



## Antifungal activity and preliminary phytochemical analysis of bark extracts of *Moringa oleifera* Lam.

Mudasser Zaffer<sup>1</sup>, Showkat Ahmad<sup>1</sup>, Rajendra Sharma<sup>1</sup>, Surabhi Mahajan<sup>2</sup>, Ankur Gupta<sup>2</sup>, Rajneesh Agnihotri<sup>1\*</sup>

<sup>1</sup>Department of Botany, School of Life Sciences, Khandari Campus, Agra, India

<sup>2</sup>Department of Microbiology, School of Life Sciences, Khandari Campus, Agra, India

Received: 04 October 2012

Revised: 18 October 2012

Accepted: 19 October 2012

**Key words:** antifungal activity, folkloric medicine, phytochemical, susceptibility.

### Abstract

The Chloroform, Ethyl acetate, Methanol and aqueous extracts of *Moringa oleifera* bark traditionally used in folkloric medicine for the treatment of various infections were investigated for their antifungal activity against 2 fungal strains viz., *Rizopus stolonifer* and *Microsporium gypseum* by disc diffusion method. The different extracts of the plant species reduced colony growth of both the fungi to a great degree compared with the control treatment. The test pathogens differed considerably with regard to their susceptibility to plant extracts. *Microsporium gypseum* was the most susceptible being inhibited by most of the extracts. Methanol was found to be the best extractive solvent for antifungal activity in the test plant. The preliminary phytochemical analysis of *Moringa oleifera* bark extract revealed the presence of alkaloids, flavanoids, phenols, tannins, saponins and steroids. The results provide justification for the use of the plant in folk medicine to treat various infectious diseases.

\*Corresponding Author: Rajneesh Agnihotri ✉ [rk\\_agnihotri@rediffmail.com](mailto:rk_agnihotri@rediffmail.com)

## Introduction

Infections due to pathogenic bacteria and fungi represent a critical problem to human health. The microorganisms have developed resistance to many antibiotics because of indiscriminate use of antimicrobial drugs that create a big problem in the treatment of infectious diseases (Davis, 1994). With the increase in resistance of many microorganisms to the currently used antimicrobials and the high cost of production of synthetic compounds, there is a need to look for the alternatives (Marjorie, 1999). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe *et al.*, 1998). Phytoconstituents confer specific characteristics and properties to plants. Therefore, the analysis of these constituents in plants would help in determining various biological activities of plants.

*M. oleifera* tree is known as a 'Miracle tree' as almost every part of this tree possess products useful for humans. The plant is also reported to be medicinally important and almost all parts of the *M. oleifera* tree are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant (Shindano and Kasase, 2009).

Considering the importance of this plant, an attempt was made in the present study to investigate the *in vitro* antifungal activity and phytochemical screening of *Moringa oleifera* bark extract to detect the phytoconstituents present in the plant.

## Materials and methods

### *Collection of plant material*

The Bark of *Moringa oleifera* was collected from different areas of Agra region. Collected material was shade dried, made to coarse powder and then packed in polythene bags for further analysis.

### *Extraction of active principles*

The weighed bark powder was extracted with chloroform, ethyl acetate, methanol and distilled

water using Soxhlet apparatus. The solvent was evaporated to obtain the crude extract using a rotary evaporator and crude extract was stored in refrigerator for antimicrobial analysis.

### *Test organisms*

The pure fungal cultures of *Rhizopus stolonifer* (MTCC 2198) and *Microsporium gypseum* (MTCC 2819) were used in the study. The test microorganisms were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. The typed cultures of fungi were sub-cultured on Sabouraud dextrose agar slants and stored at 4°C prior to use.

### *Preliminary phytochemical analysis*

Qualitative screening of various extracts of stem bark of *Moringa 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 (Raman, 2006, Harborne, 2005, Wagner and Bladt, 1996, Ahmad *et al.*, 2012).

### *Antifungal activity assay*

*In vitro* antifungal activity of selected plant extracts was determined by disc diffusion method as described by Kohner *et al.* (1994). For susceptibility testing, crude extract was made into a suspension using different solvents. The concentration of the material was made 200mg/ml and further concentrations were prepared by serial dilution. Sterile discs having a diameter of 6 mm were impregnated with 25 µl of each serial dilution of extracts and dried in an incubator to remove the solvent. 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 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.

**Statistical analysis**

The results of the experiments are given as mean  $\pm$  SD. The results given are the average of three determinations.

**Result and discussion**

The results of antifungal activity are summarized in Table 1-4 and Fig. 1-4 The study revealed that the

ethylacetate bark of *M. oleifera* at six different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml) showed considerable activity against both *R. stolonifer* and *M. gypseum*, showing zone of inhibition up to dilution of 6.25mg/ml. Bhatnagar (1961) also reported that the bark extract of *M. oleifera* have antimicrobial activity.

**Table 1.** Antimicrobial activity of *M. oleifera* ethyl acetate bark extract against different test microorganisms.

Pathogens	Zone of inhibition in (mm)							Drug
	200	100	50	25	12.5	6.25	3.125	
<i>R. stolonifer</i>	9.67 $\pm$ 1.52	8.33 $\pm$ 1.15	7.66 $\pm$ 0.58	7.33 $\pm$ 0.57	7.00 $\pm$ 0.57	6.66 $\pm$ 0.58	-	-
<i>M. gypseum</i>	10.66 $\pm$ 0.52	9.66 $\pm$ 1.15	8.66 $\pm$ 0.58	8.00 $\pm$ 1.00	7.67 $\pm$ 0.58	7.00 $\pm$ 1.00	-	-

Data given are mean of triplicates,  $\pm$ : standard deviation, concentration (mg/ml)

**Table 2.** Antimicrobial activity of *M. oleifera* aqueous bark extract against different test microorganisms.

Pathogens	Zone of inhibition in (mm)							Drug
	200	100	50	25	12.5	6.25	3.125	
<i>R. stolonifer</i>	9.66 $\pm$ 1.52	8.33 $\pm$ 1.15	8.00 $\pm$ 0.57	7.66 $\pm$ 0.58	6.66 $\pm$ 1.15	6.33 $\pm$ 0.57	-	-
<i>M. gypseum</i>	9.00 $\pm$ 1.73	8.66 $\pm$ 1.15	8.33 $\pm$ 1.00	7.67 $\pm$ 0.58	7.33 $\pm$ 0.57	7.00 $\pm$ 1.00	6.67 $\pm$ 0.57	-

Data given are mean of triplicates,  $\pm$ : standard deviation, concentration (mg/ml)

**Table 3.** Antimicrobial activity of *M. oleifera* methanol bark extract against different test microorganisms.

Pathogens	Zone of inhibition in (mm)							Drug
	200	100	50	25	12.5	6.25	3.125	
<i>R. stolonifer</i>	9.00 $\pm$ 2.00	8.66 $\pm$ 1.52	8.33 $\pm$ 1.15	7.66 $\pm$ 0.50	7.00 $\pm$ 1.00	--	-	-
<i>M. gypseum</i>	8.66 $\pm$ 1.15	8.33 $\pm$ 1.00	7.66 $\pm$ 0.58	7.33 $\pm$ 0.57	7.00 $\pm$ 1.00	6.67 $\pm$ 0.58	-	-

Data given are mean of triplicates,  $\pm$ : standard deviation, concentration (mg/ml)

**Table 4.** Antimicrobial activity of *M. oleifera* chloroform bark extract against different test microorganisms.

Pathogens	Zone of inhibition in (mm)							Drug
	200	100	50	25	12.5	6.25	3.125	
<i>R. stolonifer</i>	9.33 $\pm$ 1.51	9.00 $\pm$ 1.00	8.33 $\pm$ 0.58	7.66 $\pm$ 1.15	7.00 $\pm$ 1.00	6.67 $\pm$ 0.58	6.33 $\pm$ 0.57	-
<i>M. gypseum</i>	9.00 $\pm$ 1.51	8.66 $\pm$ 1.52	8.33 $\pm$ 0.58	7.67 $\pm$ 1.15	7.33 $\pm$ 0.57	7.00 $\pm$ 1.00	-	-

Data given are mean of triplicates,  $\pm$ : standard deviation, concentration (mg/ml)

The aqueous and methanol stem bark extracts were found comparatively more active against *M. gypseum* than *R. stolonifer*, showing zone of inhibition upto the dilution of 3.125 and 6.25mg/ml respectively.

However the chloroform stem bark extract showed maximum antifungal activity against *R. stolonifer*, showing zone of inhibition up to dilution of

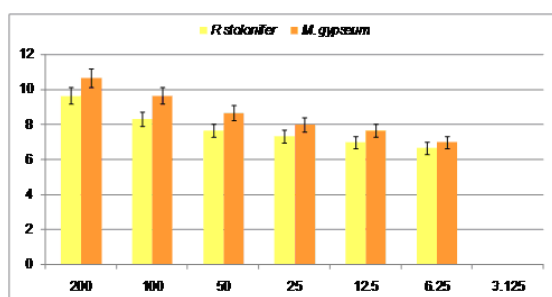
3.125mg/ml. Moderate activity was found against *M. gypseum* showing zones up to the dilution of 6.25mg/ml. The antimicrobial activity of the crude extract might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane (Body and Beveridge, 1981), and affect the growth of filamentous fungi mainly by causing membrane permeabilization (Huang *et al.*, 2000).

**Table 5.** Phytochemical analysis of different bark extract of *M. oleifera*

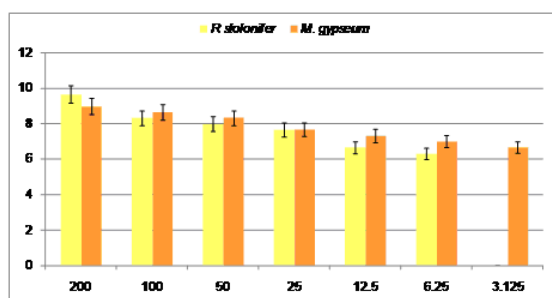
Plant	Extract	Alkaloid	Glycoside	Carbohydrate	Tanin	Flavonoid	Steroids	Triterpnoide
<i>M. oleifera</i>	Ethyl acetate	+	-	-	+	-	+	+
	Water	+	-	+	+	+	+	+
	Chloroform	+	+	-	+	-	+	+
	Methanol	+	-	+	+	+	-	+

+: Present, -: Absent

The results of preliminary phytochemical study are tabulated in Table-5. The analysis revealed the presence of alkaloid, flavonoids, phenols, saponins, steroids and tannins which could be responsible for the observed antimicrobial property.



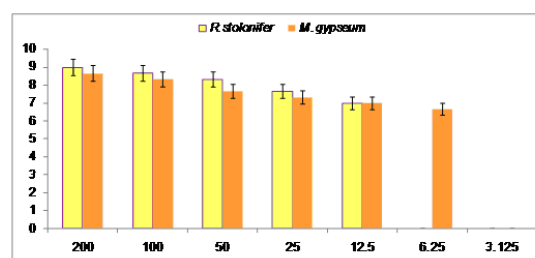
**Fig. 1.** Graphical representation of antifungal activity of *M. oleifera* ethyl acetate bark extract



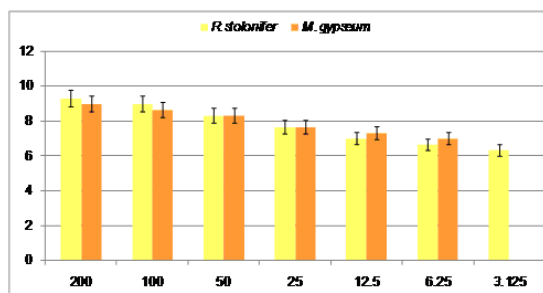
**Fig. 2.** Graphical representation of antifungal activity of *M. oleifera* aqueous bark extract.

These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Phytochemical constituents such as alkaloids,

flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against predation by many microorganisms, insects and other herbivores (Bonjar *et al.*, 2004).



**Fig. 3.** Graphical representation of antifungal activity of *M. oleifera* methanol bark extract.



**Fig. 4.** Graphical representation of antifungal activity of *M. oleifera* chloroform bark extract.

The present study provides the useful information about antimicrobial properties of *Moringa oleifera*, which is used for the therapeutic purposes. The obtained results provide a support for the use of this

plant in traditional medicine and the findings of this study support the fact that *M. oleifera* is a promising source of potential antimicrobials and suggest its further advance investigation.

#### Acknowledgements

Financial assistance provided by Govt. of U.P (Higher Education) in the form of Centre for Excellence to the Department of Botany is gratefully acknowledged.

#### References

**Ahmad S, Sharma R, Mahajan S, Agnihotri R, Gupta A.** 2012. Antibacterial evaluation and preliminary phytochemical analysis of leaf extract of *Holoptelea integrifolia*. J. Phar. Res. **5(7)**, 3823-3825.

**Bonjar GHS, Nik AK, Aghighi S.** 2004. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J. Biol. Sci. **4**, 405-412.

**Boyd I, Beveridge EG.** 1981. Antimicrobial activity of some alkyl esters of gallic acid (3, 4, 5-trihydrobenzoic acid) against *E. coli* NCTC 5933 with particular reference to n-propyl gallate. Microbios **30**, 73-85.

**Davis J.** 1994. Inactivation of antibiotics and the dissemination resistance genes. Science. **264**, 375-382

**Harborne JB.** 2005. Phytochemical Methods. Springer (India) Pvt. Ltd., New Delhi 17.

**Haug X, Xie WJ, Gong ZZ.** 2000. Characterization and antifungal activity of a chitin binding protein from *Ginkgo biloba*. FEBS. Lett. **478**, 123-126.

**Ibrahim MB.** 1997. Anti-microbial effects of extract leaf, stem and root bark of *Anogeissus leiocarpus* on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris*. J. Pharma. Devpt **2**, 20-30.

**Kohner PC, Rosenblatt JE, Cockerill FR.** 1994. Comparison of agar dilution, broth dilution and disk diffusion testing of Ampicillin against *Haemophilus* spp. by using in house and commercially prepared media J. Clin. Microbiol. **32**, 1594 -96.

**Marjorie MC.** 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews **12**, 564-582.

**Ogundipe O, Akinbiyi O, Moody JO.** 1998. Antibacterial activities of essential ornamental plants. Nigeria J. Natural Products & Medicine **2**, 46-47.

**Raman N,** 2006. Phytochemical Technique. New Indian Publishing Agencies, New Delhi. 19.

**Wagner H, Blatt S.** 1996. Drug Analysis.