

In vitro and in vivo Effects of 'Sonneratia alba' Extract against the Fish Pathogen 'Aphanomyces invadans'

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Abstract : The epizootic ulcerative syndrome (EUS) causes by the oomycete fungus, *Aphanomyces invadans*; known to be one of the infectious fish diseases for farmed and wild fishes in fresh and brackish-water from the Asia-pacific region, America and Africa. Although, EUS had been documented by the Office International des Epizooties (OIE) since 1995, hitherto, there is neither standard chemical agents that can be used for successful treatment of this destructive infection in the time of outbreak; nor available vaccine for prevention. Plant-based remedies in controlling fish diseases are gaining much attention recently as an alternative to chemical treatments, which possess negative effects to the environment and human. In present study, *Sonneratia alba*, a mangrove plant belongs to the Sonneratiaceae family, was screened in vitro and in vivo for its antifungal activity against *A. invadans* mycelium growth and its effects on fish innate immune system and disease resistant. The in vitro tests was performed using the disc diffusion methods with measurements of minimum inhibitory concentration (MIC) and inhibition zone. For in vivo study, the *S. alba* extract supplemented diets were administrated at 0.0, 1.0%, 3.0%, and 5.0% on healthy goldfish, *Carassius auratus*, which challenged with *A. invadans* zoospores (100 spores/ml). To compare the significant differences in the hematological and immunological parameters obtained from the experiments, the data were analysed using the SPSS. The methanol extract of *S. alba* effectively inhibited the mycelial growth of *A. invadans* at a minimum concentration of 1000 ppm for agar and filter paper diffusion experiments. In the agar diffusion test, 500 ppm of the extract inhibited the fungus mycelial growth up to 96 hours after exposure. The mycelial growth from the edge of the pre-inoculated *A. invadans* agar discs treated with *S. alba* extracts at concentrations of 100, 500 and 1000 ppm were 15, 8 and 0 mm respectively. The results of the filter paper disc test showed that the *S. alba* extract at its minimal inhibitory concentration (1000 ppm) has similar qualitative inhibitory effect as malachite green at 1 ppm and formalin at 250 ppm. According to the in vivo tests findings, in the infected fish fed with 3.0% and 5.0% supplementation diet, the numbers of white blood cell and myeloperoxidase activity significantly increased after the second week of treatment. Whilst the numbers of red blood cell significantly decreased in the infected fish fed with 0.0 and 1.0% supplementation diet. After the third week of feeding, significant increases in the total protein, albumin level, lysozyme activity were recorded in the infected fish fed with 3.0% and 5.0% supplementation diet. Also, the enriched diets increased the survival rate as compared to the untreated group that suffered from 90% mortality. The present study indicated that *S. alba* extract may inhibit the mycelial growth of *A. invadans* effectively, suggesting an alternative to other chemotherapeutic agents, which brought much environmental and health concerns to the public, for EUS treatment.

Keywords : fungal pathogen, goldfish, organic extract, treatment

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