



# STUDY OF ANTIFUNGAL ACTIVITY WITH NATURAL PLANT AND ANIMAL DERIVED PRODUCT IN CONTRADICTION OF DANDRUFF INSTIGATING FUNGUS *MALASSEZIA FURFUR*

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**Abstract:** Dandruff be situated very common scalp disorder by means of high prevalence in population that generally caused via *Malassezia furfur*. Most of the regularly available shampoos encompass some form of antifungal agent every so often remains chemical origin that have negative or else adverse impact that may don't gave satisfactory antifungal activity. Therefore, investigators find out a cost-effective natural product to overcome this problem. Our research based on use of natural products, is not only cost effective but also negligible side effects. In this study various animal and plant based extract such as cow urine, *Azardiractha indica* (Neem), Rice and *Citrus limon* (Lemon) were selected then assessed for antifungal activity against human dandruff causative agent (pathogen). Agent responsible for dandruff was isolated form different infected persons and characterized via morphological as well as cultural characters and identified that it may be *Malassezia furfur* fungi. The antifungal effect of extracts was assessed by measuring growth inhibition of test pathogen using three different technique pour plate, spread plate technique and disk diffusion method. Among them disk diffusion method gives promising result by using neem extract moreover, all remaining three extracts were showing less antifungal activity compared to neem extract.

**Keywords:** Antifungal, Cow urine, Dandruff, Lemon, *Malassezia*, Neem, Rice-water.

## I. INTRODUCTION

Although fungi are important for living things on earth for the reason that of their part in nutrient cycling, and their present and possible usages in pharmaceutical as well as in biotechnology, they similarly cause severe problems in many aspects of life. Some of these problems are obvious interrelated to the human beings. Human pathogenic fungi produced mycotoxin triggered serious concern to human health<sup>1</sup>. Dandruff is a scalp disorder whose distinguishing feature is disproportionate shedding of skin cells from the scalp. It is a communal problematic confronted by people of all age groups. It is one of the major cosmetic glitches as it eventually leads to hair fall. Symptoms include flaking and sometimes mild itchiness. It can result in social or self-esteem problems, seborrhoeic dermatitis, superficial fungal infections (SFIs) etc.<sup>2</sup>

*Malassezia furfur*, a lipophilic, dimorphic and yeast-like fungus, taking place in human skin as an opportunistic pathogen, reasons sicknesses such as dandruff, pityriasis versicolor and prime cause of the scalp disease known as seborrhoeic dermatitis or dandruff<sup>3,4</sup>. Alkaloids, flavonoids, saponins and tannins were existing as the major phytoconstituents in different spices. It is also detected that there was an increasing zone of inhibition with increasing concentration of extracts. Epidemiological prevalence of dandruff decreased with age: 21.6% in the age range 15–24 years, 19.7% in the age range 25–34 years, 17.4% in the age range 35–49 years, 14.3% in the age range 50–64 years, and 11.7% among people over 65 years old ( $p < 0.01$ ). Itching, prickling, tightness and pain were significantly more frequent in people reporting dandruff<sup>5</sup>.

Antique literature in Ayurveda states that there are natural preparations to treatment the ailments caused by this fungal infection. These natural remedies include effective methods such as treatment with rice-water, Neem extract, Lemon juice extract, cow's urine extract etc. <sup>6</sup>. Natural remedies execute *Malassezia* in addition other fungi and is highly effective in contradiction of the *Malassezia* species actually found on scalp. Reduction in fungi diminishes free fatty acids, thereby reducing scalp flaking by their primary and secondary metabolites. Kumar (2013) mentioned that now a day's individuals are depending on commercial shampoos encompassing antifungal compounds like miconazole, ketoconazole, selenium sulphide etc. Plant products comprehend various compounds like alkaloids, flavonoids, tannins, terpenoids etc. which have efficient antifungal activity<sup>6</sup>. Founded on this traditional and supplementary uses of *Azadirachta indica*, study was conducted to ascertain it is potentially pharmacologically active components and antibacterial activities <sup>7</sup>. Ayurveda already has immense literature that states the effectiveness of cow's urine (Sanskrit: *Gomutra*) in treating disease (Sanskrit: Panchgavya – Cowpathy or a treatment based on cow's products) <sup>8</sup>. Literature on Ayurveda states that rice-water is capable is inhibiting the growth of dandruff-causing fungi <sup>9</sup>.

## II. MATERIALS AND METHOD

### 2.1. Isolation

For the isolation of dandruff causative agent samples were collected from patients by gentle scalp and skin scrapings and preserved it. Make serial dilution (up to  $10^{3+}$ ) of the sample and 0.1 suspension spread on ampicillin contain modified oil-potato dextrose agar (Oil + PDA) medium. Incubate plates at  $28\pm 2^{\circ}\text{C}$  for 5-7 days. Fungal cultures were maintained on PDA medium and preserved culture for further experiments.

### 2.2. Cultural and morphological characterization of fungal isolates

Direct as well as microscopic examination of fungal isolates were carried out to know morphological and cultural characters. KOH test was carryout using wet mount KOH hair and scales specimens were subjected for direct examination by placing on a clean slide mounted with a drop of 10 % KOH (Merck) (to dissolved keratinized material) and covered with a cover slip. The slides were warmed gently (but not boiled to prevent crystallization of KOH) and examined under microscope (40X). Staining for microscopic observation of yeast/fungal cells was done by culture stained with lactophenol cotton blue stain on sterile glass slide.

### 2.3. Preparation of test material

#### 2.3.1. Plant ingredient extracts

Boiled white rice water – Rice were taken in sufficient quantities in a pressure cooker, and mixed with double amount of water, in 1:2 proportion. Once the rice was cooked and cooled, the boiled rice water for both varieties was drained into separate closed container and kept aside for an overnight. This was then used as the test material. Extract of neem leaves - Fresh leaves from neem tree were taken, washed well, cut to smaller pieces and mixed with 1-2 ml of water. The resultant was crushed into a thin paste used pestle and mortar. This was then centrifuged at 4000 rpm and the supernatant was used as test material.

Extract from lemon juice - fresh juice was extracted from lemons, without the seeds and stored in a closed container, this being used as the test material (Fig 1).

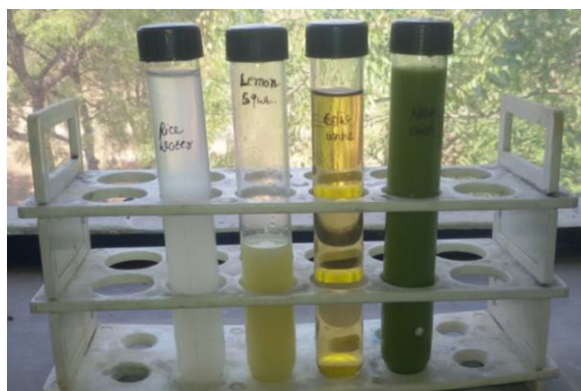


Figure 1 Rice, Water, Lemon extract and cow urine

### 2.3.2. Collection of cow's urine sample

Fresh urine sample was collected from the indigenous cow breed. This was cleared from any other particulates and used as the test material.

## 2.4. Natural remedies tests

### Pour-plate test

1ml of – boiled rice water, neem extract, lemon juice and cow's urine sample were pour in a sterile melted PDA medium and poured into petri dishes and allowed to solidify. With the help of cotton swab introduce fungal growth on PDA plate. These plates were kept aside undisturbed for 5-6 days at room temperature and growth pattern was recorded.

### Spread-plate test

Sterile PDA medium was pour in sterile petri dishes. 1ml of – boiled rice water, neem extract, lemon juice and cow's urine sample were introduced on the solidified agar surfaces, and evenly spread using glass rod. Fungi were introduced by cotton swab. These plates were kept aside undisturbed for 5-6 days at room temperature and growth pattern was recorded.

### Disc- diffusion technique test

Sterile paper disc was deep into the samples of boiled rice water, neem extract, lemon juice, cow's urine. Disc was simply dried and were placed on a sterile PDA medium. With the help of cotton swab introduce fungal growth on PDA plate. These plates were kept aside undisturbed for 5-6 days at room temperature and growth pattern was recorded.

## 2.5.Measurement of zone of inhibition

Zone of growth inhibition of *Malassezia* around the disks and on plate were measured continuously up to 5-6 day. The sensitivities of the microbial species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks. Zone of inhibition on the plate were measured and the activity for extract was scored using symbols and expressed as inactive (-), not - detectable (ND), mild (+), moderate (++) and significant (+++) and strong (++++).

## III. RESULTS AND DISCUSSION

### 3.1.Isolation

Out of the four skin samples total 4 type of fungal isolates were obtained on PDA plate. All isolates were purified and preserved for further study. All culture was well studied by their morphological and culture characters. Between them one isolate was shown to be similar to the morphology and culturally to the *Malassezia* sp. which was isolated by using potato dextrose agar from patients suffering from dandruff (Fig 1).

Findings was reported by Mehdi et al. 2016 they were isolated from patients with *Malassezia* infections. method: Twenty-six (26) *Malassezia* sp. identified by RFLP PCR, were isolated from patients with *Malassezia* infections<sup>10</sup>. Zareei et al. (2015) revealed that *M. furfur* was observed by molecular analysis with direct DNA extraction from scalp<sup>11</sup>.

### 3.2.Cultural and morphological characterization of fungal isolates

For microscopic appearance of isolate specimens were observed with lactophenol cotton blue stain separately and observed for its morphological appearance. The colonies showed oval, spherical or cylindrical shape. Morphology of isolate observed that umbonate, smooth, friable, convex, cream in colored (Fig 3). The specimens mounted with 10 % KOH showing oval or spherical shapes yeast cells under microscopic observation. The samples were also showed positive for catalase due to the presence of gas bubbles in reaction to 10% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The morphological and cultural characters indicate that isolated fungal culture may be *Malassezia furfur*.

Similar findings reported by Awad (2019) that *M. furfur* was examine cultural characters and morphological characters under microscope and informed that morphological characteristics of *M. furfur* cell are oval and cylindrical spherical while colony morphology showed that smooth umbonate, slightly folded with convex elevation, Mat and dull in appearance<sup>12</sup>.



Figure 2 Pure culture of isolate

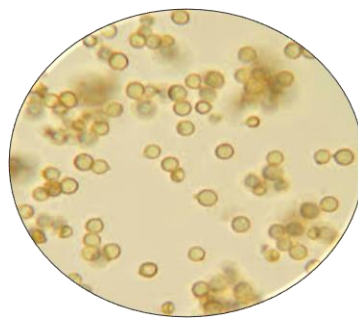


Figure 3 Spore of fungal isolate

### 3.3.Natural remedies tests

A comparative study was conducted on the levels of effectiveness, of natural remedies such as cow's urine, lemon, boiled rice water and neem extract for curing dandruff and check the inhibitory range of the growth on dandruff causative fungus *Malassezia*. Various plating techniques were applied using modified oil - potato dextrose agar as the nutrient medium for the fungal growth. The antifungal activity of plant extracts against *Malassezia* isolates was done. Results revealed that neem extract was found to be more stable and effective to inhibiting the fungus. Lemon juice was exceedingly effective though over a shorter period in inhibiting the fungus while cow urine showed least effectiveness whereas boiled rice-water did not show significant effectiveness on fungal growth (Table-1).

Table 1 Antifungal effectiveness

Remedy	Technique	Percentile of Effectiveness/ Inference
Rice Water Extract	Pour-plate Spread plate Disc diffusion	Less effective
Neem Leaves Extract	Pour-plate Spread plate Disc diffusion	More effective Neem leaf extract is highly effective in Inhibiting fungal growth and has the high stability.
Lemon Juice Extract	Pour-plate Spread plate Disc diffusion	Less effective followed by neem juice.
Cow's urine extract	Pour-plate Spread plate Disc diffusion	Least effective

All the three techniques used for the tests with the natural remedies were found effective in controlling the growth of fungi each natural therapeutic was found effective to a certain level in controlling / inhibiting the growth of the dandruff causing fungi. Comparative studies ascertained the fact that each remedy had its own characteristic level in inhibiting the growth (Table 2). Neem extract was highly capable in controlling/inhibiting the growth of fungi.

Neem Juice extract was effective in inhibiting the fungus than lemon extract beside rice water extract was not effective to controlling/ inhibiting the growth of fungi though the inhibitory/ containing action. Cow urine extract was not capable of controlling/ inhibiting the growth of fungi (Table-3).

Table 2 Effect of natural remedies on fungal isolate

Natural remedies	Antifungal activity
Neem extract	+++
Lemon extract	+++
Boiled rice water	+
Cow's urine	+

Inactive (-), Not - detectable (ND), Mild (+), Moderate (++) , Significant (+++) and Strong (++++)



Table 3 Paper disc method

Sample	Paper disc method (mm) zone of inhibition
Neem extract	18 mm
Lemon extract	15 mm
Boiled rice water	2 mm
Cow's urine	5 mm
Control	0 mm

*M. furfur* remained isolated from healthy adults and adult patients with atopic dermatitis. The growth of all the strains was inhibited by 0.5 to 32 mg/L of tacrolimus, whereas cyclosporin showed no such antifungal activity<sup>13</sup>. The results demonstrated that the aqueous extract of *Ilex paraguariensis* (1000 mg/ml) possesses inhibitory activity against *M. furfur*<sup>14</sup>. Sathishkumar et al. (2016) revealed that the most important component was identified as Thymol (phenolic rich compound) (41.3%) in *Coleus amboinicus* and in *Eucalyptus globules* the major constituents was 1-8, Cineol (91.61%). In the antidandruff activity the broad range of inhibitory zone was observed in *Eucalyptus globules* (37mm) oil compare to *Coleus amboinicus* (31mm)<sup>15</sup>.

#### IV. CONCLUSION

Crude animal and plant extracts have been included in traditional medicine and household remedies for a long time. Not all herbal preparations have been scientifically tested. Many studies are reported on the antifungal activity of plant essential oils against dandruff causing fungi *Malassezia furfur*. There are meagre studies on the effect of plant extracts on these fungi. From the comparative study, we concluded that neem juice was most effective inhibiting/ controlling the growth of fungi, whereas lemon juice extract, rice water and cow urine extract were less effective. Previous studies have also proven that natural remedies such as neem solutions can prove effective in inhibiting or controlling fungal growth.

#### V. CONFLICT OF INTEREST

Authors declared they have no conflict of interest.

#### VI. ACKNOWLEDMNET

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