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 spps. Klebsiella spps., Streptococcus pneumoniae, Sarcina lutea, Lactobacillus arabinosus, Escherichia coli, shigellae, salmonellae, Proteus spps., Pseudomonas spps. and the vibrios. Significant antibacterial effects were produced by the extract against Staphylococcus aureus, Bacillus sppa., lactobacilli, Escherichia coli, shigellae and salmonellae and the results were compared with standard antibiotic ciprofloxacin. Further the extract was proved to be bacterial 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 an astrigent 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 an 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 leaf extract 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 thorough powdering were extracted with methanol in a soxhlet apparatus 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 powered 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 thirty 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, K. ebsiella pneumoniae RM 8/98 and P. eudomonas 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. pne umoniae 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 myc oides, S. a ureus ATCC 29737 and Sarcina lutea CD/99/1 from the Central Drugs Laboratory, Kolkata, India. All the remaining strains were procured from the Division of Microbiology, Department of Pharmacaceutical 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 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 up to 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 sppa., Lactobacillus arabinosus, Escherichia coli, Shigellae and Proteus spp., but moderately active against the Klebsiellae spp., Staphylococcus pneumoniae, Sarcina lutea, Salmonellae 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 50µg/ml. At the logarithmic growth phase of the culture, when CFU count of the strain was 9.8 x 10^6 CFU/ml, 100µg/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 (µg/ml) |
|------------------------------|--------------------------------------------------|
|                              | No. of tested | 5 | 10 | 25 | 50 | 100 | 200 | >200 |
| *Staphylococcus aureus*      | 40 -          | - | -  | -  | 10 | 09  | 10  | 11   |
| *Bacillus spp.*              | 03 -          | - | -  | -  | -  | -   | -   | -    |
| *Klebsiella spp.*            | 02 -          | - | -  | -  | -  | 01  | -   | 01   |
| *Streptococcus pneumoniae*   | 01 -          | - | -  | -  | -  | -   | -   | -    |
| *Sarcina lutea*              | 01 -          | - | -  | -  | -  | -   | -   | -    |
| *Lactobacillus arabinosus*   | 01 -          | - | -  | -  | 01 | -   | -   | -    |
| *Escherichia coli*           | 06 -          | - | -  | -  | 02 | 03  | 01  | 01   |
| *Shigella spp.*              | 12 -          | - | -  | -  | 03 | 06  | 03  | 03   |
| *Salmonella spp.*            | 04 -          | - | -  | -  | 03 | -   | 01  | 01   |
| *Proteus mirabilis*          | 01 -          | - | -  | -  | 01 | -   | -   | -    |
| *Pseudomonas spp.*           | 02 -          | - | -  | -  | 01 | -   | 01  | 01   |
| *Vibrio cholerae*            | 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 Ciproflxacin.

| Bacteria                     | Extract (µg/ml) | Ciprofloxacín (µg/ml) |
|------------------------------|-----------------|-----------------------|
| *Staphylococcus aureus* ATCC 29737 | 7.00           | 10.50                 |
| *Bacillus cereus var mycoides* | 7.50 11.00      | 15.83 17.83           |
| *Lactobacillus arabinosus* CD/99/1 8.00 | 12.00         | 14.66 16.83           |
| *Escherichia coli* ROW 7/12 | 7.00 8.50       | 15.00 16.66           |
| *Shigella dysenteriae* 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        | $9.8 \times 10^6$             |
| 2           | $9.6 \times 10^3$             |
| 4           | $8.9 \times 10^2$             |
| 6           | $9.8 \times 10^2$             |
| 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