Effect of *Mesua ferrea* Linn. flower extract on *Salmonella*

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Based on its traditional uses in folk medicine, the whole flower extract of *Mesua ferrea* Linn. was tested for its *in vitro* antimicrobial efficacy against five different strains of *Salmonella* spp. All the strains were found to be highly sensitive to the extract. MIC of the extract against each organism was 50 µg/ml. The extract was tested *in vitro* for its mode of antibacterial activity against *S. Typhimurium* NCTC 74 and it was found to be bactericidal in action. *In vivo* studies of this extract offered significant protection to Swiss albino mice at doses ≥ 2 and 4 mg/mouse when challenged with 50 median lethal dose of *S. Typhimurium* NCTC 74. Further, the extract caused statistically significant reduction in viable count of the strain in liver, spleen and heart blood of challenged mice.

**Keywords:** Flower extract, *Mesua ferrea*, *Salmonella*

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Various parts of *Mesua ferrea* Linn. (Family: Guttiferae/Clusiaceae) are widely used in indigenous system of medicine. Some of the important properties of the plant have already been proved experimentally. Based on the traditional uses of the whole flower of the plant as an antityphoid agent, the present study was undertaken to determine the *in vitro* and *in vivo* antibacterial activity of the whole flower extract of the plant against various strains of *Salmonella* spp. and to prove the mode of its such antibacterial action.

**Plant material**—The flowers of *M. ferrea* were collected from Assam in April 2000, and identified by Botanical Survey of India, Botanical Garden, Shibpur, Howrah 711 103, India. One voucher specimen (CNH/II-1 (54)/2001-Tech.II) of the plant herbarium has been preserved in our laboratory for future reference. The collected whole flowers were washed, dried under shade, pulverized by mechanical grinder and passed through a 40-mesh sieve.

**Preparation of plant extract**—Dried whole flowers (71.6g) were extracted with 1500 ml methanol (90%) in a soxhlet apparatus (yield: 19.55% w/w with respect to the dried and powdered flowers). The extract was dried under reduced pressure (5 mm Hg) and stored in a vacuum desiccator (Fiber).

**Bacteria**—Two strains of *Salmonella enterica* subspecies enterica serovar Typhimurium (S. Typhimurium) namely, ATCC 6539 were collected from Institute of Microbial Technology (IMT), Chandigarh. NCTC 74 and three strains of *S. Typhi* (57, 59 and 62) were procured from Dr. Joan Taylor. *Salmonella* Reference Laboratory, London.

**In vitro tests for detection of antimicrobial action**—The methanolic whole flower extract was dissolved in 25% sterile dimethyl sulfoxide (DMSO) to prepare the stock solution (10 mg/ml). It was then added to molten nutrient agar (Oxoid) aliquots at the concentrations of 0, 5, 10, 25, 50, 100, 200 and 400 µg/ml, mixed thoroughly, adjusted to pH 7.2 to 7.4 and poured into sterile Petri dishes. Bacterial strains were grown in peptone water (PW; Oxoid brand bacteriological peptone 1.0% plus anular sodium chloride 0.5%) for 18 hr, and diluted, so that 2 mm loopful of culture would contain 10⁶ colony forming units (CFU). These were then spot inoculated by Checker-Board Technique on the plates containing increasing amount of the extract. The plates were then incubated at 37° C up to 72 hr for determination of minimum inhibitory concentrations (MIC) of the extract against various strains of *Salmonella* spp. under study by agar dilution technique.

**Mode of action of extract**—*Salmonella Typhimurium* NCTC 74 strain was grown in NB for 18 hr. This culture (2 ml) was added to 4 ml of fresh NB and incubated for 2 hr at 37° C to attain logarithmic growth phase. The viable count was determined at this stage from the culture tube and the extract was then added at concentration higher than its MIC value for that particular strain. The viable counts were determined by the method of Miles and Mishra at an interval of 2 hr up to 6 hr and then after 18 hr starting from zero hour.
In vivo tests—Virulence of the strain of *S. Typhimurium NCTC 74* was enhanced by passaging ten times through Swiss albino male mice (maintained in our own animal house) by administering intraperitoneally (ip) and recovering from heart blood. Fifty median lethal dose (50 MLD) of the strain corresponding to $1.85 \times 10^6$ CFU/ml suspended in 0.5 ml sterile nutrient broth (NB) served as the challenge dose for all animals. The reproducibility of the dose was confirmed by measuring its optical density at 640 nm in a Klett-Summerson colorimeter and by determining the viable count on nutrient agar.

Eight batches each of 20 male Swiss albino inbred strain of mice (18-20 g) were kept in separate cages. The first group of mice consisting of two such batches was administered 1 mg of the extract (by injecting, ip, 0.1 ml from a stock solution of 10 mg/ml of the extract). The second group consisting of the next two batches was given 2 mg of the extract and each mouse of the third and fourth groups (each group consisting of two batches of mice) received 4 and 8 mg of the extract, respectively. One batch of mice from each of the above mentioned four groups were challenged with 50 MLD of *S. Typhimurium NCTC 74* after 3 hr of the administration of the extract. A control group of 20 mice was also injected (ip) with *S. Typhimurium NCTC 74* and 0.1 ml sterile saline in place of the drug. Mice were observed for 30 days and mortality in each group was recorded.

In another in vivo experiment, the viable counts in blood and organ homogenates of the extract treated and untreated mice were determined. All the animals were given a 50 MLD challenge dose; 50% of the animals received the extract (4 mg/mouse) 3 hr before the challenge and the rest received sterile saline (Table 1) in place of the extract. After 18 hr, all mice were sacrificed, blood was collected individually from the heart, liver and spleen were removed aseptically and homogenized in tissue homogeniser. Viable counts of individual organs were determined separately. Statistical analysis of the data was performed using Student's t test.

In vitro study revealed that methanol extract of whole flower of *M. ferrea* inhibited growth of all the five strains at ≥ 50 μg/ml concentration.

MIC of the extract against *S. Typhimurium NCTC 74* was found to be 50 μg/ml. At the logarithmic growth phase of the culture, when viable count of the strain was $1.5 \times 10^6$ CFU/ml, 2 × MIC (100 μg/ml) of the extract was added. The viable count of the culture dropped to $1.1 \times 10^6$ after 2 hr; $2.1 \times 10^6$ at 4 hr; $8.5 \times 10^5$ after 6 hr; and 0 at the end of 18 hr. Thus, it could be concluded that the flower extract of *Mesua ferrea* L. was bactericidal in nature.

Under in vivo assays, the extract offered significant protection to mice against *S. Typhimurium NCTC 74*. At 1 mg/mouse dose, 11 mice died in the challenged batch, whereas no death was observed in the corresponding control batch that received only the extract, but no challenge. Seven out of 20 mice died in the test batch and two out of 20 mice died in the control batch (only extract but no challenge) with 2 mg/mouse dose. At 4 mg/mouse dose, 20% mortality was observed in both the challenge and the unchallenged (control) series. When 8 mg/mouse dose was administered to the test and the control (no challenge) batch of mice, all mice died in the test group within 100 hr after administration of the challenge, whereas 9

<table>
<thead>
<tr>
<th>Group</th>
<th>Treated with</th>
<th>Liver</th>
<th>Spleen</th>
<th>Heart blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanolic crude extract of whole flower of <em>M. ferrea</em> (4 mg/mouse)</td>
<td>$3.5 \times 10^7$</td>
<td>$6.6 \times 10^7$</td>
<td>$5.0 \times 10^7$</td>
</tr>
<tr>
<td>2</td>
<td>Sterile saline</td>
<td>$3.3 \times 10^7$</td>
<td>$9.5 \times 10^7$</td>
<td>$1.1 \times 10^7$</td>
</tr>
<tr>
<td>3</td>
<td>Sterile saline</td>
<td>$1.1 \times 10^8$</td>
<td>$8.2 \times 10^8$</td>
<td>$9.2 \times 10^8$</td>
</tr>
<tr>
<td>4</td>
<td>Sterile saline</td>
<td>$4.8 \times 10^7$</td>
<td>$2.0 \times 10^7$</td>
<td>$8.5 \times 10^7$</td>
</tr>
<tr>
<td>5</td>
<td>Sterile saline</td>
<td>$2.8 \times 10^7$</td>
<td>$2.0 \times 10^7$</td>
<td>$2.5 \times 10^7$</td>
</tr>
</tbody>
</table>

Table 1—Variation of CFU/ml of *S. Typhimurium NCTC 74* in blood and other organs of *M. ferrea* whole flower extract treated and untreated mice

*Viable counts between two groups significant at *P* < 0.001.

All samples were collected at 18 hr.
mice died in the control group. In a second control group of 20 mice that received the challenge and sterile saline in place of extract, 15 mice died. Chi-square ($\chi^2$) test involved the number of survival and death of mice in the respective test groups (receiving the extract and challenge) and the second control group (receiving only challenge and saline in place of extract). Thus, at dose levels 2 and 4 mg/mouse, the extract significantly ($P < 0.05 - 0.01$) reduced the number of death in mice according to $\chi^2$ test, after elimination of effect due to the extract alone. However, no significant protection was evident at 1 or 8 mg/mouse dose levels as lower dose was ineffective and the latter turned ineffective probably being toxic.

Results of the experiment for determination of effect of the extract on CFU/ml in blood and other organs after the mice were challenged with S. Typhimurium NCTC 74 are given in Table 1. There was a statistically significant reduction ($P<0.001$) in the number of viable bacteria in blood, liver and spleen in the animals treated with the extract.

_in vitro_ and _in vivo_ studies involving the methanolic whole flower extract suggested that the extract potentially had antimicrobial action against various strains of _Salmonella_ spp. The extract was found to be bactericidal in its action. Further work related to the isolation and identification of the active component(s) is underway of the whole flower of _M. ferreti_ responsible for such antimicrobial action.

**References**