Anti-microbial and Phytochemical Studies of *Mussaenda frondosa* Linn. Leaves

S.Santhi¹*, R.Radha²

**ABSTRACT**

*Mussaenda frondosa* L. (Rubiaceae) has been traditionally used in the treatment of White leprosy, eye troubles, skin infections, tuberculosis, jaundice, ulcers, wounds, cough and Bronchitis. The current study investigated antimicrobial effects of *Mussaenda frondosa* L against bacteria and fungus. In addition, Phytochemical profiling of the methanolic extract of *Mussaenda frondosa* was done using High Performance Thin Layer Chromatography (HPTLC). The antimicrobial activity of Methanol (MEMF), Ethyl acetate (EEMF), Chloroform (CEMF) and Hexane (HEMF) extracts of *Mussaenda frondosa* leaves were tested against nine bacterial and four fungal strains. The Methanol extract showed significant antibacterial and antifungal activity than hexane, Chloroform, Ethyl acetate extracts which could be attributed to the presence of phenols, flavonoids and the other bioactive compounds identified through phytochemical screening. The findings in the present study offer a scientific support to the ethno medicinal use of the plant by the traditional healers.

**Key words:** Antibacterial, Antifungal, High Performance Thin Layer Chromatography (HPTLC), *Mussaenda*, Extract.

**INTRODUCTION**

Infectious diseases are an important cause of mortality and morbidity, in all regions of the world. The increasing emergence of fannimicrobial resistance worsens the impact.¹,² Increased prevalence of resistant bacteria, together with lack and high cost of new generation drugs has escalated infection-related morbidity and mortality particularly in developing countries.³ Antibiotics are crucial in the treatment of several severe infections such as pneumonia, tuberculosis and meningitis. Thus, the emergence of the multi drug resistant strains poses a huge challenge for humanity to combat.⁴ Therefore; there is an ever growing demand for the new antibiotics that are cost effective and easily available to the common people.

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance.⁵ Therefore, a greater attention has been paid to antimicrobial activity screening from natural source.

*Mussaenda frondosa* L. belonging to the family Rubiaceae has been traditionally used in the treatment of White leprosy, eye troubles, skin infections, tuberculosis, jaundice, ulcers, wounds, cough and Bronchitis. A weak decoction of dried shoots is given to children to relieve cough.⁶ Traditional claim indicates the use of the plant against infections. Literature survey revealed that there is no scientific study on antimicrobial efficacy of the plant. Hence an attempt has been made to investigate antimicrobial effects of *Mussaenda frondosa* L against pathogenic bacteria and fungus. The methanol, chloroform, Ethyl Acetate and Hexane extracts of the leaves of the plant *Mussaenda frondosa* L. were subjected to antibacterial studies against Gram positive and Gram negative bacteria and antifungal studies.

The phytoconstituents such as rutin, quercetin, hyperin, sinapagic acid, Ferulic acid and stigluside which were isolated by droplet counter current chromatography from the methanolic extract of the sepals of *Mussaenda frondosa* L.⁷ The petals of *Mussaenda frondosa* Linn was found to have antibacterial activity against *Saccharomyces cerevisiae*, *Ustilago Mayadis*, *Escherichia coli*, *Micrococcus luteus*, *Bacillus subtilis* and *Bacillus cereus*.⁸ The methanolic extract of *M. frondosa* was found to possess hypolipidemic activity in high fat diet fed rats. The aqueous and alcoholic extract of the *Mussaenda frondosa* showed significant hepatoprotective activity in paracetamol induced liver damage model in Wistar rats.⁹

The protective effect and possible mechanism of alcoholic and aqueous extract from *Mussaenda frondosa* Linn was studied on Ethanol induced hepatic injury in Wistar rats.¹⁰ A perusal of literature revealed that no detailed study on the efficacy of the leaves of *Mussaenda frondosa* on pathogenic micro organisms.

**MATERIALS AND METHODS**

**Collection and authentication**

The leaves of *Mussaenda frondosa* L. were collected from Kodai hills, Tamil Nadu. The plant was identified, confirmed and authenticated by comparing with a Herbarium by a botanist. Dr. Jayaraman, Plant anatomical Research Centre (PARC), Tambaram, Chennai.

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Extraction

The freshly collected plant materials were cut into small pieces, shade dried and coarsely powdered. The powdered material was successively extracted with n-hexane (HEMF), chloroform (CEMF), ethyl acetate (EEMF) and methanol (MEMF) in an aspirator bottle by cold percolation method for 72 hrs. The extracts were filtered and concentrated in rotary evaporator. These extracts were subjected to antimicrobial activity, phytochemical screening and HPTLC analysis.

High performance thin layer chromatography

The MEMF, EEMF, CEMF and HEMF extracts under study, each (1 g) were dissolved in the solvents such as methanol, ethylacetate, chloroform, n-hexane individually on a water bath, filtered and made up to 10ml in a standard flask. Samples (10 µl) were applied using Aluminium sheets precoated with silica gel merck 60F 254, 0.2 mm layer thickness (10 x 10 cm) as a stationary phase. Toluene: Ethyl Acetate: Formic acid [7: 2.5: 0.5] was used as a mobile phase and the scanning was performed at 254nm using 'CAMAG' densitometer scanner.

Antibacterial activity

The organisms used were clinical samples of Madras Medical College which was identified and maintained in the Department of microbiology. The antibacterial activity was done by determining the Minimum Inhibitory Concentration (MIC) and disc diffusion assay according to the method recommended by Clinical and Laboratory Standards Institute (CLSI).

Microorganisms

The organisms used were Gram positive organisms (Coagulase negative Staphylococcus, Staphylococcus aureus) and Gram negative organisms (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B and Vibrio cholerae). The organisms were maintained on Nutrient agar slopes after confirmation by biochemical tests and stored at 4°C.

Preparation of the bacterial suspension for inoculation

Few colonies of the pathogenic strains were picked and inoculated into 4ml of peptone water. These tubes were incubated for 2 to 5 hours to produce a bacterial suspension. The suspension was then diluted, if necessary with saline solution to density visually equivalent to that of standard (0.5ml of 1% Barium chloride to 99.5ml of 1% sulphuric acid (0.36N)). This suspension was then used for seeding.

Determination of Minimum Inhibitory Concentration (MIC)

The extracts were prepared by dissolving the Methanol, Ethyl acetate, Chloroform and Hexane extract residue of the leaves of Mussaenda frondosa L. in dimethyl sulphoxide (DMSO). Mueller Hinton agar (MHA) was prepared and sterilized by autoclaving at 121°C at 15lbs for 15min. The fungi studied were filamentous in nature except for yeast, establishing a standardized inoculum for filamentous fungi. The fungi used were filamentous in nature except for yeast, establishing a standardized inoculum for filamentous fungi. The fungi, hence forth have been described but they are not widely accepted by shadomy et al., 1991. The fungi used were filamentous in nature except candida hence the inoculum could not be standardized. The fungi, hence forth were seeded directly on to the media.

The extracts were prepared by dissolving the MEMF, EEMF, CEMF and HEMF extract residue of the leaves of Mussaenda frondosa L. in Dimethyl sulphoxide (DMSO), Sabouraud’s Dextrose agar (SDA) was prepared and sterilized by autoclaving at 121°C at 15lbs for 15min.

Four fungal cultures were taken up for the study. Four slants of SDA media along with extract were prepared for each concentration (100, 150 and 200 µg / ml) and allowed to set. The different fungi were inoculated into SDA slant and incubated at 37°C for 1 to 4 weeks. The results were read by noting the presence (or) absence of growth in the slant.

RESULTS AND DISCUSSION

Mussaenda frondosa L (Family: Rubiaceae) has been traditionally used in the treatment of White leprosy, eye troubles, skin infections, tuberculosis, jaundice, ulcers, wounds, cough and Bronchitis. Based on the traditional claim the plant was evaluated for antimicrobial activity by invitro methods. The different extracts of Mussaenda frondosa L. were subjected to preliminary phytochemical investigation which shows the presence of flavonoid, saponin, Glicysides, sugar, steroid, mucilage, phenol and protein (Table 1).

HPTLC finger prints

HPTLC analyses of extracts were performed at 254 nm and the fingerprints were shown in Figure 1. MEMF showed 10 peaks at 0.11, 0.16, 0.23, 0.27, 0.37, 0.50, 0.58, 0.70, 0.81 and 0.88. The peak at 0.11 was found to have 32.65% and 0.50 was 4.07%.

EEMF indicated the presence of 9 peaks at 0.06, 0.09, 0.14, 0.21, 0.24, 0.41, 0.46, 0.55 and 0.62. The peak at 0.55 was found to have 23.21% and the peak at 0.24 was 0.02%.

Anti fungal activity

Microorganisms

The fungi studied were Trichophyton simii, Trichophyton mentagrophytes, Aspergillus niger, Rhizopus and Candida albicans. The fungi were maintained on Sabouraud’s Dextrose Agar (SDA) Slopes and stored at 4°C.

Determination of Minimum Inhibitory Concentration (MIC)

A fungus is large cells with more variation in size than bacterial preparation of a standard suspension of known CFU and is difficult to perform fungal susceptibility testing. It is much less standardized than the bacterial methods. Although possible for yeast, establishing a standardized inoculum for filamentous fungi is beyond the capability of most laboratories. Disc diffusion tests for testing antifungal antibiotics have been described but they are not widely accepted by shadomy et al., 1991. The fungi used were filamentous in nature except candida hence the inoculum could not be standardized. The fungi, hence forth were seeded directly on to the media.

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Table 1: Preliminary Phytochemical Analysis of Different Extracts of *Mussaenda Frondosa* Linn.

| Sl.No. | Phytoconstituents  | HEMF | CEMF | EEMF | MEMF |
|--------|-------------------|------|------|------|------|
| 1.     | Flavonoids        | -    | +    | +    | +    |
| 2.     | Steroids          | -    | +    | +    | +    |
| 3.     | Glycosides        | -    | -    | +    | +    |
| 4.     | Carbohydrates     | -    | +    | +    | +    |
| 5.     | Phenol            | -    | -    | +    | +    |
| 6.     | Tannin            | -    | -    | -    | +    |
| 7.     | Saponin           | -    | -    | -    | +    |
| 8.     | Alkaloid          | -    | -    | -    | -    |
| 9.     | Terpenoids        | -    | -    | -    | -    |
| 10.    | Anthraquinones    | -    | -    | -    | -    |
| 11.    | Quinones          | -    | -    | -    | -    |
| 12.    | Proteins & Amino acids | -    | -    | -    | +    |
| 13.    | Mucilage          | -    | -    | -    | +    |

MEMF-Methanol extract of *Mussaenda frondosa*, EEMF-Ethyl acetate extract of *Mussaenda frondosa*, CEMF-Chloroform extract of *Mussaenda frondosa*, HEMF-Hexane extract of *Mussaenda frondosa*.

+ indicates presence, - indicates absence

Figure 1: HPTLC Fingerprint Profile of Various Extracts.

(a) Methanol Extract  
(b) Ethyl Acetate Extract  
(c) Chloroform Extract  
(d) Hexane Extract
HEMF indicated the presence of 7 peaks with Rf 0.09, 0.23, 0.55, 0.63, 0.72, 0.78, and 0.85. The peak at 0.23 was found to have 39.71% and 0.72 was 4.05%. HPTLC fingerprinting will help to authenticate the plant in future.

**Anti bacterial studies**

The extracts viz. HEMF, CEMF, EEMF and MEMF were tested at various dose levels on the pathogenic bacteria. The results of MIC extracts of *Mussaenda frondosa* L. against the pathogenic bacteria were reported in Table 2. Sensitivity test was done by agar disc diffusion method and zone of inhibition were measured (Table 3). The results revealed that the extracts were found to be effective against all the pathogenic bacteria such as *coagulase negative staphylococcus*, *staphylococcus aureus*, *salmonella typhi*, *salmonella paratyphi A*, *salmonella paratyphi B*, *pseudomonas aeruginosa*, *klebsiella pneumoniae*, *vibrio cholerae* and *Escherichia coli*.

The MEMF inhibits the growth of *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi B* at the concentration of 100µg/ml whereas *coagulase negative Staphylococcus*, *Klebsiella pneumoniae*, *Vibrio cholerae* were inhibited at 133.33 µg/ml. The EEMF, CEMF and HEMF extract showed moderate inhibition against bacterial strains. The maximum zone of inhibition was obtained with MEMF extract against *Pseudomonas aeruginosa* (18 mm), *Salmonella typhi* (19mm) and *Salmonella paratyphi A* (18 mm).

**Antifungal studies**

Antifungal activity of MEMF, EEMF, CEMF and HEMF were observed in Sabouraud Dextrose Agar slants. The extracts were tested at different dose level against *Trichophyton mentagrophytes*, *Trichophyton simii*, *Aspergillus niger & Rhizopus* which cause *Tinea crui*, *Tinea pedis*, *Tinea capitis*, systemic aspergillosis, Invasive aspergillosis and Mucormycosis.

The extracts shows significant inhibition in growth of fungi in the agar slants. The MIC of extracts was shown in the Table 4. The MEMF inhibits *T.simii & T.mentogrophytes* at 150 µg/ml and *A. niger & Rhizopus* at 100 µg/ml. The MEMF also showed significant inhibition against *T.simii, T.mentogrophytes, A.niger & Rhizopus* whereas EEMF showed moderate inhibition.

**CONCLUSION**

The MEMF showed significant antibacterial and antifungal activity than HEMF, CEMF, EEMF extracts which could be attributed to the presence of phenolics, flavonoids and the other bioactive compounds identified through phytochemical screening. The findings in the present study offer a scientific support to the ethno medicinal use of the plant by the traditional healers. This plant can be suggested in the treatment of skin infection, enteric fever, cholera, urinary tract infection, wound infection, Nosocomial infection, Respiratory infection, Aspergillusosis and Mucormycosis.

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### Table 2: Minimum Inhibitory Concentration of the Extracts of *Mussaenda Frondosa* Linn.

| Extracts | MINIMUM INHIBITORY CONCENTRATION |
|----------|----------------------------------|
|          | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| MEMF     | 133.33 | 166.66 | 133.33 | 133.33 | 100 | 100 | 133.33 | 100 | 133.33 | 100 |
| EEMF     | 133.33 | 166.66 | 166.66 | 133.33 | 133.33 | 133.33 | 133.33 | 166.66 | 100 | 133.33 | 100 |
| CEMF     | 100 | 166.66 | 166.66 | 133.33 | 133.33 | 133.33 | 133.33 | 133.33 | 166.66 | 100 |
| HEMF     | 133.33 | 166.66 | 133.33 | 133.33 | 133.33 | 133.33 | 133.33 | 166.66 | 100 | 133.33 | 100 |

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### Table 3: Zone of Inhibition (Mm) of the Extracts of *Mussaenda Frondosa* Linn.

| Organisms | Zone diameter of ciprofloxacin (mm) | Zone diameter of MEMF (mm) | Zone diameter of EEMF (mm) | Zone diameter of CEMF (mm) | Zone diameter of HEMF (mm) |
|-----------|-----------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Coagulase negative Staphylococcus | 30 | 17 | 11 | 11 | 9 |
| Staphylococcus aureus | 19 | 15 | 12 | 11 | 9 |
| Escherichia coli | 29 | 17 | 14 | 11 | 11 |
| Klebsiella pneumoniae | 28 | 17 | 13 | 14 | 11 |
| Pseudomonas aeruginosa | 30 | 18 | 13 | 13 | 14 |
| Salmonella typhi | 38 | 19 | 16 | 12 | 12 |
| Salmonella paratyphi A | 34 | 18 | 12 | 16 | 11 |
| Salmonella paratyphi B | 23 | 16 | 11 | 13 | 9 |
| Vibrio Cholerae | 22 | 12 | 10 | 11 | 10 |

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### Table 4: Minimum Inhibitory Concentration of the Different Extracts of *Mussaenda Frondosa* Linn.

| Extracts | Trichophyton simii (µg/ml) | Trichophyton mentagrophytes (µg/ml) | Aspergillus niger (µg/ml) | Rhizopus (µg/ml) |
|----------|--------------------------|-----------------------------------|--------------------------|-----------------|
| MEMF     | 150                      | 150                               | 100                      | 100             |
| EEMF     | 200                      | 200                               | 100                      | 150             |
| CEMF     | 200                      | 200                               | 150                      | 150             |
| HEMF     | 200                      | 200                               | 150                      | 200             |

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MEMF-Methanol extract of *Mussaenda frondosa*, EEMF-Ethyl acetate extract of *Mussaenda frondosa*, CEMF-Chloroform extract of *Mussaenda frondosa*, HEMF-Hexane extract of *Mussaenda frondosa*.
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REFERENCES

1. Mulu A, Moges F, Tessema B, Kassu A. Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gonder Teaching Hospital, Northwest Ethiopia. Ethiop Med J. 2006;44(2):125-31.
2. Olivier C, Williams Jones B, Doize B, Ozdemir V. Global antibiotic resistance: ethical drug promotion in the developing world. In: De Sosa JA, Byarugaba DK, Amabile C, Hsueh PR, Kariuki S, Okeke IN, Editors. Antibiotic resistance in developing countries. New York: Springer; 2010:505-24.
3. Borkotoky R, Kalita MP, Barooah M, Bora SS, Goswami C. Evaluation and screening of antimicrobial activity of some important medicinal plants of Assam. International Journal of Advancements in Research & Technology. 2013;2(4):132-9.
4. Pallavali RR, Degati VL, Lomada D, Reddy MC, Durbaka VRP. Isolation and in vitro evaluation of bacteriophages against MDR-bacterial isolates from septic wound infections. PLoS ONE. 2017;12(7):e0179245.
5. Bonjar GHS. Screening for antibacterial properties of some Iranian plants against two strains of Escherichia coli. Asian J Plant Sci. 2004;3:310-4.
6. Anonymous. Wealth of India, Vol.VI, L-M. Publications and Information Directorate. India: Council of Scientific & Industrial Research; 1962.
7. Lakshmi DK, Girija AR, Venkata Rao D, Venkata Rao E. The phytoconstituents of methanolic extract of the sepals of Mussaenda frondosa L. Indian J Pharm Sci. 1985;47:122-3.
8. Jayasinghe ULB, Jayasooriya CP, Bandara BMR, Ekanayake SP, Mervilini L, Assante, G. Antimicrobial activity of some Sri Lankan Rubiaceae and Melliaceae. Fitoterapia. 2002;73:424-7.
9. Sambrekar SN, Patil PA, Kangsrakar VA. Protective activity of Mussaenda frondosa leaf extract against paracetamol induced hepatic damage in wistar rats. J Pharm Res. 2010;3(4):711-3.
10. Sambrekar Sudhir N, Patil PA, Patil Suhas A. Hepatoprotective activity of Mussaenda frondosa Linn extract in ethanol treated rats. International Journal of Drug Research and Technology. 2012;2(8): 446-63.
11. Harborne JB. Phytochemical Methods. 3rd ed. London: Chapman and Hall; 1984.
12. Sethi PD. Quantitative analysis of pharmaceutical formulations. 1st ed. India: CBS Publishers; 2013.
13. Hsueh PR, Ko WC, Wu JJ, Lu JJ, Wang FD. Consensus statement on the adherence to Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing Guidelines (CLSI-2010 and CLSI-2010-update) for Enterobacteriaceae in clinical microbiology laboratories in Taiwan. Journal of Microbiology, Immunology and Infection. 2010;43:452-5.
14. Anonymous. Indian Pharmacopoeia. 1st ed. India: Govt. of India, Ministry of Health and Family welfare, controller of Publication; 1996.
15. Bauer AW, Kirby WM, Sherrie JC, Turek M. Antibiotic susceptibility testing by a standard single disc method. Am J Clin Pathol. 1966;45:493.
16. Agarwal KC. Antibiotic sensitivity test by disc diffusion method, standardization and interpretation. Indian J Pathol Microbiol. 1974;17(3):149.
17. William Robert Bailey, Elvyn G Scott, Ellen Jo Baron, Sydney M Finegold. Bailey and Scott’s Diagnostic Microbiology. 9th ed. Saint Louis: Mosby; 1990.
18. Shadowy S, Pfaffer MA. Laboratory studies with antifungal agents: susceptibility tests and quantitation in body fluids. In: Balows, A., Hausler, W.J., Herrmann, K. L., Isenberg, H. D and Shadomy, H. J. (editors), Manual of clinical microbiology, American Society for Microbiology, Washington D.C.; 1991:1173-83.
19. Collee JG, Duguid, JP, Fraser AG, Marmion BP, Mackie, MC Cartney. Practical Medical Microbiology. 14th ed. India; Elsevier: 1989.

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Shanthi, et al.: Anti-microbial and Phytochemical Studies of Mussaenda frondosa Linn. Leaves

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