STUDY ON THE PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL ACTIVITY OF MEDICINAL PLANT AGAINST FUNGAL PATHOGENS OF ORYZA SATIVA

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Abstract *:* Medicinal plants are important sources of bioactive compounds with therapeutic potential and minimal toxicity. The present study evaluated the antifungal activity of ***Leucaena leucocephala*** against selected fungal pathogens, namely *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium oxysporum*. The fungal strains were isolated from infected *Citrus sinensis* fruits and cultured on Potato Dextrose Agar (PDA) and Rose Bengal agar. Identification was performed based on morphological characteristics using a compound microscope.Preliminary phytochemical screening of ***Leucaena leucocephala*** extracts revealed the presence of carbohydrates, saponins, phenols, flavonoids, tannins, terpenoids, glycosides and alkaloids . Antifungal activity was assessed using different solvent fractions, both individually and in combination. The extracts exhibited notable inhibitory effects against all tested fungal pathogens.The findings suggest that ***Leucaena leucocephala*** possesses significant antifungal properties and could serve as a potential natural source for developing alternative antifungal agents.

IndexTerms - ***Leucaena leucocephala***; Antifungal activity; Phytochemical screening; Aspergillus flavus; Aspergillus niger; Fusarium oxysporum; Medicinal plants

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world and provides the primary source of calories for more than half of the global population. It is widely cultivated in tropical and subtropical regions, particularly in Asian countries such as India, China, Bangladesh, and Thailand. Rice plays a crucial role in global food security and economic stability because millions of farmers depend on its cultivation for their livelihood. However, rice production is severely affected by various biotic and abiotic stresses, among which fungal diseases represent one of the major threats to yield and grain quality. Fungal pathogens infect different parts of the plant, especially leaves, causing serious economic losses in rice-growing regions (Savary et al., 2019).Leaf diseases in paddy crops are mainly caused by pathogenic fungi such as *Magnaporthe oryzae*, *Bipolaris oryzae*, *Rhizoctonia solani*, and *Curvularia* species. These fungi infect the leaf tissues and produce characteristic symptoms such as leaf spots, lesions, blights, and discoloration. Rice blast disease caused by *Magnaporthe oryzae* is considered one of the most destructive fungal diseases affecting paddy cultivation worldwide. The pathogen spreads rapidly under humid conditions and can infect leaves, stems, and panicles, resulting in severe yield reduction. Similarly, brown spot disease caused by *Bipolaris oryzae* leads to the

formation of brown lesions on leaves and grains, reducing both productivity and grain quality (Dean et al., 2012).

Leucaena leucocephala

Kingdom	Plantae
Class	Magnoliopsida (Dicotyledons)
Order	Fabales
Family	Fabaceae (Leguminosae)
Genus	Leucaena
Species	Leucaena leucocephala (Lam.) de Wit

Leucaena leucocephala (Family: Fabaceae) is a fast-growing tropical leguminous tree commonly known as Subabul, White leadtree**,** or Ipil-ipil in English, and in Tamil it is commonly called Subabul**.** The scientific name is *Leucaena leucocephala* (Lam.) de Wit. This plant is widely distributed in tropical and subtropical regions and is valued for its nutritional, agricultural, and medicinal properties. The leaves and seeds are rich in proteins, vitamins, minerals, and bioactive compounds such as flavonoids and tannins. Traditionally, different parts of the plant have been used in herbal medicine to treat conditions such as inflammation, skin diseases, intestinal worms, and digestive disorders, and leaf extracts have shown antimicrobial, antioxidant, and anti-inflammatory activities. The plant is also used as livestock fodder, green manure, and soil improver because it fixes atmospheric nitrogen, improving soil fertility. In folk medicine, crushed leaves are sometimes applied to wounds and skin infections, while decoctions of the bark or leaves are used for fever and pain relief (Orwa et al., 2009).

Objectives:

- To isolate and identify fungal pathogens associated with Paddy samples using standard microbiological techniques.
- To collect, authenticate, and prepare solvent extracts of ***Leucaena leucocephala*** plant materials.
- To qualitatively and/or quantitatively analyze the phytochemical constituents (alkaloids, flavonoids, tannins, saponins, phenolics, etc.) present in the extracts.
- To determine the antifungal activity of the plant extracts against isolated paddy pathogens using assay such as disc diffusion.

2.Need of the Study

Fungal pathogens such as *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium oxysporum* cause serious post-harvest losses in *paddy* and may produce harmful toxins. Increasing resistance to synthetic antifungal agents necessitates safer and eco-friendly alternatives. Therefore, this study evaluates the phytochemical constituents and antifungal potential of ***Leucaena leucocephala*** as a natural and cost-effective antifungal source.

RESEARCH METHODOLOGY

The methodology section outline the plan and method that how the study is conducted. This includes Universe of the study, sample of the study,Data and Sources of Data, study's variables and analytical framework. The detailsare as follows;

3.1 Collection of Plant Materials:

The infected leaves of *Oryza sativa* were collected from stores in and around Perambalur and brought in to the laboratory for to carry out further processes.

3.2Isolation and Identification of fungal pathogen (Aneja, 2003):

Composition of the Medium

Potato : 200 grams
Agar :15 grams
Dextrose :20 grams
Distilled water : 1000 ml

3.3 Media Preparation:

To suspend all the ingredients weighed through physical balance in 1000 ml of distilled water. The suspension was filter through Whatman filter paper for remove the impurities and then sterilized by autoclave (15 lbs pressure/121ºC). After autoclaving add chloramphenicol was added in sterile condition at 40º-50ºC of medium. The medium is stored in the refrigerator for further use.

The infected fruits are cut across lesions of 5-10 mm square, containing both the diseased and healthy-looking tissue. The surface sterilize the cut portions by dipping in a surface sterilant for solution for different times, varying from 12 to 120 seconds. Then, wash the treated pieces in three changes of sterile water and blot dry on clean, sterile paper towels to remove the sterilant. The plant pieces were aseptically transferred on a potato dextrose agar medium, usually 3-5 pieces per plate. Then, the plates were in an inverted position at 25ºC for 5-7 days. After the periods, the fungal growth was observed in each plate. The identification of fungus was performing standard straining method.

3.4 Lacto phenol cotton blue mount:

- Place a drop of lactophenol cotton blue on a clean slide.
- Transfer a small tuft of the fungus, preferably with spores and spore bearing structures, into the drop, using a flamed, cooled needle.
- Gently tease the fungal using the two mounted needles.
- Mix gently the stain with the mold structures.
- Place a cover-glass over the preparation taking care to avoid trapping air bubbles in the stain.
- Sealing lactophenol mounts: To keep the slides for many years, cover slip is sealed with nail polish as follows.
- Remove all the air bubbles from the preparation by pressure, gentle heating or the addition of more lactophenol cotton blue.
- Remove the excess mountant from around cover slip with 70 percent alcohol on a cotton swab or with blotting paper.
- Apply a thin layer of nail polish around the edge of the cover slip.
- Allow the preparation to dry overnight.
- Apply a second coat of nail polish over the first coat.

3.5 Maintenance of Fungal Inoculum:

The fungal cultures were isolated and transfer to medium containing petri plates or tubes. The isolated cultures (pure form) are stored in refrigerator for the screening purposes. The periodical changes/transfer of the fungus, helped to viable condition.

3.7 Collection of plant materials

Leucaena leucocephala leaves were collected from various areas in Perambalur, Tamil Nadu, India.

3.8 Extraction of plant materials (Harborne, 1984)

The leaves of ***Leucaena leucocephala*** were washed with dechlorinated water, dried in shade and powdered with the help of an electric blender. The test materials (1.0 kg) were extracted with different organic solvents viz., Ethanol, butanol, acetone, hexane and chloroform in a soxhlet apparatus for 8 h and the extract was concentrated in a rotary vacuum evaporator to yield crude extract (Fig. 3).

3.9 Qualitative Phytochemical Analysis

Qualitative phytochemical screening of ***Leucaena leucocephala*** extracts was carried out using standard protocols to detect the presence of major secondary metabolites. The analysis was performed following established procedures described by Kapoor et al. (1969) and Odebiyi and Sofowora (1990). The extracts were screened for alkaloids, flavonoids, carbohydrates, proteins, phenols, saponins, tannins, phytosterols, glycosides, and terpenoids.

3.9.1Test for Alkaloids (Wagner's Test) (Salehi Surmaghi et al., 1992)

Alkaloids were detected using Wagner's reagent following the method of Salehi Surmaghi et al. (1992). Wagner's reagent was prepared by dissolving 1.2 g iodine and 2.0 g potassium iodide in distilled water and making up to 100 ml. Ten milliliters of plant extract were acidified with 1.5% HCl and treated with a few drops of Wagner's reagent. Formation of a yellowish-brown precipitate indicated the presence of alkaloids

3.9.2Test for Flavonoids (Shinoda Test) (Somolenski et al., 1972)

Flavonoids were identified according to Somolenski et al. (1972). To 0.5 ml of plant extract, 5–10 drops of dilute HCl and a small quantity of magnesium or zinc were added and heated for a few minutes. Development of a reddish-pink or dirty brown colour confirmed the presence of flavonoids.

3.9.3Test for Carbohydrates (Benedict's Test) (Kapoor et al., 1969)

Carbohydrates were determined using Benedict's test. Benedict's reagent was prepared by dissolving sodium citrate and sodium carbonate in distilled water, followed by addition of copper sulphate solution. To 0.5 ml of extract, 5 ml Benedict's reagent was added and boiled for 5 minutes. A bluish-green colour or precipitate indicated the presence of carbohydrates.

3.9.4Test for Phenols (Ferric Chloride Test) (Malick and Singh, 1980)

Phenolic compounds were detected following Malick and Singh (1980). One ml of extract was mixed with distilled water and a few drops of 10% ferric chloride solution. Formation of a blue or green colour indicated the presence of phenols.

3.9.5Test for Saponins (Foam Test) (Malick and Singh, 1980)

Saponins were tested by adding a drop of sodium bicarbonate solution to 5 ml of extract and shaking vigorously. Formation of stable honeycomb-like froth persisting for several minutes confirmed the presence of saponins.

3.9.6Test for Tannins (Ferric Chloride Test) (Segelman et al., 1969)

Two ml of plant extract were treated with 5% ferric chloride solution. A bluish-black coloration that disappeared on addition of dilute sulfuric acid followed by yellowish-brown precipitate formation indicated tannins.

3.9.7Test for Terpenoids (Salkowski Test) (Odebiyi and Sofowora, 1990).

Five ml of extract were mixed with 2 ml chloroform, and 3 ml concentrated sulfuric acid were carefully added to form a layer. A reddish-brown coloration at the interface confirmed the presence of terpenoids

3.9.8 Test for Glycosides (Keller–Killiani Test) (Odebiyi and Sofowora, 1990).

Two ml of extract was treated with 1 ml glacial acetic acid containing a trace of $FeCl_3$. Concentrated H_2SO_4 was carefully added along the sides of the test tube. Formation of a brown ring at the interface indicated the presence of glycosides.

3.10 Determination of anti fungal activity using disc-diffusion method (Bauer *et al.*, 1959).

Various solvent fractions of The ***Leucaena leucocephala*** leaf extract were checked for antifungal activity using disc-diffusion method. *Aspergillus flavus ,Aspergillus niger and Fusarium oxisporum* was swabbed on the surface of the potato dextrose agar plates. The disc (Whatman No.1 filter paper with 9 mm diameter) was impregnated with the 50 µl of each test sample and it was placed on the surface of potato dextrose agar plates.To compare the antibacterial activities, nystatin (20 µg/disc) used as standard antibiotic and blank disc impregnated with water act as negative control. The plates (triplicates) were incubated 28°C for 72 h. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

IV. RESULTS AND DISCUSSION

4.1Test organism

The test organism collected from infected leaves of ***Oriza sativa*** .The ***Oriza sativa*** leaves were collected from local stores of Perambalur.

4.2 Morphological study of *A.flavus*

Microscopically, *A. flavus* colony appear as a velvety, yellow to green or brown mold with colorless or sandy beige reverse. Old colony appears as dark green. The shape is smooth and some have radial wrinkles. Conidiophores are heavy walled, uncolored, coarsely roughened, usually less than 1 mm (400-800 µm) in length and are often rough just beneath the globose vesicles. Vesicles are elongate when young, later becoming subglobose or globose, varying from 10 to 65 mm in diameter.

4.3 Morphological study of *A.niger*

Microscopically, *A.niger* can be identified by its hyaline, septate hyphae. Asexual conidiophores can be identified by being long and globose at the tip, with what appears to be a hymenial layer of structures, each "ejecting" its own spore.

4.4 Morphological study of *Fusarium oxysporum*

Microscopically *Fusarium oxysporum* hyaline, septate hyphae are observed. Conidiophores are short, simple or branched, bearing monophialides. Microconidia are abundant, oval to ellipsoid, mostly 0–1 septate, and are produced in false heads on short monophialides. Macroconidia are characteristic thin-walled, fusiform to sickle-shaped, typically 3–5 septate, with a distinct pedicellate basal cell and a tapered, curved apical cell. Chlamydospores are globose, thick-walled, formed singly or in pairs, either terminally or intercalary, and become prominent in older cultures.

4.5 Phytochemical analysis

4.5.1 Qualitative analysis

For all the medicinal plants, phytochemical screening was performed to test the presence of different secondary metabolites (Table.1). However, carbohydrates,alkaloids, saponins, phenols, flavanoids, tannins Terpenoids and Glycosides present in ***Leucaena leucocephala***.

4.6 Anti-fungal activity of plants

4.6.1 Disc diffusion method

The maximum antifungal activities observed in the crude extract prepared using ***Leucaena leucocephala***.It indicates that the extract prepared by using different solvent having various strength of plant extracts.

Leucaena leucocephala the strong antifungal activity observed ethanol (0.9 mm) and mild antifungal activities hexane (0.1mm) and moderate in butanol extracts (0.3 mm)..(Fig 5 A &B, Table.2 &3 ***Leucaena leucocephala*** the strong antifungal activity observed butanol (0.5 mm) and mild antifungal activities hexane (0.1mm) and moderate in butanol extracts (0.5 mm). ***Ixora coccinea*** the strong antifungal activity observed Ethanol (0.9 mm) and mild antifungal activities chloroform (0.1mm) and moderate in butanol extracts against Aspergillus flavus then compare to other organisms (0.7 mm)..((Fig 5, Table.4,5&6).

Fig 1:Plant with leaves of *Leucaena leucocephala*

Fig 2:Fungal infected paddy leaves

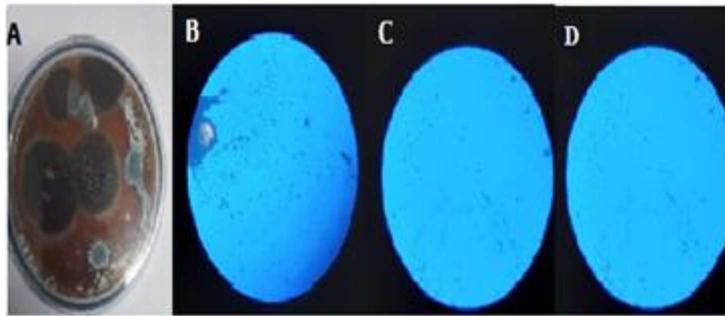

Fig 3: A.Fungal species plate viw
B.*Aspergillus niger* slide view
C.*Aspergillus flavus* slide view
D.*Fusarium oxysporum* slide view

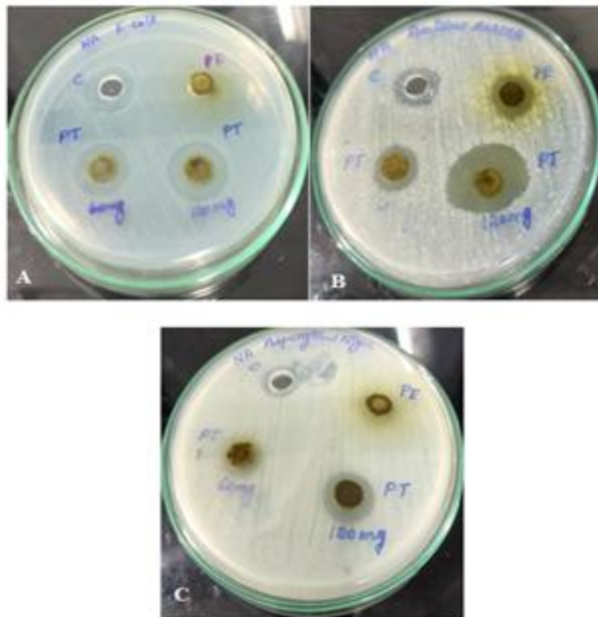

Fig 4:Maximum antifungal activity of *Leucaena leucocephala against Aspergillus flavus, Aspergillus niger and Fusarium oxysporum* by disk diffusion method.

Table 1. Qualitative profile of *Leucaena leucocephala*

S.No	Phytochemicals	Ixora coccinea
1	Alkaloids	+
2	Carbohydrates	+
3	Saponins	+
4	Phenols	+
5	Flavonoids	+
6	Terpenoids	+
7	Tannins	+
8	Glycosides	+

+ denotes present, - denotes absent

Table 2: Antifungal activity of *Leucaena leucocephala* individual fraction tested against *Aspergillus flavus* by disk diffusion method.

Plant sample/Solvent	***Leucaena leucocephala***					
	Crude	10%	25%	50%	75%	100%
Ethanol	0.4	0.3	0.4	0.3	0.6	0.9
Butanol	0.4	0.5	0.4	0.5	0.4	0.8
Acetone	0.1	0.2	0.4	0.3	0.4	0.3
Hexane	0.1	0.4	0.4	0.3	0.2	0.3
Chloroform	0.3	0.2	0.2	0.4	0.4	0.3

Table 3: Antifungal activity of *Leucaena leucocephala* individual fraction tested against *Aspergillus niger* by disk diffusion method.

Plant sample/Solvent	***Leucaena leucocephala***					
	Crude	10%	25%	50%	75%	100%
Ethanol	0.2	0.2	0.4	0.6	0.4	0.4
Butanol	0.4	0.6	0.6	0.8	0.8	0.9
Acetone	0.1	0	0.2	0.2	0.3	0.2
Hexane	0.1	0.2	0.1	0.3	0.2	0.3
Chloroform	0.3	0.1	0.1	0.2	0.1	0.2

Table 4: Antifungal activity of *Leucaena leucocephala* individual fraction tested against *Fusarium oxysporum* by disk diffusion method.

Plant sample/Solvent	***Leucaena leucocephala***					
	Crude	10%	25%	50%	75%	100%
Ethanol	0.2	0.1	0.2	0.1	0.4	0.3
Butanol	0.1	0.3	0.3	0.5	0.3	0.4
Acetone	0.1	0.1	0.2	0.2	0.3	0.2
Hexane	0.1	0.2	0.4	0.2	0.2	0.3
Chloroform	0.1	0.1	0.2	0.1	0.2	0.3

The isolation of fungal pathogens from infected Oriza sativa leaves collected from Perambalur confirms that post-harvest leavesact as major reservoirs for fungal contamination. paddyleaves are highly susceptible to fungal invasion due to their high moisture and sugar content, which provide favorable conditions for fungal growth. Market storage and transportation further increase the chances of infection due to mechanical injury and humidity fluctuations. Similar findings have been reported where Aspergillus and Fusarium species were frequently isolated from stored fruits and vegetables, indicating their strong adaptability to post-harvest environments (Snowdon, 1990).

The prevalence of ***A. flavus, A. niger,*** **and** ***F. oxysporum*** among the isolates highlights their ecological versatility and pathogenic potential. These fungi are well known for causing spoilage and producing mycotoxins that can threaten food safety. Their presence in edible fruits raises public health concerns, especially in tropical regions where temperature and humidity favor fungal proliferation. Previous studies have emphasized that improper storage conditions significantly increase fungal colonization in citrus fruits (Pitt & Hocking, 2009).

Phytochemical Composition of Plant Extracts

Qualitative phytochemical screening revealed the presence of bioactive secondary metabolites such as alkaloids, flavonoids, tannins, phenols, terpenoids, saponins, and glycosides. These compounds are known for antimicrobial and antioxidant activities. The richer phytochemical diversity observed in ***Leucaena leucocephala*** may explain its relatively higher antifungal efficacy. Secondary metabolites function as plant defense molecules and often contribute to antimicrobial effects (Cowan, 1999).

The absence of alkaloids in *Ixora coccinea* may partly explain variations in antifungal potency between the two plants. Alkaloids are often associated with strong antimicrobial properties due to their ability to interfere with DNA replication and protein synthesis. Variations in phytochemical composition can result from environmental factors, plant maturity, and extraction methods (Harborne, 1998).

Antifungal Activity of Plant Extracts

The disc diffusion assay demonstrated that ethanol extracts of plant exhibited the strongest antifungal activity. Ethanol is known to extract a broad range of polar and moderately non-polar bioactive compounds, enhancing antimicrobial effectiveness. The higher inhibition zones observed against *A. flavus* and *A. niger* indicate strong susceptibility of these fungi to ethanol-derived phytochemicals. Similar solvent-dependent extraction efficiency has been widely reported in antimicrobial studies (Das et al., 2010).Moderate and mild inhibition observed in chloroform, butanol, hexane, and acetone extracts may reflect solvent polarity and compound solubility differences. Non-polar solvents typically extract lipophilic compounds that may show lower antimicrobial activity compared to phenolic-rich polar extracts. This solvent influence aligns with established extraction principles in phytochemistry (Eloff, 1998).The overall antifungal performance of *Opuntia ficus-indica* suggests it may serve as a promising natural antifungal agent. Its efficacy against multiple fungal pathogens highlights its potential for use in agricultural disease management and food preservation. Natural plant extracts are increasingly explored as eco-friendly alternatives to synthetic fungicides (Tripathi & Dubey, 2004).

Similarly, ***Leucaena leucocephala*** showed notable antifungal activity, particularly in ethanol and butanol extracts. its activity still supports its ethnomedicinal use. The antifungal potential observed here aligns with previous reports on medicinal plants containing phenolics and flavonoids (Raut & Karuppayil, 2014).The combined findings of phytochemical richness, GC–MS profiles, and antifungal assays strongly support the therapeutic potential of the selected medicinal plants. These plants could serve as sources of biofungicides for controlling post-harvest fungal pathogens in fruits. However, further studies including MIC determination, toxicity evaluation, and compound isolation are necessary to validate practical applications. Integrating plant-based antifungals into food protection systems could reduce reliance on synthetic chemicals and promote sustainable agriculture (Tripathi & Dubey, 2004).

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