has a wide host range and has been detected in diverse groups of crustaceans (Lo et al., 1996). Ironically till now no effective treatment or prophylactic measure could be developed to manage the virus. However, different approaches to manage the pathogen in culture systems have been experimented, such as oral administration of peptidoglycan, lipopolysaccharides, β -1,3 glucan (Itami et al., 1998; Takahashi et al., 2000; Chang et al., 2003), vaccination with inactivated viral preparation and viral envelop protein VP19 and VP 28 (Singh et al., 2005; Namikoshi et al., 2004; Witteveldt et al., 2004), feeding with fucoidan extracted from *Sargassum polycysticus*, (Chotigeat et al., 2004), and Cidofovir an anti viral drug supplemented with *Spirulina platensis* (Rahman et al., 2006). The protective effect of *Cynodon dactylon* (a terrestrial plant) in *Penaeus monodon* from WSSV has also been documented (Balasubramanian et al., 2007;2008).

For centuries, mangrove plants found in the tropics have been in focus as the source of bioactive molecules (Bandaranayake, 2002), having different dimensions of activities. They have been used in folklore medicine for treatment of several diseases (Kirtikar and Basu, 1935; Chopra et al., 1956; Datta and Datta, 1982). Extracts from different parts of the plant and their associates are being widely used worldwide for medicinal purposes (Bandaranayake, 1998). The most important reports available are the anti Tobacco Mosaic Virus activity from 16 species of mangrove plants (Padmakumar et al., 1993), anti Newcastle Disease Virus, anti Encephalomyocarditis Virus, anti Semliki Forest Virus, anti Human Immunodeficiency Virus, anti Vaccinia Virus, and anti Hepatitis B Virus activities as detected in the leaves of *Bruguiera cylindrica*, and the broad spectrum antiviral activity in the bark of *Rhizophora mucronata* (Premanathan et al., 1999a,b). However, there has never been any report on the anti WSSV activity in mangrove plants. This prompted us to take up the present investigation.

2. Methods

2.1. Collection and identification of mangrove plants

Mangrove plants such as *Excoecaria agallocha*, *Acanthus ilicifolius*, *Avicennia* sp., *Rhizophora mucronata*, *Rhizophora apiculata*, *Sonneratia* sp. and *Ceriops tagal* were collected from different localities in South India (9° 58' 1.20"N, 76° 15' 0.00"E). The plants were identified

* Corresponding author. Tel.: +91 484 2381120, +91 09447631101 (Mobile).
E-mail address: bsingh@md3.vsnl.net.in (I.S.B. Singh).

following Naskar and Mandal (1999), coded and voucher specimens deposited in the herbarium collection of the National Centre for Aquatic Animal Health, Cochin University of Science and Technology.

2.2. Aqueous extract from mangrove plants

Leaves were shade dried, powdered and used for the preparation of the aqueous extract using a protocol developed here. Accordingly, 50 g of mangrove plant leaf powder was soaked in a minimum quantity of double distilled water and frozen to -20°C , thawed and frozen repeatedly for three times and extracted to 500 ml final volume of double distilled water in a Warring blender at an ambient temperature. The extract was sieved through a fine meshed ($100\ \mu\text{m}$) cloth, centrifuged at 10,000 g for 20 min and the supernatant maintained at -20°C till used. These preparations were examined for their virucidal activity and for their protective effects from WSSV on oral administration in *P. monodon*.

2.3. Virus inoculum

A composite sample of gills and soft parts of cephalothorax (500 mg) from freshly infected *P. monodon* was macerated in 10 ml cold PBS (NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g, double distilled water 100 ml) with glass wool to a homogenous slurry using mortar and pestle in an ice bath. The slurry was centrifuged at 8200 g in a refrigerated centrifuge at 4°C and the supernatant filter sterilized using a $0.22\ \mu\text{m}$ pore size PVDF membrane filter. The preparation was streaked on ZoBell's agar plates and incubated at $28 \pm 2^{\circ}\text{C}$ for 72 h to confirm the absence of bacterial contamination. Viral infectivity titre was determined as the extractable virus and expressed as LD_{50} in shrimp following Reed and Muench (1938). The virus stock thus prepared for the experiment was stored in $500\ \mu\text{l}$ aliquots at -80°C . The viral titer determined was $1 \times 10^{13.69}/\text{ml}$ inoculum as experimented by Singh et al., 2005. Viability of WSSV in suspension was checked by injecting $10\ \mu\text{l}$ to a batch of apparently healthy shrimp (6 nos) and mortality confirmed over a period of 3 to 7 days.

2.4. Preparation of mangrove extract coated feed

The aqueous extracts were lyophilized and the dry mass re suspended in the required quantity of distilled water and coated onto feed pellets to arrive at a concentration of 1% w/w. As a binder, 4% aqueous gelatin in distilled water, was surface coated at a ratio 5:40 (v/w) to immobilize the plant extract. The above preparation was dried under vacuum and used for oral administration.

2.5. Experimental animals

All animals used in this study were single spawner bred, WSSV free juveniles of *P. monodon* grown in a recirculating aquaculture system at the National Centre for Aquatic Animal Health. The WSSV free status was confirmed through frequent Nested PCR analysis using a commercial kit (Bangalore Genei, Bangalore, India) of the shrimps during the culture operation in the recirculation system and also before the start of the experiment. The shrimps weighing 4–5 g were maintained in 30 liter capacity fiber reinforced plastic (FRP) tanks with diluted sea water at salinity 15. The experiment was repeated 4 times having 5 shrimps in a batch ($5 \times 4 = 20$). Uniformly 10% water was exchanged every day.

2.6. Virucidal activity of the aqueous extract

The aqueous plant extracts (0.5 ml) were mixed with equal volumes of viral suspension and incubated for 3 h at 25°C . The controls included mixtures of WSSV and PBS (positive control) and PBS alone (negative control). From each of the preparation, aliquots of

$10\ \mu\text{l}$ each were intramuscularly administered to the animals ($5 \times 4 = 20$ animals each) and monitored for 15 days. Gill tissue was extracted from moribund animals and the controls, which survived the 15 day period of experimentation during the course of the experiment. The samples were preserved in 70% ethanol for diagnostic PCR to detect WSSV.

2.7. Oral administration of the plant extracts along with diet and challenge with WSSV

All test animals ($5 \times 4 = 20$ animals each) were fed with the plant extract coated feed at a rate of 10% of the body weight two times a day. The control animals were fed with the diet coated with 4% gelatin. Feeding continued for 15 days, and the animals were challenged by feeding with freshly generated WSSV infected tissue at a rate of 10% of the body weight, and kept under observation for 15 days on the respective diet (normal diet for the positive and negative controls and the diet coated with the plant extract in the test group). Gill tissue was extracted from moribund/dead animals and from those which survived the challenge with WSSV, and was preserved in 70% ethanol for diagnostic PCR.

2.8. Confirmation of anti WSSV activity

To confirm the antiviral activity detected in the segregated plant species (*C. tagal*) intramuscular administration of virus suspension exposed to the plant extract, and oral administration of the plant extract and subsequent oral challenge were repeated in a batch of 24 animals and assayed by way of nested PCR. On completion of the experiment, tissue homogenates were prepared from the test and control animals and passaged to a fresh batch of nested PCR negative animals as bio assay to check the presence of virions in the survived animals. The presence of WSSV DNA was further examined by way of nested PCR.

2.9. Diagnostic PCR of the extracted tissue samples

For diagnostic PCR, DNA from the gill tissue was extracted in DNAzol according to the manufacturer's protocol. A WSSV nested PCR detection kit (Bangalore Geni) that yielded 650 and 300 bp WSSV specific amplicons was used for amplification of the viral DNA. Following the instructions given with the kit, the amplified product was generated in a thermal cycler (Eppendroff). The PCR products were then analyzed on 1% W/V agarose gels using TAE (1X) buffer (Tris-HCl 0.04 M, EDTA 0.0001 M, Glacial acetic acid 5.71%), stained with ethidium bromide and visualized on a gel documentation system, Dolphin-Doc (Weal Tec, USA).

2.10. HPLC analysis of the crude aqueous extract from *C. tagal*

On realizing the aqueous extract of *C. tagal* as the most effective preparation to protect shrimp from WSSV, it was subjected for HPLC analysis to generate HPLC fingerprint. The semi preparative HPLC system employed consisted of a Dionex Ultimate 3000 high performance liquid chromatograph coupled with a UV-Visible variable detector (VWD). Lyophilized crude aqueous extract from *C. tagal* was prepared in double distilled water (100 mg/10ML) and the separation was achieved on a $4.6 \times 250\ \text{mm}$ i.d Acclaim 120 Å C18 $5\ \mu\text{m}$ (Dionex) column at an ambient temperature. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B). A linear gradient from 0 to 60% (B) was used for 85 min followed by an increase from 60 to 90% (B) for 10 min finishing at an isocratic mode at 90% (B) for 10 min. The total run time was 105 min with a flow rate of 1 ml/min, and the injection volume adjusted to $20\ \mu\text{l}$ under a monitoring wavelength of 260 nm. Subsequently, the system was brought back to the initial conditions and equilibrated for 10 min with water. The process was repeated 3 times.

2.11. Preliminary phytochemical analysis of crude aqueous extract from *C. tagal*

Preliminary qualitative assessment of phytochemicals present in the crude extract of *C. tagal* was accomplished based on the tests described by Silva et al. (1998). The stock solution containing a 0.5 g lyophilized extract was used for the analysis where, the presence of alkaloids was determined by Dragendorff, Mayer and Wagner reagents, cardiac glycosides employing Baljet reagent and flavonoids using the Shinoda test and also by the formation of deep yellow coloration in the presence of sulfuric acid. The presence of polyphenols was determined from the formation of brown precipitate in the presence of 5% ferric chloride and sterols by employing Salkowski reaction. Formation of stable froth persistent for a duration of 15 min on warming was considered as the primary evidence for saponins.

2.12. Statistical analysis

The data generated on the survival of shrimp on administering the suspension of WSSV exposed to the plant extracts, and the data generated on the survival when challenged with WSSV subsequent to oral administration of the former were statistically analyzed employing the χ^2 test. An independent t-test was performed to the per cent survived shrimp under the above situation with each plant extract separately. Data presented is \pm SD of independent experiments.

3. Results

3.1. Virus challenge experiments

Under the first category of experiments, when shrimps were challenged with WSSV exposed to the extracts from *R. mucronata*, *Sonneratia* sp. and *C. tagal* significantly higher survival (95%, 100%, and 100% respectively) ($P < 0.001$) could be obtained. Meanwhile, with the aqueous extracts from *E. agallocha*, *A. ilicifolius*, *Avicennia* sp. and *R. apiculata* the survival was significantly lower (0%, 5%, 0% and 0% respectively) ($P < 0.001$). No instances of mortality were observed in the batches of shrimp which received WSSV exposed to the extracts of *Sonneratia* sp. and *C. tagal*, even after 15 days of post challenge. Meanwhile, the batches of shrimps injected with WSSV exposed to the aqueous extracts of *E. agallocha*, *Avicennia* sp., and *R. apiculata* did not survive alike the positive control. All animals kept as a negative control survived (Fig. 1).

Under the second category of experiments, all the shrimps which were fed on the lyophilized aqueous extract of *C. tagal* could survive (100%) when orally challenged with WSSV ($P < 0.001$). Meanwhile, comparatively lower survival was observed on feeding with the extracts from the other plants subsequent to WSSV challenge (75% with *E. agallocha* and *Avicennia* sp., 50% with *A. ilicifolius*, 40% with *Sonneratia* sp., 25% with *R. apiculata*, and no shrimp survived on feeding the extract of *R. mucronata*) ($P < 0.001$, Fig. 1).

On comparing the per cent survival of animals under the above two experimental conditions with the plant extracts (independent t-test), the one from *C. tagal* alone gave a uniformly higher rate of survival, the difference between the two was least significant ($P > 0.05$, Fig. 1). With all other plant extracts significant variations between the two modes of experiments could be observed ($P < 0.05$).

3.2. Diagnostic PCR of the experimental animals

The virucidal property of the mangrove plant extracts and the protective effects of the same were assessed by PCR of WSSV extracted from the gill tissue of the experimental animals. All animals which were administered with WSSV exposed to the extract of *C. tagal* survived the challenge, but were nested PCR positive. However, all shrimps found live after administration of the virus exposed to the

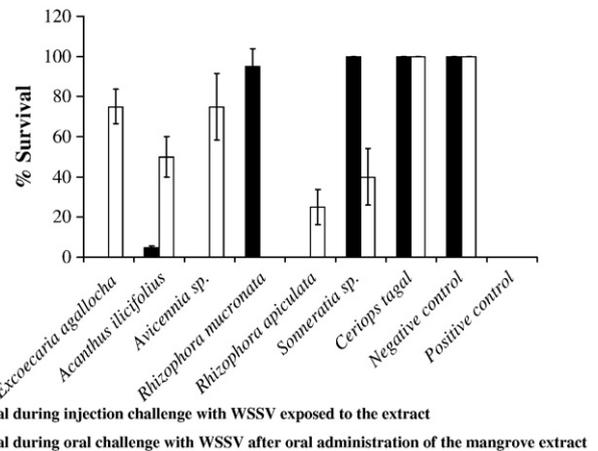


Fig. 1. Per cent survival of shrimp challenged with WSSV exposed to mangrove extracts, and challenged with WSSV after oral administration of the same.

extracts of *R. mucronata*, *Sonneratia* sp. and *A. ilicifolius* were PCR negative. Meanwhile, the animals which were found dead after the administration of WSSV exposed to the extract of *R. mucronata* were PCR negative to WSSV. In all other instances the moribund animals turned out to be PCR positive for WSSV (Fig. 2).

Meanwhile, all the animals which survived the oral challenge with WSSV subsequent to feeding with *C. tagal* were nested PCR negative. The ones which survived the WSSV challenge after receiving the diet coated with the extracts from *Sonneratia* sp., *Avicennia* sp., *E. agallocha*, and *A. ilicifolius* were also negative to WSSV, however, the dead ones altogether were PCR positive. However, the animals which received the extract from *R. apiculata* and challenged subsequently were second step PCR positive to WSSV (Fig. 3).

3.3. Confirmation of anti WSSV activity in aqueous extract of *C. tagal*

Subsequently, the antiviral activity of the aqueous extract of *C. tagal* was reexamined by repeating both the experiments in a batch of 24 animals and on completion of the experiment after 15 days the animals were nested PCR negative, and when a tissue extract was passed onto a fresh batch of animals none of them showed any

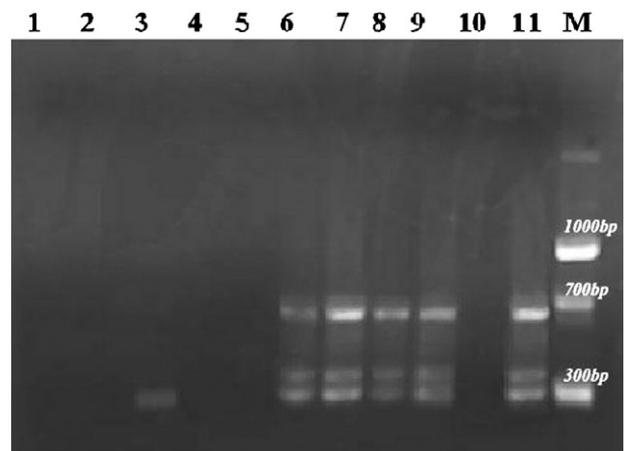


Fig. 2. Detection of the virucidal property of aqueous extracts of mangrove species. Each lane represents the PCR product of WSSV from shrimp challenged with WSSV exposed to the aqueous extracts. Lanes 1–4: Shrimp found live after challenge with WSSV exposed to *R. mucronata*, *Sonneratia* sp., *C. tagal* and *A. ilicifolius* respectively. Lanes 5–9: Shrimp found dead after challenge with WSSV exposed to the extracts of *R. mucronata*, *E. agallocha*, *A. ilicifolius*, *Avicennia* sp. and *R. apiculata*, respectively. Lane 10: Negative control. Lane 11: Positive control. M: Molecular weight marker.

clinical signs of WSSV infection and remained negative to nested PCR. The animals injected with the extract from positive control animals showed signs of WSSV and were PCR positive to the virus culminating in mortality (Table 1).

3.4. HPLC analysis and preliminary phytochemical investigation of crude aqueous extract from *C. tagal*

Repeated HPLC analysis of the crude aqueous extract generated uniformly a fingerprint of 25 peaks including 7 large well separated ones (Fig. 4). Preliminary phytochemical investigation of the crude extract from *C. tagal* revealed the presence of appreciable quantities of polyphenols, saponins, sterols and flavonoids, and lesser quantities of alkaloids and cardiac glycosides.

3.5. Efficacy of the extract

The aqueous extract (500 ml) prepared from 50 g dried leaf of *C. tagal* yielded on average 3–4 g dry matter on lyophilization which contained the active fractions. Based on a dye test conducted initially and the data on the survival of shrimp subsequent to challenge following administration of the extract along with diet, it could be realized that the binder, 4% aqueous gelatin, used in this study could effectively deliver the active fractions to shrimp.

4. Discussion

Extracts from mangrove plants and associates have been used world wide for medicinal purposes, and having been recorded around 349 metabolites it turns out to be a rich source of steroids, diterpenes and triterpenes, saponins, flavonoids, alkaloids and tannins (Bandaranayake, 2002; Zhang et al., 2005; Pakhathirathien et al., 2005; He et al., 2007; Wu et al., 2008). Pentacyclic triterpenes have been considered as the major bioactive group of compounds which could inhibit tumor cells and induce apoptosis and found useful for antiviral therapy especially for HIV (He et al., 2007).

In this context this is the first attempt to look into the possibilities of using mangrove plants as source of anti WSSV drugs. With this objective the most commonly found 7 mangrove plant species in Indian coastal zones were subjected for extraction for anti WSSV property and generated an aqueous preparation to be applied along with diet as prophylaxis. In this study aqueous extracts from *R. mucronata*, *Sonneratia* sp. and *C. tagal* were found to have virucidal property against WSSV. The same on administering along with diet

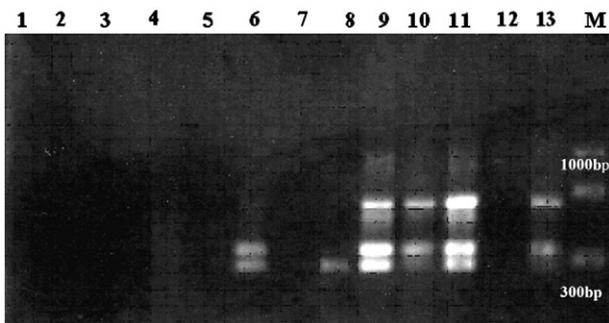


Fig. 3. PCR detection of WSSV in *P. monodon* challenged with WSSV after oral administration of the mangrove plant extracts. Each lane represents the PCR product of WSSV from shrimp. Lanes 1–6: Shrimp found live after challenge with WSSV subsequent to oral administration of the aqueous extracts of *C. tagal*, *Sonneratia* sp., *Avicennia* sp., *E. agallocha*, *A. ilicifolius* and *R. apiculata* respectively. Lanes 7–11: Shrimp found dead after challenge with WSSV subsequent to oral administration of the aqueous extracts of *E. agallocha*, *Avicennia* sp., *A. ilicifolius*, *R. apiculata* and *R. mucronata*. Lane 12: Negative control (animal fed on placebo and without WSSV challenge). Lane 13: Positive control (animal fed on placebo and WSSV challenge). M: Molecular weight marker.

Table 1

Confirmation of the protective effect of the aqueous extract from *Cerriops tagal* on *P. monodon* from WSSV.

SI No	Experimental groups	Survival on oral challenge with WSSV subsequent to oral administration of the plant extract	Survival on injection challenge with WSSV exposed to the plant extract	PCR Status	Clinical signs on passing tissue extracts from the experimental groups of animal 1, 2 and 3 to a fresh batch of apparently healthy shrimp
1	Test	24/24	24/24	– – –	
2	Positive control	0/24	0/24	+ + +	
3	Negative control	24/24	24/24	– – –	

and challenging subsequently with WSSV the preparation from *C. tagal* could accord total protection to shrimp. As the aqueous extract from *C. tagal* alone could give protection to all animals tested against WSSV under the dual experimental conditions, *C. tagal* was identified for further studies. When shrimps were fed on the aqueous extract coated feed and subsequently challenged with WSSV, the viral DNA could not be detected in the tissue suggesting that the virus had neither invaded the host tissue nor multiplied.

Several attempts were made earlier by several workers to detect anti WSSV property in plants. Citarasu et al. (2006) mixed together in equal proportion methanolic extracts of 5 medicinal plants such as *C. dactylon*, *Aeglemarmelos*, *Tinospora cordifolia*, *Picorhiza kurooa* and *Eclipta alba* and the combined extracts were supplemented through shrimp diet at different concentrations. In this experiment 74% survival of *P. monodon* was obtained on administering the extract at 800 mg/kg body weight. In a similar study the percentage survival of shrimp fed on the ethanolic leaf extract of the plant *Pongamia pinnata* was 40% on administering at 200 mg/kg of body weight and 80% on administering at 300 mg/kg of body weight per day (Rameshthangama and Ramasamy, 2007). Balasubramanian et al. (2008) on feeding 2% aqueous extract of *C. dactylon* coated feed to *P. monodon* could obtain 100% survival and the survived animals were PCR negative. There are reports of feeding *P. monodon* with diet containing extracts of herbs to improve the immune system and also to accord protection from WSSV (Citarasu et al., 2006). In our study, the quantity of the extract in the administered feed was nearly 1.0% of the total feed delivered at 500 mg/kg body weight per day.

The study could demonstrate the suitability of gelatin as a binder for delivering aqueous extract coated feed to shrimps. Since the extract is aqueous, chances of leaching out the active fractions into the surrounding water are rather high, and in this context this observation has practical implications, as one of the effective ways to deliver drugs to shrimp is through oral route.

The virucidal property of the aqueous extracts of *R. mucronata*, *Sonneratia* sp. and *C. tagal* when administered along with WSSV suspension at a 1:1 ratio after incubation for 3 h at room temperature suggested the presence of molecules in the preparation which could inactivate the virus. In a similar pattern pre incubation of WSSV with a synthetic antibacterial peptide from *Mytilus galloprovincialis* reduced mortality due to WSSV in Palaemonid shrimp *Pallamon* sp. (Dupuy et al., 2004). They suggested that this might be due to the contact of virus with Mytilin before injection onto shrimp. PCR analysis showed that the animals that survived were not accommodating the viral DNA. The virucidal property of the aqueous extract of *C. tagal* was demonstrated through the total survival obtained on a challenge with the virus suspension exposed to the extract and the non infectivity of the tissue extracts of the ones that survived. Moreover, on the oral administration of the extract and subsequent challenge with WSSV the shrimps altogether were found surviving.

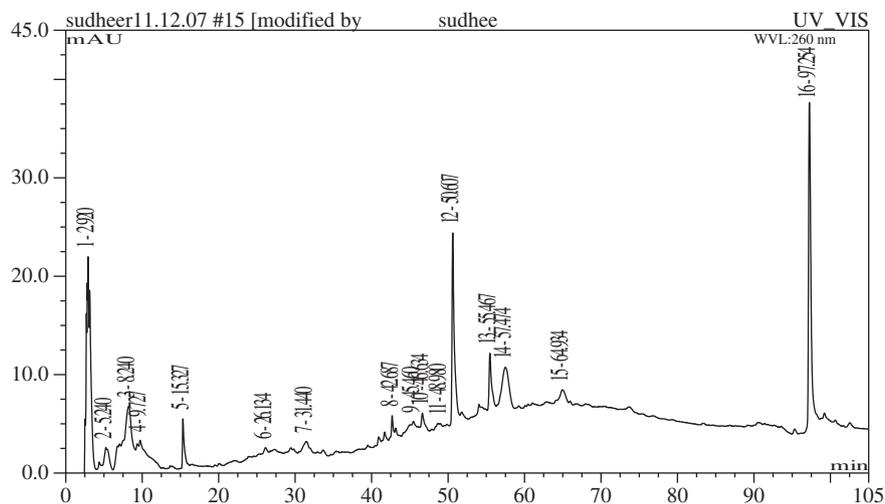


Fig. 4. HPLC chromatogram of the crude aqueous extract of *C. tagal*. Column: 4.6×250 mm i.d 5 μ m Acclaim 120 Å C18 (Dionex); Eluent: water (solvent A) and acetonitrile (solvent B). Linear gradient from 0 to 60% (B) for 85 min, 85%–90% (B) for 10 min, and isocratic at 90% (B) for 10 min; Flow rate 1 ml/min; Detection: 260 nm.

Diterpenes and triterpenes are the main natural products isolated from *C. tagal* (Zhang et al., 2005, Pakhathirathien et al., 2005, He et al., 2007). Pentacyclic triterpenes have been considered as a major bioactive group used as inhibitor of tumor cells, induction of apoptosis and also in antiviral therapy of AIDS (He et al., 2007). Premanathan et al. (1999b) on carrying out an extensive study of 17 mangrove plants belonging to 8 families against both DNA and RNA viral pathogens of human, could find that mangrove plants belonging to family Rhizophoraceae were the potential source of antiviral substances. An anti HIV sulfated polysaccharide has been isolated from *R. apiculata* (Premanathan et al., 1999a). *C. tagal* belongs to the family Rhizophoraceae.

The HPLC fingerprint generated from the aqueous crude extract of *C. tagal* was reproducible under the conditions described. It was a rich source of alkaloids, flavonoids, polyphenols, cardiac glycosides, saponins and sterols as observed here as well as described earlier by Bandaranayake (2002), Zhang et al. (2005), Pakhathirathien et al. (2005), He et al. (2007), and Wu et al. (2008) in mangrove plants. In general, all of them were reported to be antiviral (Jassim and Naji, 2003). Accordingly, polyphenols from Rhizophoraceae have been reported to have anti HIV and anti Hepatitis B virus activities (Premanathan et al., 1999a,b). Saponins (triterpenoid saponin oleanolic acid) inhibit HIV-1 replication by inhibiting HIV-1 protease (Mengoni et al., 2002). Sterols have a virus inhibitory property against tobamoviruses (Khan et al., 1991). Flavonoid chrysoferol C is one of the groups of compounds known to be a potent and specific inhibitor of picornaviruses and rhinoviruses (Semple et al., 1999). Total Alkaloids from the medicinal plant *Tripterygium* showed in vitro Anti Herpes Simplex Virus Type 1 activity (Ren et al., 2010). Cardiac glycosides have been demonstrated to have an inhibitory activity on the multiplication of herpes simplex virus (Dodson et al., 2007). Based on these evidences we conclude that the antiviral activity of the *C. tagal* aqueous extract might be due to one of the above compounds or due to their synergistic action. Further investigations are necessary in this direction.

Precisely, the study has brought to light the presence of the anti WSSV property in *C. tagal*, a mangrove plant found growing in several tropical countries. A protocol to extract water soluble bioactive metabolites from the mangrove plant *C. tagal* which could protect *P. monodon* from white spot syndrome virus could be developed. This preparation could effectively be delivered to shrimp through diet by coating with 4% aqueous gelatin as demonstrated earlier by Selvin and Lipton (2003) for delivering antibacterial and immunostimulatory substances to shrimp. The aqueous extract from *C. tagal* shall find

application as a prophylactic in the management of white spot syndrome in *P. monodon*.

Acknowledgements

This work was carried out with financial assistance from the Department of Biotechnology under the projects BT/PR3509/AAQ/03/173/2000 and BT/PR7419/AAQ/03/274/2006. The Department of Science and Technology, Lakshadweep, is acknowledged for the partial supply of mangrove plants and Mr. Krishna Iyer, Scientist, CIFT, Cochin (Rtd) for statistical analysis.

References

- Balasubramanian, G., Sarathi, M., Kumar, S.R., Hameed, A.S.S., 2007. Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. *Aquaculture* 263, 15–19.
- Balasubramanian, G., Sarathi, M., Venkatesan, C., Thomas, J., Hameed, A.S.S., 2008. Oral administration of antiviral plant extract of *Cynodon dactylon* on a large scale production against White spot syndrome virus (WSSV) in *Penaeus monodon*. *Aquaculture* 279, 2–5.
- Bandaranayake, W.M., 1998. Traditional and medicinal uses of mangroves. *Mangroves Salt Marshes* 2, 133–148.
- Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecol. Manage.* 10, 421–452.
- Chang, C.F., Su, M.S., Chen, H.Y., Liao, I.C., 2003. Dietary β -1, 3-glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. *Fish Shellfish Immunol.* 15, 297–310.
- Chopra, R.N., Nayar, S.L., Chopra, I.C., 1956. *Glossary of Indian Medicinal Plants*. Public and Information Directorate, CSIR, New Delhi, India, pp. 1–330.
- Chotigeat, W., Tongsupa, S., Supamataya, K., Phongdara, A., 2004. Effect of fucoïdan on disease resistance of black tiger shrimp. *Aquaculture* 233, 23–30.
- Citarasu, T., Sivaram, V., Immanuel, G., Rout, N., Murugan, V., 2006. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, *Penaeus monodon* with reference to haematological, biochemical and immunological changes. *Fish Shellfish Immunol.* 21, 372–384.
- Datta, S.K., Datta, P.C., 1982. *Pharmacognosy of Excoecaria agallocha* L. *Bark. Q. J. Crude Drug Res.* 19, 131–139.
- Dodson, A.W., Taylor, T.J., Knipe, D.M., Coen, D.M., 2007. Inhibitors of the sodium potassium ATPase that impair herpes simplex virus replication identified via a chemical screening approach. *Virology* 366, 340–348.
- Dupuy, J.W., Bonami, J.R., Roch, P., 2004. A synthetic antibacterial peptide from *Mytilus galloprovincialis* reduces mortality due to white spot syndrome virus in palaemonid shrimp. *J. Fish Dis.* 27, 57–64.
- He, L., Wang, Y.S., Wang, Q.J., 2007. In vitro antitumor activity of triterpenes from *Cerriops tagal*. *Nat. Prod. Res.* 21, 1228–1233.
- Itami, T., Asano, M., Tokushige, K., Kubono, K., Nakagawa, A., Takeno, N., Nishimura, H., Maeda, M., Kondo, M., Takahashi, Y., 1998. Enhancement of disease resistance of kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. *Aquaculture* 164, 277–288.
- Jassim, S.A.A., Naji, M.A., 2003. Novel antiviral agents: a medicinal plant perspective. *J. Appl. Microbiol.* 95, 412–427.

- Khan, A.M.M., Jain, D.C., Bhakuni, R.S., Zaim, M., Thakur, R.S., 1991. Occurrence of some antiviral sterols in *Artemisia annua*. *Plant Sci.* 75, 161–165.
- Kirtikar, K.R., Basu, B.D., 1935. *Indian Medicinal Plants*. Lalit Mohan Basu Publishers, Allhabad, India, p. 2793.
- Lightner, D.V., 1996. *A Hand Book of Shrimp Pathology and Diagnostic Procedures for Disease of Cultured Penaeid Shrimp*. World Aquaculture Society, Baton Rouge, Louisiana, USA, p. 304.
- Lo, C.F., Ho, C.H., Peng, S.E., Chen, C.H., Hsu, H.C., Chiu, Y.L., Chang, C.F., Kuan, F.L., Su, M.S., Wang, C.-H., Kou, G.-H., 1996. White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. *Dis. Aquat. Org.* 27, 215–225.
- Mayo, M.A., 2002. A summary of taxonomic changes recently approved by ICTV. *Arch. Virol.* 147, 1655–1656.
- Mengoni, F., Lichtner, M., Battinelli, L., Marzi, M., Mastroianni, C.M., Vullo, V., Mazzanti, G., 2002. In vitro anti-HIV activity of oleanolic acid on infected human mononuclear cells. *Planta Med.* 68, 111–114.
- Namikoshi, A., Wu, J.L., Yamashita, T., Nishizawa, T., Nishioka, T., Arimoto, M., Muroga, K., 2004. Vaccination trials with *Penaeus japonicus* to induce resistance to white spot syndrome virus. *Aquaculture* 229, 25–35.
- Naskar, K., Mandal, R., 1999. *Ecology and Bio Diversity of Indian Mangroves*, vol. 2. Daya Publishing House, Delhi, pp. 397–400.
- Padmakumar, K., Ramaswamy, S., Ayyakkannu, K., Nair, P., 1993. Analgesic activity of marine plants. In: Devadsan, K., Mukundan, M.K., Antony, P.D., Nair, P.G.V., Perigreen, P.A., and Joseph, J. (Eds.), *Nutrients and Bioactive Substances in Aquatic Organisms*. Cochin, India: Society of Fisheries Technologists (India), Cochin Publishers, pp. 25–30.
- Pakthathirathien, C., Karalai, C., Ponglimanot, C., Subhadhirasakul, S., Chantrapromma, K., 2005. Dammarane triterpenes from the hypocotyls and fruits of *Ceriops tagal*. *J. Nat. Prod.* 68, 1787–1789.
- Premanathan, M., Arakaki, R., Izumi, H., Kathiresan, K., Nakano, M., Yamamoto, N., Nakashima, H., 1999a. Antiviral properties of a mangrove plant, *Rhizophora apiculata* Blume, against human immunodeficiency virus. *Antiviral. Res.* 44, 113–122.
- Premanathan, M., Kathiresan, K., Nakahhima, H., 1999b. Mangrove halophytes: a source of antiviral substances. *S. Pac. Study* 19, 49–57.
- Rahman, M.M., Escobedo-Bonilla, C.M., Wille, M., Alday Sanz, V., Audoorn, L., Neyts, J., Pensaert, M.B., Sorgeloos, P., Nauwynck, H.J., 2006. Clinical effect of cidofovir and a diet supplemented with *Spirulina platensis* in white spot syndrome virus (WSSV) infected specific pathogen-free *Litopenaeus vannamei* juveniles. *Aquaculture* 255, 600–605.
- Rameshthangama, P., Ramasamy, P., 2007. Antiviral activity of bis (2-methylheptyl) phthalate isolated from *Pongamia pinnata* leaves against white spot syndrome virus of *Penaeus monodon* Fabricius. *Virus Res.* 126, 38–44.
- Reed, L.J., Muench, H., 1938. A simple method of estimating 50 per cent end point. *Am. J. Hyg.* 27, 493–497.
- Ren, Z., Zhang, C.H., Wang, L.J., Cui, Y.X., Qi, R.B., Yang, C.R., Zhang, Y.J., Wei, X.Y., Lu, D.X., Wang, Y.F., 2010. In vitro anti-viral activity of the total alkaloids from *Tripterygium hypoglaucum* against herpes simplex virus type 1. *Virol. Sin.* 25, 107–114.
- Selvin, J., Lipton, A.P., 2003. Shrimp disease management using bioactive marine secondary metabolites: an eco-friendly approach. *NAGA World Fish Cent. Q.* 26, 11–13.
- Semple, S.J., Nobbs, S.F., Pyke, S.M., Reynolds, G.D., Flower, R.L., 1999. Antiviral flavonoid from *Pterocaulon sphacelatum*, an Australian aboriginal medicine. *J. Ethnopharmacol.* 68, 283–288.
- Silva, G.L., Lee, I., Kinghorn, A.D., 1998. Special problems with the extraction of plants. In: Cannell, R.J.P. (Ed.), *Methods in Biotechnology: Natural Product Isolation*, vol. 4. Humana Press Inc, Totowa, N.J, pp. 343–363.
- Singh, I.S.B., Manjusha, M., Pai, S.S., Philip, R., 2005. *Fenneropenaeus indicus* is protected from white spot disease by oral administration of inactivated white spot syndrome virus. *Dis. Aquat. Org.* 66, 265–270.
- Takahashi, Y., Kondo, M., Itami, T., Honda, T., Inagawa, H., Nishizawa, T., Soma, G.I., Yokomizo, Y., 2000. Enhancement of disease resistance against penaeid acute viraemia and induction of virus-inactivating activity in haemolymph of kuruma shrimp, *Penaeus japonicus*, by oral administration of *Pantoea agglomerans* lipopolysaccharide (LPS). *Fish Shellfish Immunol.* 10, 555–558.
- Witteveldt, J., Vlask, J.M., Hulten, M.C.W.V., 2004. Protection of *Penaeus monodon* against white spot syndrome virus using a WSSV subunit vaccine. *Fish Shellfish Immunol.* 16, 571–579.
- Wu, J.Y., Xiao, Q., Xu, J., Li, M.Y., Pan, J.Y., Yang, M., 2008. Natural products from true mangrove flora: source, chemistry and bioactivities. *Nat. Prod. Rep.* 25, 955–981.
- Zhang, Y., Lu, Y., Mao, L., Proksch, P., Lin, W., 2005. Tagalsins I and J, two novel tetraterpenoids from the mangrove plant, *Ceriops tagal*. *Org. Lett.* 7, 3037–3040.