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ANTIFUNGAL ACTIVITY OF DIFFERENT LEAF EXTRACTS OF *MUSSAENDA FRONDOSA* LINN. ON *MALASSEZIA FURFUR.*

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ABSTRACT

Malassezia furfur is the primary fungal agent responsible for dandruff, a prevalent scalp disorder. Folk practitioners have long utilised *Mussaenda frondosa* Linn. (*M. frondosa*) for its medicinal properties, including its use in treating dandruff. This study evaluates the antifungal activity of Aqueous, Hydro-Alcohol, and Chloroform leaf extracts of *M. frondosa* Linn. against the dandruff-causing fungus *M. furfur*.

Methods: The leaf extracts were obtained by cold maceration and evaporation extraction. The well diffusion method was used to investigate antifungal activity. The standard drug used in the study was Fluconazole.

Results: Chloroform extract showed significant antifungal activity in higher concentrations against *M. furfur*.

Keywords: *M. frondosa* Linn leaf, *M.furfur* , Antifungal activity, Chloroform extract.

INTRODUCTION

Antifungals are substances that either eliminate or inhibit the development of the fungi or spores responsible for infection. An antifungal medication is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm infections, candidiasis, and severe systemic infections

such as cryptococcal meningitis.[1] *M. furfur* is a member of a monophyletic genus of fungi normally found on human and animal skin. These lipid-dependent, commensal yeasts normally constitute more significant than 80% of the total fungal population of human skin and are frequently isolated in both healthy and diseased hosts. *Malassezia spp*. has been implicated in several common dermatologic disorders, including seborrheic dermatitis (SD), pityriasis versicolor (PV), and Malassezia folliculitis. Researchers discovered a yeast that correlated with PV as early as 1846, later named *M. furfur* in 1853. First designated as a distinct genus in 1889, over the years, 17 species of *Malassezia* have since been isolated from human and animal skin, which are classified by molecular biology, morphology, phenotype, and ultrastructure.[2]

Organism: [3]

The genus *Malassezia* belongs to the Kingdom Fungi, the order *Malasseziales*, and the family Malasseziaceae. M. furfur is a unicellular organism that varies in size between 1.5 and 4.5 × 2.0–6.5 micrometres. The cells have a bottle-like shape due to a small protrusion visible at the end of each cell. Cells are challenging to grow in a lab since they require specific conditions.

Epidemiology: [4]

M. furfur ubiquitously colonises adults and even infants by age 3 to 6 months, and it does not have a predilection for any particular age or sex.

Drug

The trial drug *M.frondosa* Linn. The Rubiaceae family is an erect shrub with grey bark, leaves simple, opposite, ovate, acuminate at the apex, soft white tomentose beneath, flowers yellowish green and orange in colour, one of the calyx lobes becomes enlarged into a white foliaceous structure [5]. This shrub is mainly found on the Malabar coast. There is no classical reference to this plant, but we get references based on research that its flowers are diuretic, anti-asthmatic and antipyretic. Leaves are used for external applications in ulcers. Root is used to treat white leprosy and white petiolate bract in jaundice.[6] According to IMP of India, the whole plant is anti-inflammatory, demulcent, febrifuge, etc. Many tribes in western ghats use *M. frondosa* Linn. leaf juice in Dandruff. [8] A literature survey indicated that there is limited scientific research on the antimicrobial efficacy of *M. frondosa* L. Therefore, an effort has been made to investigate the antifungal effects of *M. frondosa* leaf extracts against the pathogenic fungus *M. furfur*.

AIMS AND OBJECTIVES

To assess the Antifungal activity of *M. frondosa* Linn. Leaf extracts on *M. furfur* by "Well Diffusion Method".

MATERIALS AND METHODS:

Test performed: Antifungal activity test

Collection and sample preparation

M. frondosa Linn. Leaves were collected from the wild, i.e., near Kunjarugiri hill, Udupi district. The leaves were thoroughly washed and shade dried. After proper drying, the known weight of the leaves was measured; it was around 300 grams.

Leaves were subjected to fine powdering according to the standard method for preparing Aqueous, Hydro-alcohol, and Chloroform extracts.

Materials used for the study.

1. Cotton swabs
2. Sterile borer
3. Micropipette
4. Micropipette tips
5. Inoculation loop
6. Standard stains
7. Petri plates
8. Test tubes
9. Beaker
10. pH meter

Medias

1. Yeast
2. Peptone
3. Dextrose
4. Agar

Microorganism tested: *Malassezia furfur*

Test Drug: leaf extracts of *M. frondosa*

Standard drug: Fluconazole 150 mg/ml

METHODOLOGY

1. Preparation of different extracts (Aqueous, Hydro-alcohol, Chloroform.)
2. Preparation of Dixon's agar medium

3. Preparation of inoculum
4. Introducing inoculum in the medium through the well diffusion method.

1. Preparation of Extract [9]

Aqueous

A 10g dried powder of *M. frondosa* Linn. leaf was weighed and dissolved in 200 ml of water in a 500 ml beaker, then covered with aluminium foil.

The flask was kept shaking uniformly overnight using an orbital shaker and allowed to stand still for 18 hours to ensure thorough mixing and complete elucidation of the active materials' evaporation in the respective solvent.

The solvent was filtered using Whatman filter paper and dried over a water bath. The final volume was carefully dried using a crucible.

This obtained extract was weighed (6.36g) in an air-tight bottle and stored at -20˚ C for further studies.

Hydro-alcohol

8 g of dried powder of *M. frondosa* Linn. leaf was weighed and dissolved in 80 ml of Hydro-alcohol in a 500 ml beaker covered with aluminium foil.

The flask was shaken frequently for 6 hours and allowed to stand still for 18 hours for thorough mixing and complete elucidation of active materials' dissolution in the respective solvent.

The solvent was filtered using Whatman filter paper and dried over a water bath. The final volume was carefully dried using a crucible.

This obtained extract was weighed (1.096g) in an air-tight bottle and stored at -20˚ C for further studies.

Chloroform

8 g of dried powder of *M. frondosa* Linn. leaf was weighed and dissolved in 80 ml of Chloroform in a 500 ml beaker covered with aluminium foil.

The flask was shaken frequently for 6 hours and allowed to stand still for 18 hours for thorough mixing and complete elucidation of active materials' dissolution in the respective solvent.

The solvent was filtered using Whatman filter paper and dried over a water bath. The final volume was carefully dried using a crucible.

This obtained extract was weighed (0.297g), kept in an air-tight bottle, and stored at -20˚ C for further studies.

2. Preparation of Dixon's agar medium

15 mL of fluid (Part B) was suspended in 800 mL of double distilled water, and 106.5 g of Part A was added. The above mixture was mixed well and heated till boiling to dissolve the medium completely. The pH was adjusted to 5.6-6.0, and the volume was adjusted to 1000 mL by adding double distilled water. The medium was sterilised by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

3. Preparation of inoculum:

M. furfur was procured from the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. One loopful of 7-day-old culture from the slants was transferred to sterile water and mixed well to prepare a homogeneous inoculum.

4. Well diffusion method:

The medium was cooled to around 45-55˚C, and 20 ml of medium was poured into 3 sterile petri plates for three different extracts. 1mL of the inoculum was immediately added to each plate and swirled for uniform distribution. The wells were bored using a sterile borer. All three test samples, i.e Aqueous, Hydro-alcohol, Chloroform extracts of *M. frondosa* Linn. leaf, vehicle control and antibiotic, were dispensed separately into the respective wells of the petri plates. The plates were incubated overnight at 27˚C at BOD and observed after 7 days.

OBSERVATIONS AND RESULTS

Table no. 1 *In vitro* antifungal activity **of Aqueous extrac**t of *M. frondosa* leaf against *M. furfur*.

Sample	Volumes	Zone of inhibition (Radius in mm)	
Aqueous extract of *M. frondosa* leaves	25 µl	0	0
	50 µl	0	0

(10 mg/ml)	75 µl	0	0
	100 µl	0	0
Control (DD water)	50 µl	0	0
Standard (Fluconazole) 150 mg/ml	30µl	17	16

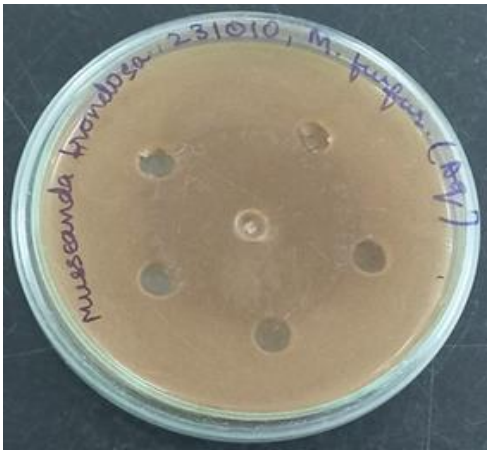

Fig no. 1 Showing the effect of Aqueous extract of *M. frondosa* Linn. leaf on *M. furfur*.

Table no. 2. *In vitro* antifungal activity of Hydro-alcoholic extract of *M. frondosa* leaf against *M. furfur*.

Sample	Volumes	Zone of inhibition (Radius in mm)	
Hydro-alcoholic extract of *M. frondosa* leaves (10 mg/ml)	25 µl	0	0
	50 µl	0	0
	75 µl	0	0
	100 µl	0	0
Control (Hydro-alcohol)	50 µl	0	0
Standard (Fluconazole) 150 mg/ml	30 µl	15	16

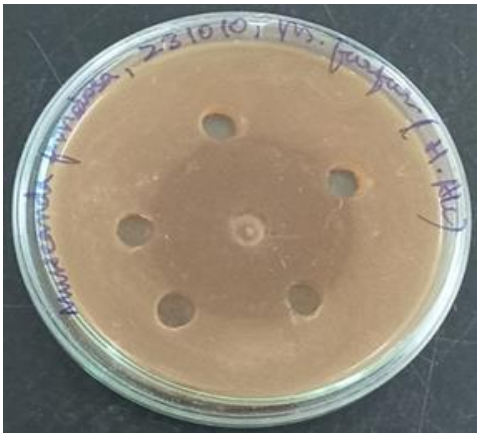

Fig no. 2 Showing the effect of Hydro-alcoholic extract of *M. frondosa* Linn. leaf on *M.furfur*

Table no. 3 *In vitro* antifungal activity of Chloroform extract of *M. frondosa* leaf against *M. furfur*.

Sample	Volumes	Zone of inhibition (Radius in mm)	
Chloroform extract of *M. frondosa* Leaves (10 mg/ml)	25 µl	0	0
	50 µl	0	0
	75 µl	0	0
	100 µl	6	6
Control (Chloroform)	50 µl	0	0
Standard (Fluconazole) 150 mg/ml	30 µl	20	20

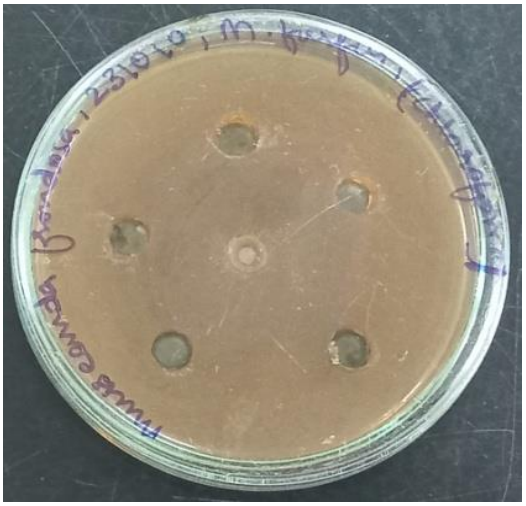

Fig no. 3 Showing the effect of Chloroform extract of *M. frondosa* Linn. leaf on *M.furfur*

DISCUSSION

Crude herbal remedies have been utilised in traditional medicine and home treatments for an extended period. However, not all herbal formulations have undergone scientific evaluation. Numerous studies have documented the antifungal properties of plants against the dandruff-inducing fungus *M. furfur*. In contrast, there is a scarcity of research concerning the impact of plant extracts on these fungi. The effects of different plant extracts on M. furfur, a yeast linked to dandruff, were investigated to assess the potential advantages of various herbal extracts—three different extracts of *M. frondosa.* Linn. leaves were prepared, i.e Aqueous, Hydro-alcohol and Chloroform; these extracts were studied for their potential antifungal activity against *M. furfur*. The study found that the aqueous and hydro-alcoholic extracts of the plant did not exhibit any antifungal activity against *M. furfur*. The chloroform extract of the plant exhibited antifungal activity against *M. furfur* at higher concentrations, possibly due to the presence of phenolics, flavonoids, and various other bioactive compounds.

CONCLUSION

The study demonstrates that the leaf extracts of *M. frondosa* exhibit significant antifungal activity against *M. furfur*, the primary fungus responsible for dandruff. Among the various extracts, the chloroform extract showed the most promising results, particularly at higher concentrations. The antifungal activity was attributed to the presence of bioactive compounds, specifically phenols and flavonoids, which are known for their antimicrobial properties. This research provides scientific validation for using *M. frondosa* in dermatological treatments, aligning with traditional practices while offering a promising alternative to synthetic antifungal agents.

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