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Full Length Research Article

ANTIMICROBIAL PLANT EXTRACT ACTIVITY TEST AGAINST ISOLATED FUNGI FROM INFECTED FRESHWATER FISH *LABEO ROHITA*

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The present study was aimed to evaluate the growth inhibitory effect of *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* leaves extracts on isolated fungi *Aspergillus niger, Candida albicans, Cunnighamella bertholatiae* and *Penicillium* sp.,. Aquous plant extracts were tested against 4 Fungi. Gel diffusion method, were used in this investigation. The *Azadirachta indica* medicinal plant extract 12 mm (Mean value in Dia.) were showed enormous antimicrobial activity against *Aspergillus niger, Candida albicans* and *Cunnighamella bertholatiae. Ocimum sanctum* medicinal plant extract showed the maximum zone of inhibition 12 mm (Mean value in Dia.) against *Penicillium notatum.* All the *pathogenic fungi* were more resistance to *Ficus religiosa* medicinal plant extract. The *Azadirachta indica* and *Ocimum sanctum* medicinal plants extracts were showed very promising antifungal activity.

Key words:

INTRODUCTION

Indian aquaculture has demonstrated a six and half fold growth over the last two decades, with freshwater aquaculture contributing over 95 percent of the total aquaculture production. The production of carp in freshwater and shrimps in brackish water form the major areas of activity. The three Indian major carps, namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contribute the bulk of production with over 1.8 million tonnes (FAO, 2003); followed by silver carp, grass carp and common carp forming a second important group. Freshwater microbiological studies in the environments, mainly on the occurrence of species composition and abundance of viable bacteria have been helpful in realizing the importance of heterotrophic prokaryotes in freshwater biological process. In aquatic ecosystem the water column is rapidly colonized by a variety of microorganisms. In the heterogeneous and complex marine environment waste recycling is particularly important. As far as carp farming is concerned, the impact of microbial community on these systems is appreciable, whether it is beneficial or harmful. Being confined systems, carp farms provide a favourable environment to flourish the microbial heterotrophic activity. The fungal pathogens first attack the body surface of the carps and progressively grows deeper into the tissues. Histopathological studies indicate the growth of mycelia over epidermis, destroying the body muscles and gradually passing through dermis causing necrosis of muscle layers. The fungal pathogens are caused severe problems in carps and complete loss of fish production. The fungi are two types namely saprophytes and parasites.

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Saprophytes depend on dead on dead organic matter whereas parasites live on or in the living bodies of other organisms. In fungi reproduction is of three kinds vegetative, bisexual and sexual. However, of late the species has been observed to be fungal pathogens that have resulted in the recurrence of India from Andhra Pradesh, Tamil Nadu and Kerala by devasting the carp culture industry in India and inflicting losses amounting to approximately USS 10 million. Fungi are known to attack fish eggs, fry, fingerlings and adult fish. Water molds infections cause losses of freshwater fishes and their eggs in both natural and commercial fish farms (Bangyeekhun and Sylvie, 2001). The fungal diseases occur in brood stock and all life stages of fish and eggs. Fungal infection cause low productivity of fry and low production in fish culture (Kwanprasert *et al*., 2007). The mortality rate due to fungal infection may reach some time up to 80-100% in incubated eggs (Chukanhom and Hatai, 2004). According to post-harvest handling of fishes may also result in infection with microorganisms such as bacteria and fungi (Akande and Tobor, 1992). However Refai *et al*. (2010) has characterized *Aspergillus* spp*., Penicillium* spp., and *Rhizopus* spp., as normal mycoflora and these spp.

May be regarded as opportunistic pathogens (Refai *et al*., 2004) as many of them possess virulence factors which enable them to cause disease (Refai *et al.,* 2010), especially under favorable predisposing conditions. Ecological differences play an important role in species diversity of fungi that develop on both fish and eggs (Hussein *et al*., 2001). Interaction of physiochemical factors generally has influence on the diversity of water molds (Paliwal and Sati, 2009). After that numerous water mould species parasiting in different species of fishes and their eggs have been described by different researchers. The fungal pathogens first attacks the body surface of the fishes and progressively grow deeper into the tissues have been reported by Phyllis (2007).

METHODOLOGY

Plants were collected between the month of January and February 2010 in the Chidambaram area Tamil Nadu, India. Plant leaves were initially dried in an airconditioned, dehumidified room, then further dried in an oven at ca. 40°C for a total of seven days, and then finally ground to a fine powder. Antimicrobial activity test was determined by the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966). The Antimicrobial activity was tested against isolated 4 bacterial strains. The medicinal plants of *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* leaves extract were tested by the disc diffusion method. The extracts were prepared by reconstituting with aquous. The test microorganisms were seeded into respective Mueller - Hinton agar medium by spread plate method 10 µl (10 cells/ml) with the 72h cultures of Fungi growth in Mueller - Hinton agar broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates., *Aspergillus niger, Candida albicans, Cunnighamella bertholatiae* and *Penicillium* sp., were used for antibacterial test.

The antibacterial assay plates were incubated at 37°C for 27 h. After incubation, the results were observed and measured the diameter of inhibition zone (mm) around the each well.

RESULTS

In the present observation, antifungal activity of medicinal plant extracts such as *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* (Leaves) were tested against some pathogenic fungi such as *Aspergillus niger*, *Candida albican*s, *Cunninghamella bertholatiae* and *Penicillium notatum*. The different pathogenic fungi were quantitatively assessed for zone of inhibition (Table 1, Fig. 2 and Plate 1). The antimicrobial acitivity test with medicinal plant extracts such as *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* (Leaves) were studied against selected fungal pathogens. The selected fungal strains such as *Aspergillus niger*, *Cunninghamella bertholatiae, Candida albicans* and *Pencillium notatum.* The *Azadirachta indica* medicinal plant extract 12 mm (Mean value in Dia.) were showed enormous antimicrobial activity against *Aspergillus niger, Candida albicans* and

Table 1. Statistical analysis of antimicrobial activity test in medicinal plants extract against fungus (± S. D. of Mean).

Name of the Species	Zone of inhibition (dia in mm)			
	S1. *Hibiscus rosasinensis*	**S2. *Azadirachta indica***	**S3. *Ficus religiosa***	**S4. *Ocimum sanctum***
Aspergillus niger	8.67 ± 1.53	12.67 ± 0.58	10.00 ± 3.60	9.67 ± 2.52
Candida albicans	12.00 ± 2.00	12.33 ± 0.58	9.67 ± 3.51	11.33 ± 4.04
Cunnighamella bertholatiae	11.67 ± 1.52	12.67 ± 0.58	10.67 ± 4.51	9.33 ± 3.05
Penicillium notatum	10.67 ± 0.58	11.33 ± 2.08	11.67 ± 2.52	12.00 ± 3.60

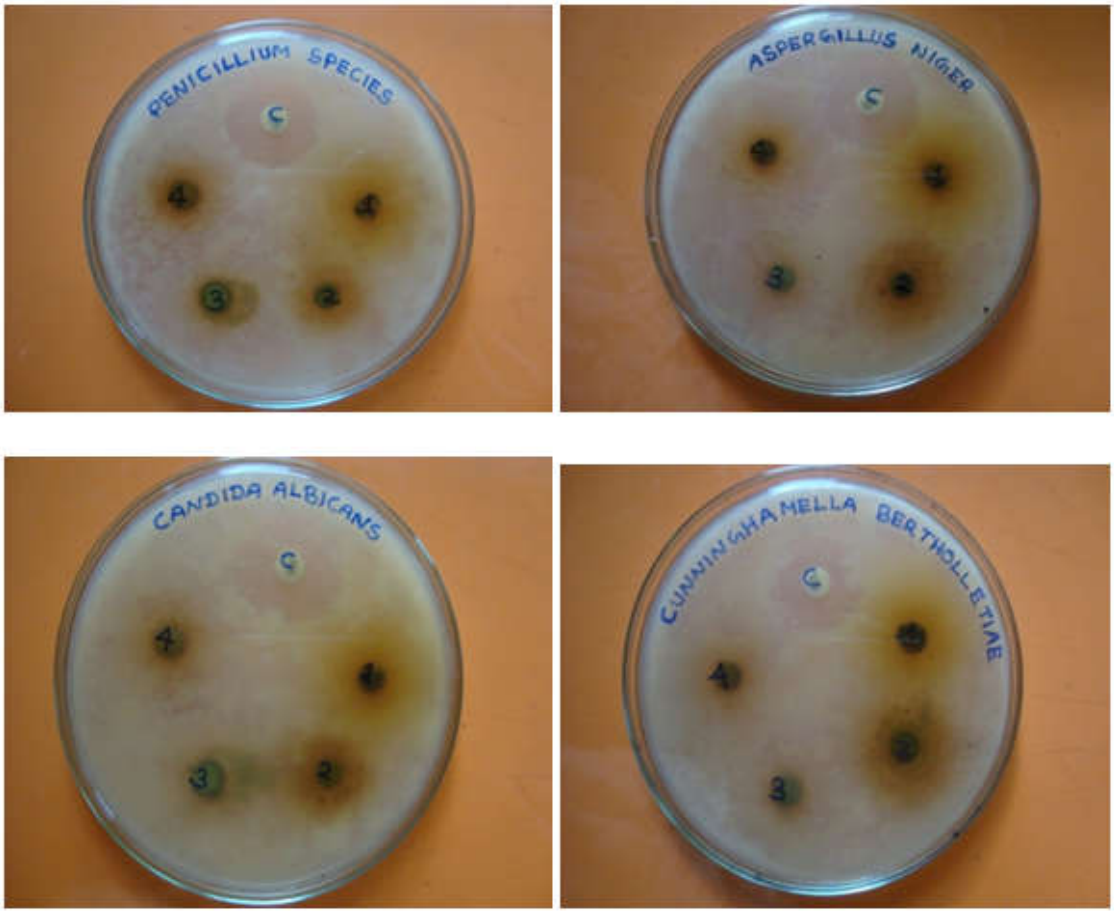
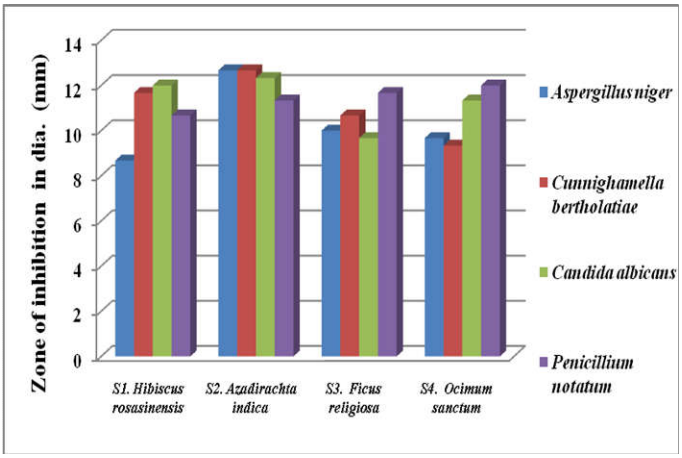


Fig. 1. Antimicrobial acitivity test against isolated fungi from infected carp *Lobeo rohita.*

Cunnighamella bertholatiae. *Ocimum sanctum* medicinal plant extract showed the maximum zone of inhibition 12 mm (Mean value in Dia.) against *Penicillium notatum*. Moderate amount of antifungal activity was observed on *Hibiscus rosasinensis* medicinal plant extract, the maximum zone of inhibition 8 mm



(Mean value in Dia.) against *Aspergillus niger*. At the same time *Hibiscus rosasinensis* plant extract was highly sensitive 12 mm (Mean value in Dia.) against *Candida albicans*. All the *pathogenic fungi* were more resistance to *Ficus religiosa* medicinal plant extract. The *Azadirachta indica* and *Ocimum sanctum* medicinal plants extracts were showed very promising antifungal activity.

DISCUSSION

Antifungal activity of some medicinal plants extracts such as *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* (Leaves) were tested against isolated fungal strains *Aspergillus niger*, *Candida albicans*, *Cunninghamella bertholatiae* and *Penicillium notatum*. The *Azadirachta indica* medicinal plant extract were showed enormous antimicrobial activity against *Aspergillus niger, Candida albicans* and *Cunnighamella bertholatiae,* when compared with other medicinal plant extracts. According to Nishant Rai *et al*., (2011) were studied the petroleum ether and methanolic extracts of *Azadirachta indica* exhibited high activity against *Candida albicans* (15-18mm). Lalit Mohan *et al*., (2011) were reported that the *Ocimum sanctum* also possesses antifungal activity against *Asperigillus niger* and aqueous extract of it was found to be effective in patients suffering from viral encephalitis.

In the treatment of ring worm infections, Tulsi leaves paste is indeed found to be very effective. Tulsi has significant natural antibacterial, antiviral and antifungal activities and is helpful in treating many serious systemic diseases, as well as localized infections. Many similar studies reported differences in antibacterial and antifungal activity of different medicinal plant extracts and the differences were rationalized as due to differend in morphological structure of the cell membranes (Mazutti *et al*., 2008 and Rang *et al*., 2001). Different plant extracts have been reported for their antifungal properties (Al-Fatimi *et al*., 2007; Afolayan *et al*., 2002; Bagamboula *et al*., 2004), which supports our present findings.

There is very little information available on the activity of medicinal plants aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity). The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. The present study similar workers reported (Chopra *et al*., 1992; Bruneton, 1995).

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