

# Antifungal Efficacy of *Moringa oleifera* Lam.

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## ABSTRACT

**Background:** The success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant microbes. *Moringa oleifera* has been traditionally used in Indian folklore medicine for the treatment of various microbial infections. **Objective:** To investigate antifungal activity and explore the phytochemistry of methanolic and ethyl acetate leaf extracts of *M. oleifera*.

**Methods:** Disc diffusion and broth dilution methods were used for antifungal activity and standard qualitative chemical tests for the identification of phytoconstituents.

**Results:** Both the plant extracts showed antifungal activity against *Rizopus stolonifer* and *Microsporum gypsum*. Ethyl acetate extract was more active against *M. gypsum*, while *R. stolonifer* was more sensitive to methanolic extract. MIC ranged from 1.56 to 6.25mg/ml for both the extracts. The phytochemical analysis revealed the presence of alkaloids flavonoids, glycosides, tannins, triterpenoids and steroids.

**Conclusion:** The study showed potential antifungal activity of *M. oleifera* extracts, particularly against dermatophytic fungi, *M. gypsum*, therefore, provides justification for the use of the plant species in folk medicine to treat skin and other infectious diseases.

**Keywords:** Antifungal activity, Chemotherapy, Folklore medicine, *Moringa oleifera*, Phytochemistry.

## INTRODUCTION

Plants remained an important resource to combat serious diseases in the world. The traditional medicine still plays a vital role to cover the basic health needs in the developing countries. In the last few

decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in both developing and developed countries, owing to its natural origin and lesser side effects<sup>1</sup>. In recent

years considerable attention has been devoted to medicinal plants with antimicrobial properties. The antimicrobial studies are commonly postulated to play an important role in preventing diseases caused by resistant microbes.

The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds<sup>2</sup>. Phenolic compounds are known to possess different pharmacological activities, among which antioxidant and antimicrobial effects have recently received more attention. Plants generally contain 10 phytoconstituents *viz.* anthraglycosides, arbutin, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenols, carboxylic acids, terpenes and valepotriates. These phytoconstituents confer specific characteristics and properties to plants. Therefore, the analysis of these constituents would help in determining various biological activities of plants.

There is much literature concerning the antimicrobial properties of many species from Moringaceae family. *Moringa oleifera* is a tree and belongs to the family Moringaceae. It is known as a 'Miracle tree' as almost every part of it possesses products useful for humans. The leaves and pods are eaten. The plant is medicinally important and is traditionally used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant<sup>3</sup>. Leaves are also known to have antioxidant properties and are known to cure hallucinations, dry tumors, hiccups and asthma<sup>4</sup>. Considering therapeutic potential of this plant, the present study was carried out to explore the antifungal activity of *M. oleifera* leaf against fungi normally implicated in many diseases and

phytochemical screening to determine the phytoconstituents present in the plant.

## MATERIALS AND METHODS

### Plant material

Fresh leaves of *Moringa oleifera* were collected from different areas of Agra region. Collected material was thoroughly washed, shade dried, made to coarse powder, packed in polythene bags and stored for further analysis. The plant species was identified in the Department of Botany, School of Life Sciences, Khandari Campus, Dr. B. R. Ambedkar University, Agra and by other renowned taxonomists of the area.

### Extraction of active principles

Plant material was extracted in ethyl acetate and methanol using soxhlet extraction method. After extraction, the traces of solvent was evaporated to get the crude extract using a rotary evaporator. The crude extract was stored in refrigerator at 4°C for antifungal activity.

### Test organisms

The pure fungal culture of *Rhizopus stolonifer* (MTCC No. 2198) and *Microsporum gypseum* (MTCC No. 2819) were used in the study. The test microorganisms were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The fungi were periodically subcultured and maintained in Potato Dextrose Agar (PDA) and Sabroux's Dextrose (SDA) Agar media.

### Antifungal assay

*In vitro* antifungal activity of selected plant extracts was determined by disc diffusion method<sup>5</sup>. For susceptibility testing, crude extract was made into a suspension using Dimethyl sulphoxide (DMSO). The concentration of the material was made to 200mg/ml and further

concentrations were prepared by serial dilution. Sterile discs having a diameter of 6 mm were impregnated with 25µl of each serial dilution of extracts and dried in an incubator to remove the solvent. On the other hand fungal strains were spread on the surface of agar plate aseptically by sterile cotton swab. The sterile discs loaded with extracts were placed on inoculated surface of agar plate with the help of sterile forceps. These plates were incubated for 24-72 hours at 25°C. The diameter of the zones of inhibition around each of the disc was taken as measure of the antifungal activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was measured in millimeter.

#### Phytochemical analysis

Qualitative screening of leaf extracts of *M. oleifera* was performed for the identification of various classes of active chemical constituents like alkaloids, carbohydrates, glycosides, proteins, amino acids and steroids using standard procedures<sup>6-8</sup>.

#### Statistical analysis

Each experiment has three replicates and three determinations were conducted. Means of variable and standard deviations were recorded.

## RESULTS AND DISCUSSION

#### Antifungal activity

Methanolic and ethyl acetate extracts of *Moringa oleifera* leaves were found to be effective against both the fungi. The ethyl acetate extract showed maximum activity (9.67±1.57mm) and (8.67±151mm) against *M. gypseum* and *R. stolonifer*, respectively (Table 1). Methanolic extract was more effective against *R. stolonifer*, showed activity at all the test extracts, with highest activity of 9.66mm (Table 2).

The MIC was found to be 1.56mg/ml (ethyl acetate extract against *M. gypseum* and methanolic extract against *R. stolonifer*) and 6.25mg/ml (ethyl acetate extract against *R. stolonifer* and methanolic extract against *M. gypseum*).

The findings of the present study reveal that both the plant extracts were active against test fungi. The activity was both solvent dependent and pathogen dependent. Similar trend was also observed in some previous reports on antimicrobial activity of different parts of *M. oleifera*<sup>9-11</sup>. Ethyl acetate extract was more active against *M. gypseum*, while *R. stolonifer* was more sensitive to methanolic extract. The antifungal activity of the crude extract might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane<sup>12</sup>, and affect the growth of filamentous fungi mainly by causing membrane permeabilization<sup>13</sup>. Both the plant extracts worked in dose dependent manner, as the concentration of the extract was decreased the activity also decreased. This is due to susceptibility of the pathogen towards concentration of the extracts, after which the extract damages that microbe which is not tolerable for it<sup>14</sup>.

#### Phytochemical analysis

The result of the phytochemical screening of *M. oleifera* leaf extracts is presented in Table 3. The screening showed that the plant contains alkaloids, flavonoids, glycosides, tannins, triterpenoids and steroids. However, the tannins were found to be absent in methanolic leaf extract.

Phytochemical components are responsible for both pharmacological and toxic activities in plants. These medicinally bioactive components exert antimicrobial action through different mechanisms. Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes

with proline rich protein<sup>15</sup>. The saponins have the ability to cause leakage of proteins and certain enzymes from the cell<sup>16</sup>. Terpenoids are responsible for dissolution of the cell wall of microorganism by weakening the membranous tissue<sup>17</sup>. Flavonoids which have been found to be effective antimicrobial substances against a wide array of microorganisms *in vitro* are known to be synthesized in response to microbial infection by plants. They have the ability to bind with extracellular and soluble proteins and complexes with bacterial cell walls. Steroids are known for their antibacterial activity specifically associated with membrane lipids and cause leakage from liposomes<sup>18</sup>. According to Dahot<sup>19</sup> *M. oleifera* leaf extracts contain small peptides which could play an important role in the plant's antimicrobial defense system. The proteins/peptides are believed to be involved in a defense mechanism against phytopathogenic fungi by inhibiting the growth of micro-organisms through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall<sup>20</sup>.

The presence of these chemical constituents in this plant is an indication that the plant, if properly screened, could yield drugs of pharmaceutical significance. This is better supported by the fact that members of the family of this plant have been known to be involved in ethnomedicine in the management of various ailments<sup>21,22</sup>.

A large number of phytoconstituents were detected in *Moringa oleifera* leaves in previous studies. These phytochemical compounds are known to be responsible for bioactivities in medicinal plants and thus support the antifungal activity of the plant extract used in this study.

## CONCLUSIONS

Based on the findings, it may be concluded that antifungal activity is both

solvent and pathogen dependent. Both methanolic and ethyl acetate extracts showed dose dependent antifungal activity. The results of this study are very encouraging, however, further study on chemical constituents and their mechanisms in exhibiting certain biological activities are needed to understand the complex pharmacological effects of the plant species. The plant is active against *M. gypseum*, a dermatophytic fungus, therefore, it could be used as an alternate source for the treatment of skin diseases caused by the dermatophytes.

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**Table 1.** Antifungal activity of *M. oleifera* ethyl acetate leaf extract

Test organism	Zone of inhibition (mm)						
	200	100	50	25	12.5	6.25	3.125
<i>R. stolonifer</i>	8.67±1.51	8.33±1.08	7.67±0.52	7.33±0.58	7.00±1.00	6.67±0.58	-
<i>M. gypseum</i>	9.67±1.57	8.66±0.58	8.33±0.57	8.00±1.00	7.67±0.58	7.33±0.57	6.67±0.58

**Table 2.** Antifungal activity of *M. oleifera* methanolic leaf extract

Microorganism	Zone of inhibition (mm)						
	200	100	50	25	12.5	6.25	3.125
<i>R. stolonifer</i>	9.66±2.30	8.67±1.15	8.33±0.58	7.66±0.58	7.33±0.57	6.67±0.58	6.33±0.57
<i>M. gypseum</i>	9.67±2.25	8.67±1.16	8.33±1.00	7.67±0.58	6.66±0.58	6.33±0.57	-

**Table 3.** Phytochemical analysis of different leaf extracts of *M. oleifera*

Plant Part	Extract	Alkaloid	Glycoside	Carbohydrate	Tannin	Flavonoid	Steroid	Triterpnoid
Leaf	Ethylacetate	+	-	+	+	+	-	+
	Methanolic	+	+	+	-	+	+	+

+: Present, - : Absent