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Calliandra haematocephala's phytochemical analysis and antifungal effectiveness against fish pathogen in the lake of Bhopal

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Abstract

Aquaculture has been a major part of our Economy from ancient times and antimycotic resistant fish illnesses have emerged as a major threat in aquaculture from a very long time. As routinely used anti-fungal medications have become less effective and traditional medicinal herbs have been identified as a possible medication source that can counteract antifungal resistance. The current study will look at the antifungal efficacy of leaf and flower extracts of the nutritional and traditional medicinal plant called *Calliandra haematocephala* against 3 common fish fungal pathogens (*Achlya*, *Saprolegnia* and *Aphanomyces*) that were isolated from Lower Lake of Bhopal (M.P.) and causes illness in fishes present in Lower Lake, Bhopal (M.P.). The leaves and flowers of *Calliandra haematocephala* were extracted sequentially with ethanol as a solvent, and the presence of possible phytoconstituents was qualitatively evaluated. Flavonoids, phenol, alkaloids, carbohydrates, tannins and saponins were present in both flowers and leaves and steroids were found exclusively in the ethanolic extract of the leaf only not in flower extract. The antifungal activity of ethanolic extract was determined using the agar well diffusion technique. The ethanolic fraction of the flower demonstrated a wide range of activity against fish pathogens, with a maximal inhibitory or clearance activity against *Saprolegnia* in a amount of 20 μ L (4 mm) and in leaves it showed maximum clearance zone in *Achlya* and *Saprolegnia* in 20 μ L (4 mm). In conclusion, *Calliandra haematocephala's* extracts consist of potential secondary metabolites that might be employed as antifungal treatments for 3 prevalent fish Pathogens, and the leaf ethanolic extracts have more inhibitory efficacy than the flower ethanolic extract.

Keywords *Calliandra haematocephala*; Fish fungal pathogen; Phytoconstituents; Antifungal resistance; Fish illness

1. Introduction

On Earth, there are roughly two million different kinds of living things, of which there are about 100,000 species of fungus, with many more still undiscovered. Fungi are among the most common members on Earth and are crucial to both the environment and human health.

Some are parasites of plants that spread illnesses including canker, rust, scabs, and mildew (Smith & Read, 1997). A relatively tiny percentage of fungus can make fishes sick. Water contains a wide variety of fungus, including many Mastigomycotina (zoosporic fungi). On Earth there is 71% of water this large percentage of water contain large number of life forms which we now a day's call aquatic life or aquatic system. Aquatic means "water". An aquatic fungus is one of the life forms reproducing and surviving in water. Chhote Khan, the Nawab Hayat Muhammad Khan Bahadur's minister, gave the order to build Lower Lake in 1794 to improve the aesthetics of the Bhopal city (M.P.).

The fish illness brought on by a fungus is widespread worldwide in both farmed and wild fish. The bulk of the aquatic fungus that have been linked to the illness caused in fish and shellfish, including *Aphanomyces*, *Saprolegnia* and *Achlya*,

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are members of the family Saprolegniaceae (Srivastava, 1980). *Aphanomyces invadans* fungus is the cause of **Epizootic Ulcerative Syndrome** a fish disease. Saprolegniasis is a condition that affects fish and is caused on by Saprolegnia and Achlya sp.

Several commercially significant plants are members of the huge plant family known as the Fabaceae. Leaves of *Calliandra haematocephala* are compound, bipinnate and lanceolate, green in color and floral parts are lacking fragrance (Zeid et al., 2007). It is found to bloom from December to March.

Ethanol solvent was used for extraction. And this extract was also used for Phytochemical analysis. The efficacy of *Calliandra haematocephala*'s ethanolic leaf and flower extract was tested by Agar well diffusion method for testing effectiveness on fish fungal pathogen like *Achlya*, *Saprolegnia* and *Aphanomyces* which was isolated from water of Lower lake of Bhopal because of which the fish being highly susceptible to different infectious diseases and these aquatic fungus which is now major problem in the Lower Lake Bhopal. The *Calliandra haematocephala* plant's leaf and flower ethanolic extract will also be used for phytochemical analysis.

2. Materials and Methods

2.1. Sample collection and storage

In 1-litre plastic bottle or can that had already been sterilized is used for collection of water sample from the Lower Lake, Bhopal (M.P.), India. The coordinates of location is Latitude - 23.24051'08"N and Longitude - 77.40032'53"E. After that, the sample bottle was taken to the lab for further isolation and research on the water. The cap of bottle should be open.

2.2. Isolation of aquatic fungus

2.2.1. Baiting technique

The distilled and sample water was poured (in 1:4 measurement) in sterilized petri dishes. The baits, include Till seeds (*Sesamum indicum*) (Nahar, B., 2020). They are roasted in a hot air oven and then it can be employed as bait in each petri dish. In each petri dish 0.5 g of antibiotic streptomycin is introduced. After that, leave the petri plates in isolation for a week or more.

2.2.2. Culture and identification of aquatic fungus

The petri dish which is baited with till (*Sesamum indicum*) and kept in BOD Incubator at 16-18°C for a week or more until the growth is seen in water sample. After the growth is seen the petri plates with growth is taken out of Incubator. These fungal forms were identified under microscope with the help of various books, reviews, manuals, monographs, relevant research papers and published work on taxonomy of aquatic fungi by various famous scientists like Sparrow (1960), Khulbe (2001) etc. The fungus was identified on the basis of morphological characters.

2.2.3. Slide preparation of aquatic fungus

With use of forceps, aquatic fungus hyphae were removed after identification under microscope from a petri dish and placed on slides where they were dyed with lactophenol cotton blue. Then fungi were identified by looking at them under a microscope.

2.3. Collection of plant material

Before collecting the plant we should identify the plant carefully by the help of Herbarium and other Taxonomic tools. The leaves and flower was collected from the *Calliandra haematocephala* plant present in Bhopal, (M.P.), India. The leaves and flowers were plucked from *Calliandra haematocephala* plant, was collected in a bag and brought to laboratory for further process.

2.4. Preparation of crude extract

The leaves and flowers of the *Calliandra haematocephala* plant are bought to laboratory and washed with clean water, rinsed with sterilized distilled and spread it on tissue paper. Then pick the leaves and flowers and spread it on a plain sheet or newspaper in a shady place in a dark room where sunlight couldn't enter then allow it to get shade dried (Susmitha et al., 2013) and for a time period of one week.

Maceration method described by (Abubakar, A. et al., 2020) was performed. A conical flask is filled with fifty gram (50g) of finely powdered drug material. The menstruum or solvent which we took was 50% ethanol to make an ethanolic plant extract and to dissolve the active component present in the shade dried leaves and flowers. The solvent is then poured on top, covering the drug material or powdered material entirely. After that, the container is sealed. To achieve excellent extraction, the substance is mixed or stirred occasionally. Filtration is done on the resultant solvent extract, after the extraction process is over and when the bioactive components are extracted in the solvent, the liquid in the conical flask was filtered using Whatman No. 1 filter paper (Rahman, T., et al., 2013). In a clean and sterilized conical flask, the filtrate was collected. For the purpose of evaluating the antifungal impact, the filtrate containing the active ingredient was utilized. Then, through evaporation the filtrate obtained was concentrated by evaporation of solvent to a large extent and by placing on top of a water bath at 50°C. Then take small sterilized bottles and then fill the leftover or the crude extract of leaf and flower extract in those bottles.

2.5. Phytochemical screening of plant extract-

2.5.1. Test for Alkaloids (Wagner's reagent test)

- 1 ml of plant extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water).
- Then we waited for the formation of reddish brown precipitate or coloration in plant extract (Hait et al., 2019).

2.5.2. Test for Phenols (Ferric chloride test)

- 1ml of the extracts was treated with 5% ferric chloride solution.
- Then we observed for formation of deep blue or black color which indicates the presence of phenol (Hait et al., 2019).

2.5.3. Test for Tannins

- 1ml of the extract was treated with few drops of 10% lead acetate solution.
- Then we observed the formation of white precipitate confirms the presence of Tannins in plant extract.

2.5.4. Test for Carbohydrates

- 1ml of the extracts was treated with few drops of Molisch reagent.
- Then Conc. H₂SO₄ was added.
- The formation of violet ring that confirms the presence of carbohydrates.

2.5.5. Test for Protein

- 1ml of the extracts was treated with and Copper sulphate in small amount.
- Then by adding NaOH solution gives violet color.

2.5.6. Test for Steroids (Liebermann Burchard test)

- 1ml of the extract treated with 3ml acetic anhydride.
- Then add Glacial acetic acid few drops.
- Then few drops of Conc. H₂SO₄ that gives green or green blue color.

2.5.7. Test for Sterols

- One ml of the extract was treated with acetic anhydride.
- Add chloroform.
- Then add concentrated H₂SO₄ and checked for the development of a dark pink or red color.

2.5.8. Test for Glycosides

- 1 ml of plant extract treated with few drops of ferric chloride.
- Then add 0.5 ml of glacial acetic acid.
- After that add Conc. HCl few drops gives the development of green color ring.

2.5.9. Test for Flavonoids

- 1ml of plant extract treated with 10% of H₂SO₄ then wait for it to cool down for some time.

- Then add 1ml of dilute Sodium carbonate that gives yellowish red or yellow color which confirms the presence of flavonoids.

2.5.10. Test for Terpenoids

- 1 ml of chloroform was added to 1ml of plant extract
- Followed by a few drops of concentrated Sulfuric acid a reddish brown precipitate was produced that indicates the presence of terpenoids.

2.5.11. Test for Saponins

- 3 ml of H₂O were added to 1 ml of extract in a test tube.
- After vigorously shaking the mixture, it was examined to see whether any persistent foam formed, which would indicate the presence of saponins.

3. Results and Discussion

To determine the presence of various active components, the crude extracts of *C. haematocephala*'s leaves and flowers were fractionated using successive polar extraction techniques. The solvent utilized was ethanol. The usual testing approach was used to identify the main phytoconstituents that were present in each fraction (Deepthi et al., 2022). Following is a summary of the findings from investigations on *C. haematocephala* leaf and flower extracts' phytochemical screening, aquatic fungus that is identified and antifungal activity or effect.



Figure 1 (a) Leaves of *Calliandra haematocephala*, (b) flower of *Calliandra haematocephala*

3.1. Phytochemical screening -

The presence of significant bioactive metabolites such favonoids, steroids, alkaloids, glycosides and phenols was examined since *Calliandra haematocephala* is rich in a variety of bioactive components. The result is outlined below.

Table 1 Phytochemical screening of plant *Calliandra haematocephala* leaf and flower

S.no.	Phytoconstituents	Leaf extract	Flower extract
1	Alkaloids	+	+
2	Phenol	+	+
3	Tannins	+	+
4	Carbohydrate	+	+
5	Protein	-	+
6	Steroids	+	-
7	Sterol	-	-
8	Glycosides	+	-
9	Flavonoids	+	+
10	Terpenoids	Nil	+
11	Saponins	+	+

+ = PRESENT, - = ABSENT

3.2. Antifungal effect of crude extract on fish pathogens

Calliandra haematocephala was checked against fish pathogens that are *Achlya*, *Saprolegnia* and *Aphanomyces*. We used agar well diffusion method (Sen, A., & Batra, A., 2012) to assess the antifungal activity in vitro. The Malachite green (Rahaman et al., 2008) of 4 µl was employed as the standard in well. 10 µL and 20 µL of each ethanolic leaf and flower extract were added to the well. Below are figures of each ethanolic leaf and floral extract's specific inhibitory zones on all 3 aquatic fungus or fungal fish pathogen. Different amounts of *Calliandra haematocephala* leaf and flower extracts have demonstrated an inhibitory zone against all three fish pathogens. All 6 petri plates (three of the leaves and three of the flowers) exhibited an inhibitory effect. With regard to fish pathogens, the ethanolic fraction of flowers and leaves has demonstrated a variety of activities in terms of Zone of inhibition. The activities of the leaf extract on *Achlya* 10 µL (3 mm) and 20 µL (4 mm), *Saprolegnia* 10 µL (3.5 mm) and 20 µL (4 mm) and *Aphanomyces* 10 µL (1 mm) and 20 µL (2 mm). The activities of flower extract on *Achlya* 10 µL (2 mm) and 20 µL (3 mm), *Saprolegnia* 10 µL (2 mm) and 20 µL (4 mm) and *Aphanomyces* 10 µL (2 mm) and 20 µL (3 mm). The Malachite green that was tested with leaf extract as standard also has shown wide range of activities on *Achlya* 4 µL (4.5 mm), *Saprolegnia* 4 µL (5.5 mm) and *Aphanomyces* 4 µL (3 mm). When Malachite green was tested with flower extract as standard it showed following activities on *Achlya* 4 µL (3.5 mm), *Saprolegnia* 4 µL (9 mm) and *Aphanomyces* 4 µL (4 mm). Leaf extract was more effective on *Saprolegnia* and *Achlya* in amount 20 µL the zone of clearance was (4 mm) and (4 mm) respectively and flower extract was found to be more effective on *Saprolegnia* in amount 20 µL the zone of inhibition was (4 mm). The Malachite green that we used as standard shown activity to large extent on *Saprolegnia* when used as standard in both leaf extract (5.5 mm) and flower extract (5 mm).

Table 2 Zone of clearance of different amounts of leaf extract on 3 fish fungal pathogens.

S.no.	Fungal pathogen fish	Effect of leaf extract 10 µl (mm)	Effect of leaf extract 20µl (mm)	Effect of standard (malachite green) 4 µl (mm)
1	<i>Achlya</i>	3 mm	4 mm	4.5 mm
2	<i>Saprolegnia</i>	3.5 mm	4 mm	5.5 mm
3	<i>Aphanomyces</i>	1 mm	2 mm	3mm

Table 3 Zones of clearance of different amounts of flower extract on 3 fish fungal pathogens.

S.no.	Fungal pathogen fish	Effect of flower extract 10 µl(mm)	Effect of flower extract 20 µl (mm)	Effect of standard (malachite green) 4 µl (mm)
1	<i>Achlya</i>	2 mm	3 mm	3.5 mm
2	<i>Saprolegnia</i>	2 mm	4 mm	5 mm
3	<i>Aphanomyces</i>	2 mm	3 mm	4 mm

4. Conclusion

This conventional medicinal plant (*Calliandra haematocephala*) can be found as a possible therapeutic source capable of combating fungal resistance in fish. In our results the *Calliandra haematocephala*'s phytochemical analysis indicated that there is presence of Flavonoids, Phenol, Alkaloids, Carbohydrates, Tannins and Saponins in both flowers and leaves. Steroids were found exclusively in the ethanolic extract of the leaf only not in flower extract.

In our investigation we found out that extracts of *C. haematocephala* leaves and flowers demonstrated significant inhibitory effect with varying diameters of inhibition against all three major fish pathogens. The leaf's ethanolic fraction demonstrated an array of spectrum of anti-fungal fish pathogen activity, with 20 µL on *Saprolegnia* and *Achlya* (5 mm) and (5mm) showing its greatest inhibitory or clearance effectiveness. The ethanolic fraction of the flower demonstrated a wide range of activity against fish pathogens, with a maximal inhibitory or clearance activity against *Saprolegnia* in amount of 20 µL (4 mm).

Compliance with ethical standards

Disclosure of Conflict of Interest

The corresponding author states that there is no conflict of interest.

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