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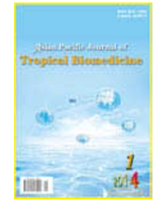

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Screening, isolation and optimization of anti–white spot syndrome virus drug derived from terrestrial plants

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PEER REVIEW

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Comments
This is a good study in which the authors have proved the efficacy of the terrestrial plants derived anti–WSSV drug. The formulation of the drug is inexpensive and at the same time cost effective for the use of the marginal farmers.
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ABSTRACT

Objective: To screen, isolate and optimize anti–white spot syndrome virus (WSSV) drug derived from various terrestrial plants and to evaluate the efficacy of the same in host–pathogen interaction model.

Methods: Thirty plants were subjected to Soxhlet extraction using water, ethanol, methanol and hexane as solvents. The 120 plant isolates thus obtained were screened for their *in vivo* anti–WSSV property in *Litopenaeus vannamei*. The best anti–WSSV plant isolate, TP22C was isolated and further analyzed. The drug was optimized at various concentrations. Viral and immune genes were analysed using reverse transcriptase PCR to confirm the potency of the drug.

Results: Seven plant isolates exhibited significant survivability in host. The drug TP22C thus formulated showed 86% survivability in host. The surviving shrimps were nested PCR negative at the end of the 15 d experimentation. The lowest concentration of TP22C required intramuscularly for virucidal property was 10 mg/mL. The oral dosage of 750 mg/kg body weight/day survived at the rate of 86%. Neither VP28 nor *ie 1* was expressed in the test samples at 42nd hour and 84th hour post viral infection.

Conclusions: The drug TP22C derived from *Momordica charantia* is a potent anti–white spot syndrome virus drug.

KEYWORDS
Shrimps, *Litopenaeus vannamei*, Anti–WSSV, Terrestrial plants, TP22C, White spot syndrome virus

1. Introduction

In the aquaculture industrial sectors, shrimp farming is not an exceptional case with a steady increase of cultivated shrimp production since the early 1980s[1–3]. Shrimp products are the largest single seafood commodity by value, which accounts for about 17% of the total internationally traded fishery products. The black tiger shrimp [*Penaeus monodon* (*P. monodon*)] and the white leg shrimp [*Litopenaeus vannamei* (*L. vannamei*)] are the two species which dominate production and account for approximately 75% of global shrimp aquaculture

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production[4]. However, shrimp farms are often infected by bacterial diseases such as, vibriosis caused by *Vibrio alginolyticus* and *Vibrio harveyi*, *etc.* and viral diseases like monodon baculovirus virus, white-spot syndrome virus (WSSV), and Taura syndrome virus[5-9]. Among these viral diseases, WSSV is the most pathogenic and the longest existing virus without any preventive or control measures[10]. This virus is considered to be an extremely virulent one, and may cause 100% mortality within 3-7 d as it initiates an infective necrotising condition in *P. monodon*, *Penaeus chinensis*, *L. vannamei* and *Fenneropenaeus indicus*[11], the most important shrimp species in the aquaculture sector. WSSV is a species of the genus *Whispovirus* and belongs to the new family Nimaviridae[12]. Previous researches have focused on the pathogenicity and development of diagnostic methods to detect the virus[13-16]. WSSV has been isolated and characterized from *Penaeus indicus* and *P. monodon* and found to be similar to that described earlier[13,15]. WSSV has been found to be highly pathogenic not only to penaeid shrimp, but also to a wide host range, which includes marine crabs and copepods, freshwater crabs and prawns[16-19].

Previous researchers have tried to eradicate aquaculture viruses through various approaches, such as by treating viral infection with common chemotherapeutants that have turned out to be ineffective and at the same time the development of virus-resistant species is time-consuming[20]. The inactivation of such viruses, including shrimp viruses, by physical and chemical agents have been reported by various authors, who have determined the effects of heat, ultraviolet light, pH, desiccation and chemical disinfectants on the inactivation of baculoviral mid-gut gland necrosis virus and WSSV[21-24]. Earlier experimentation has been done with natural adjuvants, synthetic agents, that were used as immunosuppressive and immunostimulative agents. Many herbals have been used for millennia for their potent anti-viral properties. Among them, a few have been found to have anti-viral activity against fish viruses in tissue culture and some have been investigated for their ability against shrimp viruses[25,26]. These natural plant products have been reported to have various activities like antistress, growth promoters, appetiser, tonic, non-toxic, biodegradable and biocompatible immunostimulants and antimicrobials[27,28]. Several plants of both terrestrial and marine origin have already been tested against viral diseases to judge its immunostimulant efficacy. *Cynodon dactylon* (*C. dactylon*) (terrestrial plant) and *Ceriops tagal* (*C. tagal*) (mangrove) exhibited protective effect against WSSV in *P. monodon*[29-31]. The seaweed, *Sargassum weighti*, showed significant anti-WSSV property against marine shrimp, *Penaeus indicus* and freshwater crab, *Paratelphusa hydrodomous*[20]. The extract of *Phyllanthus amarus* (*P. amarus*) and *Psidium gugajava* has shown antiviral activity against yellow head baculovirus in *P. monodon*[25]. The extract of *Clinacanthus nutans* has been tested against yellow head virus (YHV) in shrimp and the results indicated an effective control of YHV infection in shrimp[32]. Other control measures that have been undertaken against the WSSV virus in the culture systems are oral administration of peptidoglycan, lipopolysacharides, β-1,3 glucan, vaccination with inactivated viral preparation and viral envelop protein, VP19 and VP28, feeding with fucoidan extracted from *Sargassm polycysticus* and antiviral drug supplemented with *Spirulina platensis*[33-40]. *P. amarus* was very effective against the fish viruses such as INHV and OMV and shrimp virus YHV and its water and alcohol extracts blocked HIV-1[41,42]. The methanol extract of *Aegle marmelos* showed little antiviral activity against WSSV whereas it revealed strong antiviral activity against herpes simplex virus-1[29,43]. Glycoproteins derived from *Celosia cristata* similar to the antiviral proteins of *Bougainvillea spectabilis*, exhibited active antiviral property by inhibiting the mechanical transmission of two tobamoviruses, tobacco mosaic virus and sunnhemp rosette virus, and citrus ring spot virus into their hosts[44,45].

These previous literatures motivated us to work with the commonly available terrestrial plants, for the eradication of WSSV from the shrimp aquaculture industry and at the same time the protocol should be inexpensive for the marginal farmers. The leaves from each of these plants were studied for their anti-WSSV property in *L. vannamei*.

2. Materials and methods

2.1. *In vivo* anti-WSSV activity of terrestrial plants

2.1.1. Selection of terrestrial plants

The terrestrial plants selected for the present study were collected from different parts of West Bengal, namely Bongaon, Basirhat, Contai, Sundarban, Kolkata, Kharagpur and from some of the places from Tamil Nadu, namely Pichavaram, Parangipettai and Chidambaram. Thirty terrestrial plants such as, *Aloe barbadensis*, *Amaranthuus* sp., *Bougainvillea spectabilis*, *Carica papaya*, *Celosia cristata*, *Centella asiatica*, *Cinnamomum verum*, *Citrus aurantifolia*, *Citrus maxima*, *Cocos nucifera*, *Coriandrum sativum*, *Eclipta alba*, *Elettaria cardamomum*, *Ficus religiosa*, *Hibiscus rosa-sinensis*, *Impatiens balsamina*, *Justicia adhatoda*, *Lactuca sativa*, *Lantana camara*, *Manilkara zapota*, *Mentha spicata*, *Momordica charantia*, *Moringa oleifera*, *Murraya koenigii*, *Musa* sp., *Nicotiana tabacum*, *Piper betle*, *Polyalthia longifolia*, *Withania somnifera* and *Zingiber officinale* were selected for preliminary screening against WSSV based on the criteria such as, random collection of plants followed by mass screening; selection based on ethnomedical and ethnopharmacological uses in the management of diseases; follow-up of existing literature leads; chemotaxonomic approaches and easy availability of the plants for its evaluation[46,47]. All the plants were personally identified by Dr. Kumudranjan Naskar, National Fellow, ICAR, Govt. of India. The voucher specimens were preserved in the