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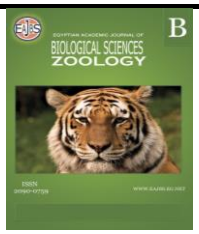

Antifungal Potential of *Parthenium hysterophorus, Achyranthes aspera* and *Catharanthus roseus* against *Aspergillus sp., Candida sp.* and *Penicillium sp.* of Aquarium Fish.

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ABSTRACT

Fungi affect the physiological conditions of animals as well as plants by decreasing their activities. Fishes are one of the victims of the fungal infections. In aquariums, ornamental fishes get infected with different species of the fungi due to several factors.

To avoid such infections to fishes, several commercial chemicals are available in the market. The current study is to replace these chemicals with plant extracts. The number of bioactive molecules present in plants makes them an affluent source of variants of medicines. In the present study, antifungal activity of medicinal plants *Parthenium hysterophorus, Achyrathes aspera,* and *Catharanthus roseus* was assessed against *Candida sp., Penicillium sp.,* and *Aspergillus sp.* isolated from infected aquarium fish. In the present study it was found that as compared with the zone of inhibition obtained in the standard tests, the maximum inhibition was exhibited by ethanolic extract of *Parthenium hysterophorus* and *Catharanthus roseus* against *Aspergillus sp,* chloroform extract of *Parthenium hysterophorus* and *Catharanthus roseus* and ethanolic extract of *Achyranthes aspera* against *Candida sp* while ethanolic extract of *Parthenium hysterophorus, Catharanthus roseus* and *Achyranthes aspera* against Penicillium *sp.*

INTRODUCTION

Aquarium fish are inclined to fungal infections which is a high hazard to the aquarium business. Furthermore, *Aspergillus sp., Candida sp.,* and *Penicillium sp.* become increasingly common in fish contaminations. *Aspergillus sp., Candida sp. and Penicillium sp.* genera are prominent for their creation of mycotoxins (Simon G. Edwards *et.al.*2002) which cause serious impacts on aquarium fishes. To intercept these contaminations traditional synthetics are being utilized like Lotrimin AF, Gyne-Lotrimin, Malachite, etc. These chemicals are recognized for perilous effects on the environment. The plants have manifested effective resistance against fungal infections with natural compounds present within them which may dispense prospective substitutes to the implementations of synthetic chemicals as fungicides.

An undertaking has been made to utilize plant extracts, which are viewed as sheltered with a natural viewpoint, as an antifungal specialist. Numerous plants have been contemplated as of late for their bug spray, bactericide, fungicide, and different impacts. The plants selected for this work are *Parthenium hysterophorus, Achyranthes aspera,* and *Catharanthus roseus*. *Parthenium hysterophorus* is an undesirable plant i.e. weeds in agribusiness. *Parthenium hysterophorus* is an annual herb that forcefully colonizes irritated locales. "It is highly extended with vivacious amplification propensity, fragrant, yearly (or a vaporous lasting), an erect, herbaceous plant with a profound taproot. These species replicate by seed. It develops to 30-90 cm in stature (Lorenzi,1982; Kissmann and Groth,1992), can be up to 1.5m or even 2.5m, in extraordinary circumstances (Haseler,1976; Navie *et.al.,*1996). The reasons for the plant being dangerous is 'Parthenin' and other phenolic acids, for example, Caffeic, Vanillic, Ansic, Panisic, Chlorogenic, and parahydroxy benozoic are deadly to people and creatures (Mahadevappa,1997; Oudhia,2000). describe *Parthenium hysterophorus L*. as a restorative plant since it utilized in numerous diseases. Indeed, even *Parthenium hysterophorus* has appeared antimicrobial movement (Terefe Tafese Bezuneh,2015). *Catharanthus roseus* is a consequential medicinal plant. This enduring plant develops as a herb or a subshrub, spreading along the ground or standing erect up to a meter I stature. It has charming bloom in white or pink shading with five petals while the weathered, dim green leaves are orchestrated in direct opposite sets. Phytochemicals have medicinal properties. Muhammad *et al.* (2009) revealed that *Catharanthus roseus* demonstrated the antibacterial potential in crude extracts of various plants (viz., leaves, bloom, root and stem) against clinically critical bacterial strains. *Catharanthus roseus* has known anticancer, antifungal, antibacterial, antidiabetic and antiviral exercises (Prajakta j. Patil and Jai S. Ghosh, 2010). *Achyranthes aspera* is an erect, yearly or perpetual herb, frequently with a woody base, which develops as no man's land herb all over the place. Since time immemorial, it is being used as people's medication. It holds a rumored position as a therapeutic herb in various frameworks of the drug in India.

Leaf-cutting edges elliptic, praise or extensively applaud to orbiculate, obovate-orbiculate, or comprehensively rhombate, adpressed-pubescent abaxially and adaxially. This plant has the notoriety of being a ground-breaking charm and is currently famously expected to go about as a shield against scorpions and snakes by incapacitating them. Acetone, ethanol, chloroform, methanol and aqueous extract of these plants were evaluated for its antifungal action against *Aspergillus sp., Candida sp*. and *Penicillium sp.* In comparison with methylene blue liquid which is well known as an antifungal solution in the local market. These extracts can additionally be examined for its dynamic part and be incorporated into the system to deal with the contagious contaminations. *Parthenium hysterophorus, Achyranthes aspera,* and *Catharanthus roseus* are the plants that are used amid the investigation. These are effortlessly accessible.

MATERIALS AND METHODS

Extract Collection and Preservation:

1.Crude Extract:

Fresh plant leaves of *Parthenium hysterophorus, Achyranthes aspera,* and *Catharanthus roseus*(Table 1 and Figs.2-4) were amassed from Manjari Budruk, Pune, Maharshtra 411028 (MS) (18.5042°N, 73.9539°E) and washed with distilled water. The samples were homogenized physically with mortar and piston. With the end goal

to acquire separate hoard 25ml of solvents (Ethanol, Mehanol, Chloroform, Acetone, and Dihydrogen monoxide) was coordinated with 15g crisp samples. At that point, the extracts were discretely separated and amassed from paste by using Whatman filter paper no.1. Amassed unrefined extracts were then safeguarded in the icebox at low temperatures for further tests.

2.Soxhlet Extracts:

Crude extracts were presented in soxhlet mechanical assembly flushed with separate solvents previously continuing to begin the procedure. Subsequent to setting up the powder shape from crude extracts by utilizing "Rotational Evaporator", 1 gm. of powder was presented in soxhlet device with 30ml of solvents. And obtained extracts were put away in the refrigerator at low temperature for further analysis.

3.Standard Solution:

Methylene blue liquid is being used in the experiment as a standard solution as this solution is well known as "Antifungal Liquid" in the local market (Fig,1).

Fig.1. Commercially available product in the market

Table 1.: Names of selected plants

Local Name	English Name	Scientific Name	Used part
Gajar Gavat (Fig.2)	Carrot grass	*Parthenium hysterophorus*	Leaves
Pandhri Sadaphuli (Fig.3)	Tiny periwinkle	*Catharanthus roseus*	Leaves
Aghada (Fig.4)	Prickly Chaff Flower	*Achyranthes aspera*	Leaves

Fig. 2 .: *Parthenium hysterophorus*

Fig.3.: *Catharanthus roseus*

Fig.4.: *Achyranthes aspera*

Test Organisms:

With the end goal to research the impacts of plants on contagious pathogens of fish fungus growth detached from ailing fish from an aquarium.

Isolation of Fungi:

The cotton plug was adjusted to aggregate the organisms from the skin of the contaminated fish. At that point, this cotton plug was acquainted with saline water for further preparation. After collection of organisms, these were acquainted with sterile petri dish a PDA media. The Petri dish was hatched until the event of organisms for around 24 hours at 25°C. After recognizable proof of growth; they were isolated to various Petri dishes. Sub cultivations on Petri dishes were done and used for further tests.

Antifungal Assay:

Antifungal activities of the plants were seen by using Disc Diffusion method (Kerby Bauer Technique) on a sterile petri dish of PDA media by presenting every fungus in independent Petri dishes. At that point, Petri dishes were incubated for 24 hours at 25˚C.

Investigation of Effects of Selected Plants:

Culture media and plant extracts were used in the disc diffusion technique discretely. Fungi cultures were spread on the surface of the potato dextrose agar plates and discs (Whatmann No.1 channel paper with 9mm breadth) impregnated with the 10 µl of *P.hysterophorus,A. aspera* and *C.roseus* leaf extracts. Plants extracts were put at first glance separately. The plates were incubated at 25˚C for 24 hours. The antifungal capability of the test samples was evaluated by deciding the width of the zones of inhibition in centimetres.

Determination of Minimum Inhibitory Concentration (MIC):

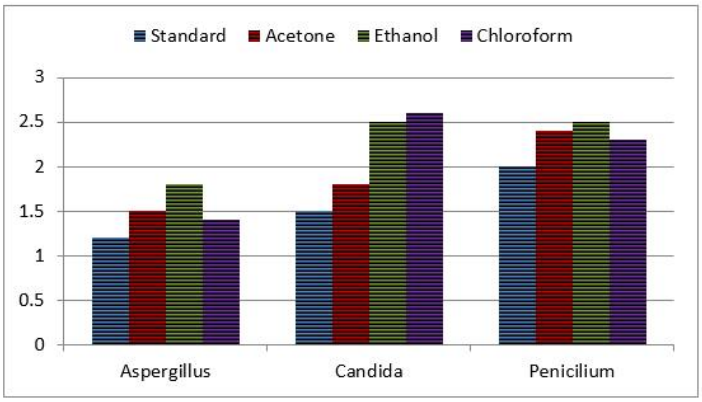
The MIC for each test organism was determined by following the modified agar dilution method. A sequential dilution of each extract was set up by first reconstituting the dried extracts (0.1 mg mL^{-1} obtained by dilution in sterile distilled water (1:1) to accomplish a diminishing fixation scope of 100 µg mL^{-1} to 10 µg mL^{-1}. A 100 µL volume of every dilution was brought in to test tubes (in triplicate) in the agar stock effectively seeded with 10 µL of institutionalized inoculum (106 CFU mL^{-1}) of the test microbial strain. All test tubes were incubated vigorously at 37˚C for 24 hours and watched for the inhibition zones. The MIC, taken as the last grouping of the test extract that totally inhibited the development of the microorganism, appeared by a reasonable zone of inhibition, was recorded for each test organism.

RESULTS

Extracts of *Parthenium hysterophorus* with cold acetone, ethanol, and chloroform demonstrated the famous antifungal activity against *Candida sp.* in for all intents and purposes double zone of inhibition than that of standard. Moreover, against *Aspergillus* and *Penicillium* sp. impact was great with a greater zone of inhibition than standard (Table 2 and Fig, 5).

Table 2.: Zone of inhibition (cm), *Parthenium hysterophorus*

Fungi	*Aspergillus*	*Candida*	*Penicilium*
Standard	1.2	1.5	2.0
Acetone	1.5	1.8	2.4
Ethanol	1.8	2.5	2.5
Chloroform	1.4	2.6	2.3



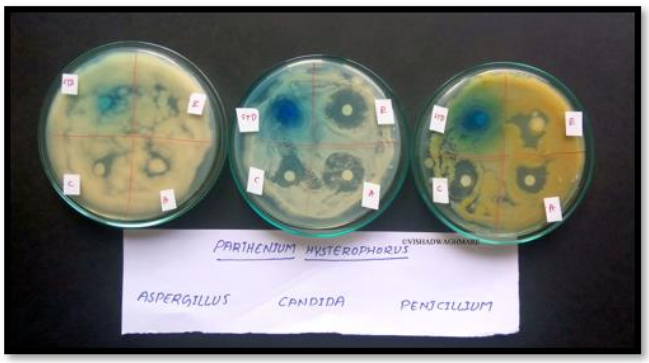


Fig.5: Zone of Inhibition exhibited by *Parthenium hysterophorus* against the selected fungi strains

Extracts of the *Catharanthus roseus* with cold ethanol showed a notable antifungal activity against *Candida sp.* as well as *Penicillium sp.* and with cold chloroform it showed activity against *Candida sp.* and *Penicillium sp.*in almost double zone of inhibition than that of standard. And against *Aspergillus sp.* it showed little activity (Table 3 and Fig 6).

Table 3: Zone of inhibition (cm) *Catharanthus roseus*

Fungi	***Aspergillus***	***Candida***	***Penicillium***
Standard	1.2	1.5	2.0
Ethanol	1.8	2.5	2.5
Chloroform	1.4	2.6	2.3

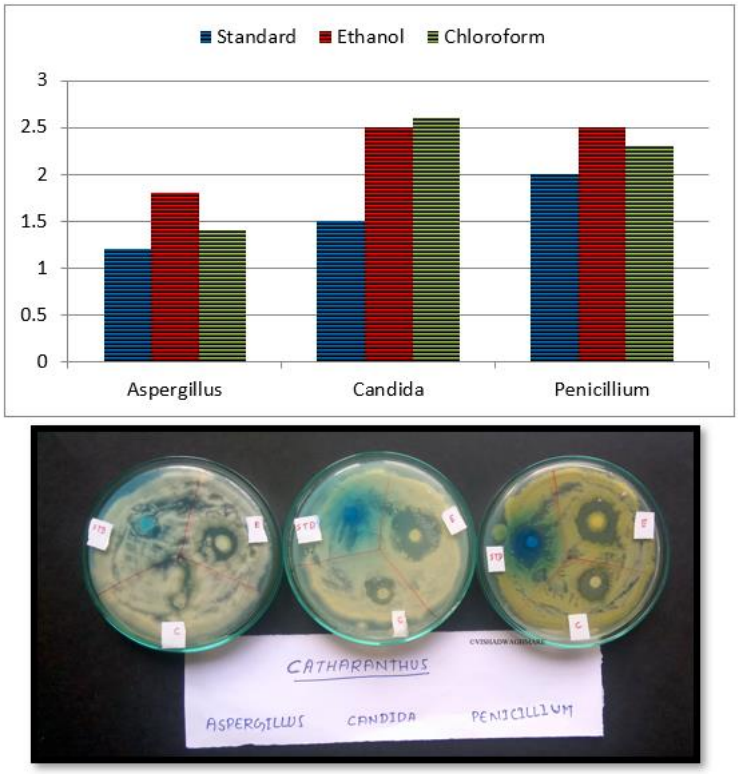


Fig.6: Zone of Inhibition exhibited by *Catharanthus roseus* against the selected fungi strains

Extract of the *Achyranthes aspera* with cold ethanol demonstrated the prominent antifungal action against *Aspergillus sp.*in for all intents and purposes double zone of inhibition than that of standard. Against *Penicillium sp.* also *Candida sp.* impact was great with very more zone of inhibition than standard with cold ethanol extract.

Table 4 Zone of inhibition (cm) *Achyranthes aspera*

Fungi	***Aspergillus***	***Candida***	***Penicillium***
Standard	1.5	1.5	2.0
Ethanol	2.6	2.5	3.0

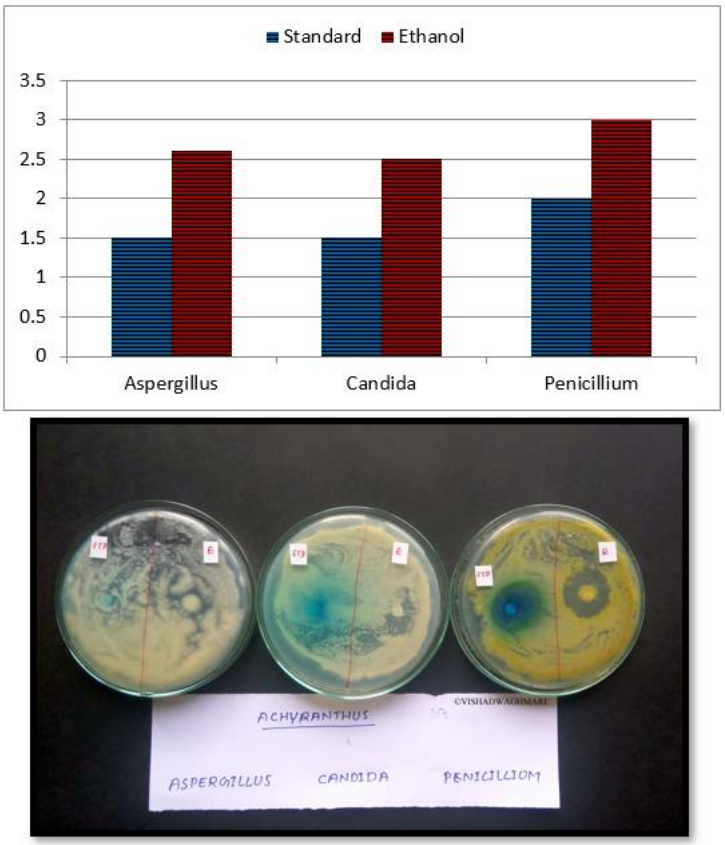


Fig. 7: Zone of Inhibition exhibited by *Achyranthes aspera* against the selected fungi strains

DISCUSSION

The fungal infections in fish have been of concern to the aquarium business and those who keep fish as pets. Several attempts have been made to find some alternatives to control the infections. The present study emphasizes on the use of plant extracts as potential agents to be employed as antifungal agents. As compared with the zone of inhibition obtained in the standard tests, the maximum inhibition was exhibited by ethanolic extract of *Parthenium hysterophorus* and *Catharanthus roseus* against *Aspergillus sp,* chloroform extract of *Parthenium hysterophorus* and *Catharanthus roseus* and ethanolic extract of *Achyranthes aspera* against *Candida sp* while ethanolic extract of *Parthenium hysterophorus, Catharanthus roseus* and *Achyranthes aspera* against Penicillium *sp.*

The obtained results are in accordance with those reported by Terefe Tafese Bezuneh 2015 as quoted "Antifungal property of *P.hysterophorus* has been accounted against the basic growths that we utilized on plants and animals". As per Devkota A.and Sahu A. (2017), "methanol unrefined leaf extracts had higher antifungal potential" yet in present examination, it has been seen that *P.hysterophorus* is progressively effectual with acetone and chloroform. In Kratika Kumari, Sharmita Gupta (2013) examinations *C.roseus* indicated 1.62 cm and 1.5 cm zone of inhibition against *Aspergillus sp.* Moreover, *Candida sp.* with acetone though in current examinations we have outwardly seen *C.roseus* demonstrated 2.2 cm and 1.5 cm zone of inhibition separately. *C.roseus* has demonstrated great action against fungal pathogens with ethanol and chloroform with the most extreme zone of inhibition (Divya Pailkara *et.al.,* 2017). *Achyranthes aspera* moderate action was appeared by

the chloroform fraction (50% inhibition) against *Microsporum canis* (Khuda F *et.al.*, 2013) while in present examination *Achyranthes aspera* demonstrated the higher antifungal movement against each of the three growths species in contrast with the standard. The present study indicates the importance of the selected plant extracts as antifungal agents with extensive field trials.

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