

# Antiparasitic, Antibacterial, and Antifungal Activities Derived from a *Terminalia catappa* Solution against Some Tilapia (*Oreochromis niloticus*) Pathogens

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## Abstract

Tilapia, *Oreochromis niloticus*, is one of the most economically important fishery products of Thailand with export viability. Unfortunately, disease losses cause a major problem in the production of farmed tilapia. Most farmers have been using chemicals and antibiotics to treat fish pathogens which leads to the creation of antibiotic resistant pathogens and undesired residues in the fish and in the environment. Food safety is currently a great concern worldwide and Thailand's inspectors are now finding antibiotic residues in exported fish products. The purpose of the present research is to apply the Indian almond, *Terminalia catappa*, as an alternative to the use of chemicals and antibiotics in the aquaculture industry. Dried leaves of Indian almond were ground and dissolved in water. A variety of concentrations of this solution were used to determine resulting activities against tilapia pathogens. The results indicated that *Trichodina*, fish ectoparasites, were eradicated at 800 ppm. The growth of two strains of *Aeromonas hydrophila* was also inhibited at a concentration of 0.5 mg/ml Indian almond leaves upward. In addition, this solution can reduce the fungal infection in tilapia eggs. Research is underway to determine the toxicity of this solution, if any, on tilapia and the isolation of the active ingredients in the Indian almond for fish pathogen treatment.

## INTRODUCTION

Under intensive aquaculture conditions, fish parasites, bacteria, and fungi have been recorded as a cause of serious economic losses. There is an increasing concern about the toxic residues in agricultural foods though farmers still need to use chemical and antibiotic treatments for pathogen prevention. This continued use of chemical and antibiotic substances leads to a growing interest in screening antiparasitic, antibacterial, and antifungal substances from plants to replace chemical and antibiotic alternatives. Indian almond, *Terminalia catappa*, is commonly used as an herbal drug in Taiwan (Liu et al., 1996). It has been locally claimed to be a wound healing substance for Siamese fighting fish hurt after matches in Thailand as well. Burapadaja (1997) showed the crude ethanol extract from the leaves of Indian almond was active against *Staphylococcus aureus* with a minimum inhibitory concentration of 512 µg/ml. In addition, the crude extracts of *T. catappa* had in vitro antifungal properties against *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Aspergillus fumigatus* (Goun et al., 2003). However, no information about using Indian almond leaves to treat tilapia pathogens has been published. This paper is a report on the antifungal, antibacterial, and antiparasitic activities derived from Indian almond leaves for alternative treatment as opposed to chemicals and antibiotics.

## MATERIALS AND METHODS

Indian almond leaves, *Terminalia catappa*, were collected from a local area, baked at 50°C for 18 hours, and ground. The water extract of the leaves of Indian almond was passed through No. 1 Whatman filter paper and kept in 4°C until use.

One thousand and two hundreds tilapia eggs were randomly divided into four treatment groups with three replications each. The experiment was conducted in a cold season in order to make the eggs more susceptible to fungal infection. The antifungal activity was evaluated by treating tilapia eggs at concentrations of 0 and 200 ppm *Terminalia* extract compared with 200 ppm of garlic and formalin until the eggs were hatched. The percentage of fungal infection was daily determined by counting the number of dead eggs.

The experimental design was based on the concentration of *Terminalia* extract established to reduce the bacteria growth. The antibacterial activity was conducted in triplicate using tube-serial two-fold dilution MIC assay in which two strains of *Aeromonas hydrophila* was used as the indicator fish pathogen as described by Christofilogiannis (2001) with some modification. The MIC was recorded as the lowest concentration of extract in a given series where there was no bacterial growth.

Tilapia fingerlings (*Oreochromis niloticus*) with average weight of  $3.62 \pm 0.06$  g were obtained from a commercial farm in Chiang Mai, Thailand and acclimated in the laboratory for 2 weeks before experimentation. Fish were randomly divided into four groups of 30 each in triplicates. The parasiticidal effect was determined against natural infections with *Trichodina* sp. on the skin and gills of tilapia using a water extract of Indian almond varying from 0-800 ppm. The numbers of *Trichodina* sp. were then counted under microscope after 48 h exposure.

### Statistical Analysis

Data was reported as means  $\pm$  SD of three independent replications and evaluated by one-way ANOVA. Turkey multiple comparison test to determine significant differences between treatment was used. Significant differences were established at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Antifungal Activity on Tilapia Eggs

The treatment with 200 ppm of water extract derived from Indian almond showed the highest antifungal effect (Table 1). Referring to the result, the effectiveness of reference substances including garlic and formalin is quite low. One possible reason to explain this phenomenon is the variation of active ingredients in the raw material. It appeared to be the best potential alternative for fungal treatment to malachite green or acriflavin, both of which are carcinogens. As fungal infections were observed in all treatment groups, further study about the optimal doses for fungi-infection eradication is in progress. In addition, the toxicity of this substance and any side effects on tilapia need to be determined before applying the preparation to fish farms. In this experiment, eggs of the negative control group had an approximately 10% fungal infection. Schreier et al. (1996) demonstrated that with fungal infected rainbow trout eggs (10% infection), hydrogen peroxide of 500  $\mu$ l/l seemed to be the most effective treatment for controlling the spread of fungus and improving hatch percentage. This finding suggests that active substances from Indian almond need to be purified to avoid the variation of crude extracts and then it can be used in small amounts.

### In Vitro Antibacterial Activity on *Aeromonas Hydrophila*

The minimum inhibitory concentration (MIC) for Indian almond solution against *A. hydrophila* was 0.5 mg/ml while the MIC of Oxytetracycline (Fluka) was 0.125 mg/ml. These bacteria strains are considered as oxytetracycline-resistant since growth occurred at higher than 50  $\mu$ g/ml (Kelch and Lee, 1978). Further studies in vivo for this extract are needed. The terminalia extract may be added to the rearing water, and thus may help to control bacteria infection in fish eggs or larval fish in hatchery which are prone to bacteria infection. We assume that this extract may have an effect against other pathogenic bacteria; however, the research is underway to determine if that statement is true.

### **Antiparasitic Activity on *Trichodina* sp.**

Indian almond water extract treatment of 800 ppm was the most effective in treating *Trichodina* infection (Table 2). This result might indicate that Indian almond solution, properly prepared, can be used as an alternative treatment of trichodiniasis since formalin has been found insufficient. Previously, 50-120 ppm formalin was effective in controlling *Trichodina* sp. infection, but it now appears insufficient to control this infection (Madsen et al., 2000). Moreover, formaldehyde is suspected to have some carcinogenic risk to humans as indicated by U.S. Department of Health and Human Services (1994). The Indian almond residues in fish flesh and any deleterious effects must also be examined before its introduction to aquaculture industry. In addition, Madsen et al. (2000) suggested 200 ppm of raw and squeezed garlic as an alternative preparation to the use of the presently used formaldehyde treatment for trichodiniasis in eel.

### **CONCLUSIONS**

Evidence to date has suggested that the water extract of the leaves of Indian almond contains the positive effects for the treatment of fungi, bacteria, and fish ectoparasite. Therefore, this could be used as an alternative treatment in place of chemicals and antibiotics in treating fish pathogens. The immune response mechanisms of these activities and the advanced methods to purify active ingredients should be further studied. Additional toxicological and efficacy studies must be elucidated before commercial use is accepted because this herbal extract should be recognized as safe for both fish itself and consumers. Full-scale trials in farms subsequently will be conducted. These results might be applied for other aquatic organisms.

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## Tables

Table 1. The percent of infected tilapia eggs treated with formalin, garlic, and Indian almond. (These data are representative of two experiments with similar results.)

<b>Treatment</b>	<b>*Fungal infection (%)</b>
Control	9.89 ± 2.98 <sup>a</sup>
200 ppm formalin	4.10 ± 3.02 <sup>b</sup>
200 ppm garlic	5.80 ± 1.00 <sup>ab</sup>
200 ppm Indian almond	1.54 ± 0.53 <sup>b</sup>

\*Mean values with the same letter are not significantly different ( $P \leq 0.05$ ).

Table 2. Parasiticidal effect of Indian almond water extract on *Trichodina* sp. in tilapia.

<b>Indian almond water extract</b>	<b>*Number of <i>Trichodina</i> sp.</b>
0 ppm	211.11 ± 61.34 <sup>a</sup>
200 ppm	90.66 ± 73.23 <sup>b</sup>
400 ppm	20.66 ± 15.15 <sup>c</sup>
800 ppm	0 <sup>c</sup>

\*Mean values with the same letter are not significantly different ( $P \leq 0.05$ ).  
(Trials were conducted as 48 h water bath exposure of infected tilapia.)