Efficacy of traditional herbs of Bangladesh against fish pathogenic fungi

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ABSTRACT
Twenty-six species of traditional herbs of Bangladesh were selected to examine their anti-fungal activity against the common fish pathogenic fungi in aquaculture. Extracts of these herbs were applied under in vitro condition against individual fungal pathogen, viz., Achlya sp., Aphanomyces invadans and Saprolegnia sp. Initially a high dose was used to detect the antifungal effects of the herbs and categorized as high, medium and low effective herbs. Sixteen species of the herbs tested showed anti-fungal effect. Among these, the extracts of C. zedoaria (Bulb), C. longa (Bulb), C. zedoaria (Bulb) plus A. indica (Leaves) and C. longa (Bulb) plus A. indica (Leaves) were high effective against all of the fungi exposed. The minimal inhibitory dose (MID) of the high effective herbs was determined with respect to three fish pathogenic fungi tested.

Introduction
Fungal disease is one of the serious problems in grow-out fish farming and hatchery operations in Bangladesh. It can be fatal if it is not treated early. Different types of chemicals such as malachite green, copper sulfate and formalin are sometimes applied as treatments. In the recent years, there have been strong moves against malachite green application, especially with respect to use in food fish. This is because the chemical has a moiety that is known to be carcinogenic (Shariff et al., 1996). Meinertz et al. (1995) estimated that residues of malachite green could be found in fry after eggs were disinfected. Formalin can induce nausea if used for prolonged periods. The disadvantages of copper sulfate are that it is extremely toxic, particularly in water of low alkalinity (< 50mg/l) and may cause severe oxygen depletions (Francis-Floyd and Reed, 1997). So, the chemicals used in aquaculture sometimes might have negative effect on aquatic environment and fisheries products.

In the past, vegetable drugs were used in many parts of the world as folklore remedies, and claims have thus been made that certain plants have the power to heal wounds and remove inflammation. Disyaboot (1975) reported that out of 63 Thai medicinal plants, 62 species could inhibit the growth of bacteria. Direkbusarakom et al. (1998) tested 16 species of Thai traditional herbs of which 11 species showed antibacterial activity. Kraus (1995) found that extract of neem fruit, seeds, seed kernel, twigs, stem bark and
root have fungicidal and bactericidal properties. Since many species of herbs are used as human food/medicine such as, C. longa, C. zedoaria, A. sativum it is probable that their use would be safe for aquatic animals and for human consumers of aquatic animal products. Different parts of A. indica (neem tree) are used as medicine for various diseases (Dastur, 1977; Anawer, 2001). Different kinds of traditional medicinal herbs are available but research on application of herbs in aquaculture is not yet done in Bangladesh. Considering the importance of ecofriendly health management in aquaculture, it is of great interest to study this aspect step by step whether herbs might be used as an alternative treatment for fungal fish disease in Bangladesh. So, the objective of the study was to observe the efficacy of traditional herbs of Bangladesh against common fish pathogenic fungi.

**Materials and methods**

**Selection of fungi**

Fish pathogenic fungi Achlya sp. FAC-1, Aphanomyces invadans FAP-11 and Saprolegnia sp. FSA-39 from laboratory stock were selected for this study based on their previous pathogenic studies.

**Preparation of herbal extracts**

Twenty-six species of traditional herbs of Bangladesh were selected for the study based on the recognized medicinal properties described by Dastur, 1977 and Anawer, 2001. These were Acacia arabica, Allium cepa, Allium sativum, Andrographis paniculata, Azadirachta indica, Basella alba, Calotropis gigantea, Cephalaria indica, Curcuma zedoaria, Curcuma longa, Cuscuta reflexa, Cynodon dactylon, Datura metel, Enhydra fluence, Heliotropium indicum, Hydrocotyle asiatica, Mimosa pudica, Momordica charantia, Ocimum sanctum, Piper betle, Polygonum hydropiper, Psidium guajava, Sesbania grandiflora, Sesbania sesban, Tagetes erecta, and Tamarindus indica. Fresh leaves/bulb (a few cases infusion/decoction/bark infusion was used) were collected and immediately brought to Fish Disease Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh. These were washed with sterilized distilled water and paste prepared by stone made homogeniser. Further no water was added to maintain same concentration of the extract. Extract was collected from the paste by pressing with hand and stocked into the sterilized glass bottles. Collected extract was filtered through Whatman 541 filter paper and then centrifuged by BR 401 Bench Refrigerated Centrifuge. Finally extract was collected and preserved at 4°C for further studies.

**Antifungal tests of herbal extracts**

Fungal isolates were taken from the preserved bottles and transferred to fresh plates of GP-PenStrep (Glucose-Peptone Penicillin Streptomycin) agar until cultures become free from bacterial contamination. The fungi were subcultured aseptically on GP agar plates and incubated at 22°C for 18h. Then circular agar block of 3-4 mm in diameter containing fungal hyphae were cut by sterile Cork Borer and placed these into individual vial containing 10 ml GP broth (capacity 20 ml). Herbal extracts using different doses was then mixed with 10 ml GP broth containing one fungal block. All the vials were then incubated at 22°C. For the control, no herbal extract was mixed. Initially a high dose (100 µl/ml GP broth) was used to detect the antifungal effects of the herbs and categorized as high (fungi
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disappeared within 3 days), medium (fungi disappeared within 5 days) and low (fungi disappeared but were in suppressed condition) effective herbs. It may be mentioned that the extracts of leaves of the herb, A. indica was mixed with C. aromatica and C. longa at a ratio of 1:1. Pattern of fungal growth was observed daily up to 10 days experimental periods. The fungal growth was recorded by grading system based on the intensity of fungal growth to different doses and prevalence of growth inhibition was detected. After 10 days, treated fungal blocks were recultured on GP agar plate and observed the growth performance after incubation at 22°C for 24h to confirm either alive or dead. Thus the antifungal effects of herbal extracts were observed and minimal inhibitory dose (MID) was determined using only high effective herbs. Each test was replicated three times.

Results

Out of 26 herbs tested, 16 were found to be effective against Aphanomyces invadans, 15 against Saprolegnia sp. and 17 against Achlya sp. inhibiting their growth at a dose of 100 µl/ml GP broth but varied from species to species. Among these, the extracts from the bulb (B) of C. zedoaria, C. longa, C. zedoaria plus the extracts from the leaves (L) of A. indica and C. longa plus the extracts from the leaves of A. indica were highly effective and only the extract of leaves of H. indicum showed medium effect to inhibit the growth of the fungi exposed i.e., Achlya sp., A. invadans and Saprolegnia sp. The extracts from the leaves of H. asiatica and M. pudica showed medium effect on Achlya sp., low effect on A. invadans but no effect was observed on Saprolegnia sp. The leaves extracts of C. indica displayed medium effect on A. invadans, low effect on Achlya sp. and no effect was observed on Saprolegnia sp. Although the infusion of leaves of T. erecta had no effect on Achlya sp., showed medium effect on A. invadans and Saprolegnia sp. The extracts collected from the bulb of A. sativum showed low inhibitory effect on Achlya sp. and A. invadans, but medium effect on Saprolegnia sp. The extracts of leaves of A. indica showed low effect against all of the fungi tested, whereas the decoction (D) of leaves of this herb showed low effect except on Saprolegnia sp. The leaf extracts of C. gigantea, C. indica, C. longa, C. reflexa, C. dactylon, P. guajava and S. sesban were less effective against Achlya sp. In the case of A. invadans, the less effective herbs were C. reflexa (whole), P. hydropiper (L) and S. sesban (L) whereas P. hydropiper (L) and P. guajava (L) were low effective against Saprolegnia sp. On the other hand, the bark infusion of A. arabica, bulb extracts of A. cepa, leaf extracts of C. zedoaria, M. charantia, O. sanctum, P. betle, T. erecta, T. indicus, root (R) extracts of H. asiatica, decoction of P. hydropiper and bark infusion of S. grandiflora did not show any antifungal effect against all of the fungi tested, rather A. arabica (bark inf.), M. pudica (D), P. hydropiper (D), H. asiatica (L), A. indica (D), O. sanctum (L), P. betle (L) were most suitable for the growth of fungi and more growth was observed than that of control ones. The leaves extracts of D. metel and E. fluctuence displayed medium effect on Saprolegnia sp. though there was no effect on Achlya sp. and A. invadans.

Out of the 16 herbs which showed anti-fungal effect against A. invadans, high, medium and low effect were 4 (25%), 3(18.75%) and 9(56.25%), respectively (Fig. 1). In the case of Saprolegnia sp., high, medium and low effective herbs were 4 (26.67%), 8
(53.33%) and 3 (20%), respectively. Out of the 17 herbs which were effective against Achlya sp., high, medium and low effective were 4 (23.53%), 3 (17.65%) and 9 (56.25%), respectively.

Among the high effective herbs, the bulb extracts of C. zedoaria along with the leaf extracts of A. indica at a ratio of 1:1 showed the highest inhibitory effect on Saprolegnia sp. at minimum inhibitory dose (MID) of 1.56 µl/ml GP broth. The fungus, A. invadans was inhibited by the bulb extracts of C. zedoaria, C. longa and C. longa plus A. indica (L) at MID of 3.12 µl/ml GP broth. In contrast, these were found to be low effective on Achlya sp. In this case, the leaf extracts of A. indica combined with the bulb extracts of C. zedoaria and C. longa at a ratio of 1:1 showed effect at MID of 25 µl/ml whereas C. zedoaria and C. longa showed effect at MID of 50 µl/ml.

Prevalence of inhibition by extracts from the effective herbs tested is shown in Table 3. In the case of Saprolegnia sp., the bulb extracts of C. arometica combined with the leaf extracts of A. indica at a ratio of 1:1 was the most effective since it inhibited 30% of the treated fungi at MID of 1.56 µl/ml and 80 and 100% were inhibited at MID 3.12 and 6.25 µl/ml, respectively. In contrast, the extracts of C. longa combined with A. indica at a ratio of 1:1 was the second most effective which inhibited 80% fungus at MID of 3.12 µl/ml and 100% at MID of 6.25 µl/ml. The bulb extracts of C. zedoaria and C. longa inhibited 70 and 100% at MID of 3.12 and 6.25 µl/ml, respectively. On the other hand, A. invadans was be inhibited 60 and 100% by the bulb extracts of C. zedoaria plus A. indica (L) and C. longa plus A. indica (L) at MID of 3.12 and 6.25 µl/ml, respectively. Whereas Achlya sp. was inhibited 50 and 100% with same extract at MID 50 and 100 µl/ml, respectively. In case of control, rapid fungal growth was observed.

Discussion

The results provide useful information for the first time in Bangladesh regarding the efficacy of traditional herbs of Bangladesh against some common fish pathogenic fungi. Efficacy of herbs varied from species of the fungi exposed and also doses to dose of the herbs. Among the twenty-six species of traditional herbs C. zedoaria, C. longa, C. zedoaria plus A. indica and C. longa plus A. indica showed the highest inhibitory effect at MID of 100 µl/ml. Liping (1994) used the leaf extracts of P. hydropiper to prevent and treat bacterial and fungal diseases in Chinese fish farms. In the present study, the leaf extracts of this herb showed anti-fungal effect to inhibit Saprolegnia sp. and A. invadans, whereas the decoction of the leaves did not show any anti-fungal effect. A. sativum had low to medium effect on the fungi tested. Although the leaf extracts of H. indicum was effective against Saprolegnia sp. and A. invadans, the root extracts of H.
indicum did not show any anti-fungal activity. It suggests that antifungal activity of this herb does not reside in the root. Anon (1994) suggested that a paste prepared from ground neem (Azadirachta indica) leaves and turmeric was effective against EUS by inhibiting the spread of infection as well as influencing the growth and survival of the recovered fish. Neem extracts exhibit anti-fungal effect against plant pathogenic fungi, insects and pests (Immaraju et al., 1996; Singh and Pritiviraj 1997). In in vitro trial, Campbell et al. (2001) found that neem extracts as well as malachite green, ash and potassium permanganate had a strong anti-fungal property against A. invadans. In the present study, the decoction of neem leaves (A. indica) had no effect on Saprolegnia sp. but leaf extracts combined with C. zedoaria (B) and C. longa (B) was effective against all of the fungi.

The essential oil of holy basil (O. sanctum) has anti-fungal and antibacterial activities (Dey and Choudhuri, 1981). However, in this study, the extract of O. sanctum had no inhibitory effect on any of the fungi. M. charantia was the most effective herb against fish and shrimp pathogenic bacteria (Direkbusarakom et al., 1998). In Thailand, farmers used the bark of Cork wood (Sesbania grandiflora) for the treatment of haemorrhagic lesions and obtained satisfactory result (Direkbusarakom, 2000). But in this experiment, these two herbs could not inhibit any of the fungi tested. A. arabica (Bark inf.), A. cepa (B), P. betle (L) and T. erecta (L) also had no inhibitory effect, rather fungal growth was observed more than that of control ones. It proves that extract/infusion of these herbs was enriched with fungal nutrients. In the present study, the bulb extracts of C. zedoaria and C. longa individually and along with the leaves extracts of A. indica at a ratio of 1:1 were effective on Saprolegnia sp. and A. invadans and no significant difference was observed in effectiveness of these herbs. However, in the efficacy test, the bulb extracts of C. zedoaria combined with the leaf extracts of A. indica at a ratio 1:1 seemed to be the most promising herbs with minimum inhibitory dose (1.56 µl/ml) for the control of fish pathogenic Saprolegnia sp. Anyway, on the basis of the present information, further detailed studies are necessary under in vivo condition to observe the efficacy and effect of these herbs before applying at the field level.

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References


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