

EMERGENCE OF *MESUA FERREA* LINN. LEAF EXTRACT AS A POTENT BACTERICIDE

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ABSTRACT : *The methanolic extract of leaves of Mesua ferrea Linn. were tested for its antibacterial potentiality against 103 various strains of bacteria including Staphylococcus aureus, Bacillus spp., Klebsiella spp., Streptococcus pneumoniae, Sarcina lutea, Lactobacillus arabinosus, Escherichia coli, shigellae, salmonellae, Proteus spp., Pseudomonas spp. and the vibrios. Significant antibacterial effects were produced by the extract against Staphylococcus aureus, Bacillus spp., lactobacilli, Escherichia coli, shigellae and salmonellae and the results were compared with standard antibiotic ciprofloxacin. Further the extract was proved to be bactericidal in its action.*

INTRODUCTION

Mesua ferrea Linn. (Family : Clusiaceae : Guttiferae) commonly known as ‘ Naagkesar’ (Bengali, Hindi and Punjabi), ‘ Naagchampa’ (Gujarat, Kon. and Mar) and ‘ Naagakeshara’ (Sanskrit) is a well known medium – sized or large evergreen tree with its various parts having tremendous use in the Indian traditional system of medicine for the treatment of various diseases. The barks are used as astringent and in combination with ginger as a sudorific. The leaves and flowers are used in snake bite and scorpion stings, flower buds are used in dysentery, flowers are used as astringent, stomachic and expectorant, unripe fruits have sudorific effects, seed oil is used externally for cutaneous affections as an embrocation in rheumatism (1,2).

Antibacterial and antifungal activities of the flowers of the flowers of *Mesua ferrea* Linn. Have already been reported (3). The present study was undertaken to investigate the

antibacterial activity of the leaf extract of *Mesua ferrea* Linn.

MATERIALS AND METHODS

Plant Material

The leaves of *Mesua ferrea* Linn (Family : Clusiaceae : Guttiferae) were collected from Assam, India. In November, 2000. The plant part was authenticated by Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah – 711 103, West Bengal, India [CNH/I – I (54)/2001 – Tech.II].

Preparation of the plant extract

The washed and dried matured leaves after collection were coarsely powdered 9114.1 gm and extracted with methanol in a Soxhlet at below 60°C. The extract was evaporated to dryness at low temperature under vacuum in a vacuum desiccator. The yield of the methanol

extract with respect to dry powdered material was calculated to be 24.72% w/w.

Preparation of samples

Dimethyl sulfoxide (DMSO) was used as the solvent to dissolve the dry powdered extract for the antibacterial tests. Ciprofloxacin solutions were prepared by using sterile distilled water and were used as standard for the comparison of the antibacterial potency of the leaf extract.

Chemicals

All chemicals and solvents used in this experiment were of AR grade and obtained from BDH (Poole, UK).

Microorganisms

One hundred three strains of bacteria belonging to 12 different genera were tested in this study. *S. aureus* AM 8/98, *E. coli* AM 8/98, *P. mirabilis* AM 8/98, *Klebsiella pneumoniae* RM 8/98 and *Pseudomonas* spp. Were collected from S.C.B. Medical College, Cuttack, Orissa, India; *E. coli* VC Sona wave 3:37C, *S. typhi* ATCC 6539, *S. aureus* NCTC 7447 AND *S. pneumoniae* NCTC 7465 were collected from Institute of Microbial Technology, Chandigarh, India. We had collected *B. subtilis* CD/99/1, *Lactobacillus arabinosus* CD/99/1, *E. coli* CD/99/1, *B. cereus* var *mycoides*, *S. aureus* ATCC 29737 and *Sarcina lutea* CD/99/1 from Central Drugs Laboratory, Kolkata, India. All the remaining strains were procured from the Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

In vitro tests for antibacterial efficacy of the extract.

The minimum inhibitory concentrations (MIC) of the extract against the various tested strains were determined by agar dilution technique (4). The antibacterial potentiality of the extract was assayed by disc diffusion method (5,6) and the results so obtained were

compared with those obtained with standard antibiotic Ciprofloxacin.

Determination of mode of antibacterial action of the extract

A highly sensitive bacterial strain, *S. aureus* ML 161, to the extract was grown in sterile nutrient broth medium overnight, 2 ml from which were added to 4 ml of sterile nutrient broth and incubated for 2 hr at 37 °C, so that the culture attained logarithmic phase of growth. After 2 hr incubation the extract was added at a higher concentration than its MIC value for that particular strain. The number of colony forming unit (CFU/ml) was determined by Miles and Mishra's method (7) at an interval of 2 hr upto 6 hr and then after 18 hr starting from zero hour.

RESULTS AND DISCUSSION

The results of the determination of MIC of the extract against 103 various tested bacterial strains are recorded in Table 1. This shows that the extract was mostly active against the *Staphylococcus aureus*, *Bacillus* spp., *Lactobacillus arabinosus*, *Escherichia coli*, *Shigellae* and *Proteus* spp., but moderately active against the *Klebsiella* spp., *Streptococcus pneumoniae*, *Sarcina lutea*, *Salmonella typhimurium*, *Pseudomonas* spp. and the vibrios.

The comparative results of the antibacterial assay of the extract and Ciprofloxacin are depicted in Table 2.

The MIC of the extract against the most sensitive strain *S. aureus* ML 161 was found to be 50 µg/ml. At the logarithmic growth phase of the culture, when CFU count of the strain was 9.8×10^6 CFU/ml, 100 µg/ml of the extract was added. Subsequently, the CFU count of the culture was found to decrease after 2, 4 and 6 hr and it ultimately

reduced to zero at the end of 18 hr. Thus it can be concluded that the methanolic extract of the leaf of *M. ferrae* (Linn) is bactericidal in its action. (Table 3 and Fig.1).

In summary, the methanolic extract of the leaves of *Mesua ferrea* Linn appears to have potent bacterial effect against both gram positive and gram negative strains, mostly against the organisms causing dysentery. An attempt to identify and isolate the chemical component(s) which is responsible for this activity is being carried out.

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Table 1. Bacterial inhibitory spectrum of the methanolic extract of leaves of *Mesua ferrea* Linn.

| Bacteria | No. of bacteria inhibited by the extract of ($\mu\text{g/ml}$) | | | | | | | |
|---------------------------------|--|---|-----|----|----|-------|-----|------|
| | No. of tested | 5 | 10 | 25 | 50 | 100 | 200 | >200 |
| <i>Staphylococcus aureus</i> | 40 - | | - | - | 10 | 09 | 10 | 11 |
| <i>Bacillus spp.</i> | 03 | - | --- | | | - 03 | | - |
| <i>Klebsiella spp.</i> | 02 | - | --- | | | 01 - | | 01 |
| <i>Streptococcus pneumoniae</i> | 01 | - | --- | | | - 01 | | - |
| <i>Sarcina lutea</i> | 01 | - | --- | | | - 01 | | - |
| <i>Lactobacillus arabinosus</i> | 01 | - | --- | | | 01 - | | - |
| <i>Escherichia coli</i> | 06 | - | --- | | | 02 03 | | 01 |
| <i>Shigella spp.</i> | 12 | - | --- | | | 03 06 | | 03 |
| <i>Salmonella spp.</i> | 04 | - | --- | | | - 03 | | 01 |
| <i>Proteus mirabilis</i> | 01 | - | --- | | | 01 - | | - |
| <i>Pseudomonas spp.</i> | 02 | - | --- | | | - 01 | | 01 |
| <i>Vibrio cholerae</i> | 30 - | | - | - | 03 | 07 | 07 | 13 |
| Total Strains | 103 | | | | | | | |

Table 2. A comparative account of the assay results (in terms of diameters of zones of inhibition) of methanolic extract of *Mesua ferrea* Linn. leaf and Ciprofloxacin.

| Bacteria | Extract ($\mu\text{g/ml}$) | | Ciprofloxacin ($\mu\text{g/ml}$) | |
|---|------------------------------|-------|------------------------------------|-------|
| <i>Staphylococcus aureus</i> ATCC 29737 | 7.00 | 10.50 | 14.83 | 17.66 |
| <i>Bacillus cereus var mycoides</i> | 7.50 | 11.00 | 15.83 | 17.83 |
| <i>Lactobacillus arabinosua</i> CD/99/1 | 8.00 | 12.00 | 14.66 | 16.83 |
| <i>Escherichia coli</i> ROW 7/12 | 7.00 | 8.50 | 15.00 | 16.66 |
| <i>Shigella dysenteriae</i> 6 | 8.50 | 11.50 | 17.00 | 21.00 |

Table 3. Mode of antibacterial activity of the methanolic extract *Mesua ferrea* Linn. leaf and *S. aureus* ML161.

| Time (hours) | CFU count /ml for the extract |
|--------------|-------------------------------|
| Zero | 9.8×10^6 |
| 2 | 9.6×10^4 |
| 4 | 8.9×10^2 |
| 6 | 9.8×10^2 |
| 18 0 | |

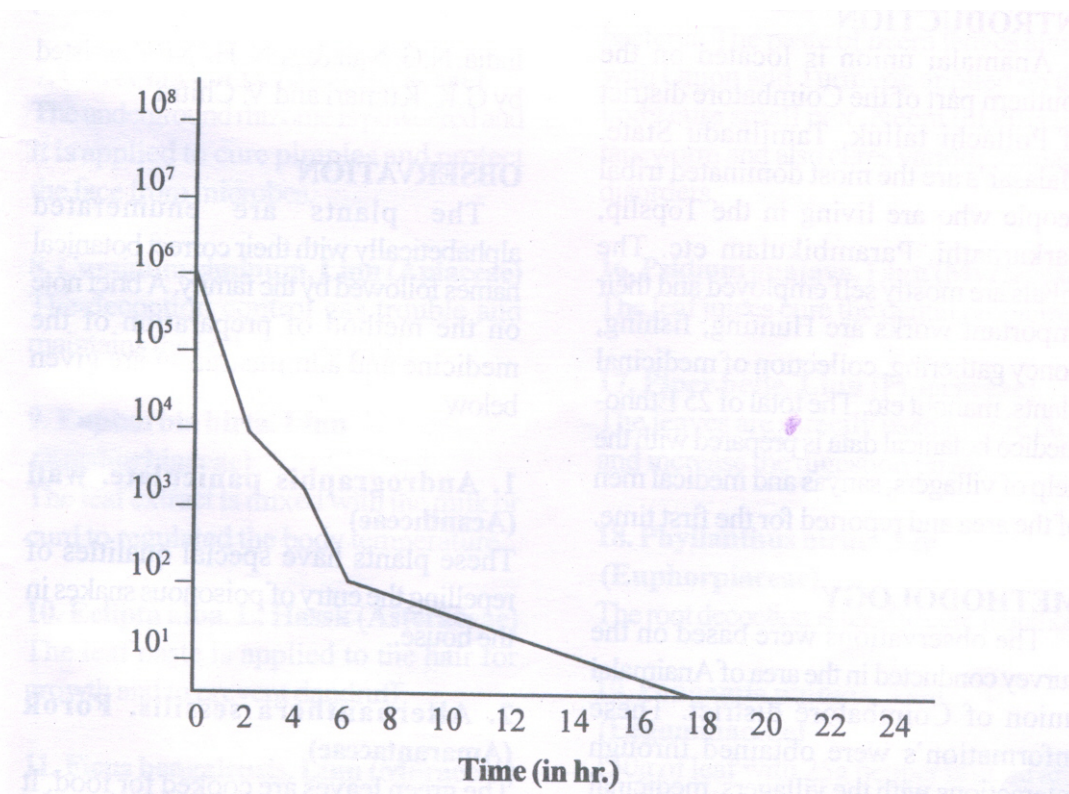


Fig . 1 : Graphical representation of the mode of antibacterial activity of the methanol extract of *Mesua ferrea* Linn. leaf extract against *S. aureus* ML 161