In-vitro Anti-Microbial and Brine-Shrimp Lethality Potential of the Leaves Extract of Nahar (*Mesua ferrea*) Plant

Presented by
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Introduction

*Mesua ferrea* L. (Guttiferae)

**COMMON NAMES:** Ceylon ironwood, Indian rose chestnut, Cobra's saffron, Penaga Lilin, Na, Nahar/Nahor, Nāga

**FAMILY:** Clusiaceae (Guttiferae).

**NATIVITY:** Tropical Sri Lanka (also Assam, Southern Nepal, Indochina, the Malay Peninsula)

**VEGETATION:** Bears flowers between April and July. Fruits between October and November (Dennis and Kumar, 1998).

**USES:** Well-known medicinal plant (used in indigenous system of medicine for the treatment of fever, dyspepsia, and renal diseases) (Nadkarni, 1954)

**RESOUCEFULNESS:** Antimicrobial activity of plant oils other extracts applications (raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis–Balchin and Deans, 1997)
Problem statement and its significance

Increasing interest in human health, concern over pathogenic and spoilage microorganisms in foods and increase in outbreaks of food borne disease

Infectious diseases represent an important cause of morbidity and mortality among humans

Bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento et al. 2000)

Efforts are being encouraged towards the substitution of synthetic non-biodegradable polymers by fully or partially biodegradable polymers of semi-synthetic or bio-origin due to several environmental concerns (Suvangshu et al., 2010)

Increasing need to search for natural antimicrobials from non-conventional sources to augment the available ones and also to meet specific applications.

The use of medicinal plants as screening pool for novel antibiotics has several advantages related to safety, availability, and minimizing the risk of side effects and addiction (Lee et al., 2003).

The importance of identifying new effective antimicrobial agents cannot be overemphasized (Ghaleb et.al, 2009)
Research Objectives

1. Evaluation of the antimicrobial activity of the leaves extract

2. Cytotoxicity analysis of the extract
Methodology

Leaves pretreatment and sample preparation (oven drying @ 45°C for 2 days)

Extraction of Nahar leaves: oven shaker set at 37°C and 200rpm for 24 hours
Methodology...cont’d

Anti-microbial assay of Nahar leaves extracts on *E. coli, P. aeruginosa, B. subtilis and S. aureus* using disc diffusion methods

Determination of Minimum Inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC) using broth dilution

Cytotoxicity test using Brine Shrimps Lethality Bioassay
RESULTS

AND

DISCUSSIONS
NL crude extract yields

Ethanol gave higher crude extract’s yield (about 10.2%) than methanol.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Yields of crude extract (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>6.71±0.25</td>
</tr>
<tr>
<td>Methanol</td>
<td>6.09±0.22</td>
</tr>
</tbody>
</table>

This confirmed the earlier report of Wang and Helliwell [23], that ethanol is superior to methanol and acetone for extracting biologically-active components (e.g., flavonoids) from tea. Besides, ethanol is considered as safe (GRAS solvent).
Zone of inhibition (mm) of Nahar leaves extract

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Ethanol extract</th>
<th>Methanol extract</th>
<th>Chl.</th>
<th>Tet.</th>
<th>Str.</th>
<th>Gen.</th>
<th>Van.</th>
<th>DMSO</th>
<th>Methanol</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>17.5±0.5</td>
<td>18.0±0.5</td>
<td>23.0</td>
<td>20.0</td>
<td>24.0</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>17.0±0.5</td>
<td>17.5±0.5</td>
<td>23.0</td>
<td>23.0</td>
<td>20.0</td>
<td>21.0</td>
<td>19.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>17.0±0.5</td>
<td>16.0±0.5</td>
<td>25.0</td>
<td>26.0</td>
<td>20.0</td>
<td>23.0</td>
<td>20.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>18.0±0.5</td>
<td>18.0±0.5</td>
<td>24.0</td>
<td>26.0</td>
<td>21.0</td>
<td>24.0</td>
<td>20.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Antibacterial activity**

The extract showed a remarkable antibacterial property against all the selected microbes (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus*) with the inhibition zones ranging from 16.0±0.5mm to 18.0±0.5mm for all the tested bacteria.
MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF NAHAR SEED KERNELS’ OIL
The result obtained from the MIC and MBC determinations showed that the active extracts were found to be both bacteriostatic and bactericidal with the gram-positive bacteria showing less resistance.

The MIC range of 0.625 - 2.5 mg/ml with MBC value of 5 mg/ml was obtained for the gram-negative bacteria while MIC range of 0.313 - 1.3 mg/ml with MBC value of 2.5 mg/ml was obtained for the gram-positive bacteria.

It could be deduced from this that the Gram-positive bacteria appeared to be more sensitive, more susceptible and less resistant, while the Gram negative bacteria are less sensitive, less susceptible and more resistant.

This also justifies the fact that is generally known, which is that Gram negative bacteria have an outer membrane consisting of lipoprotein and lyopolysaccharide, which is selectively permeable and thus regulates access to the underlying structures.
### Brine shrimp lethality bioassay of methanol extract of NL

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>1000</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.50</th>
<th>31.25</th>
<th>15.63</th>
<th>7.81</th>
<th>3.91</th>
<th>1.95</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of shrimps per test sample</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. of survivors</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. of death</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Percentage mortality (%)</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

LC$_{50}$ = 500ppm (µg/ml)
The extract was found to be moderately cytotoxic to the Brine shrimps at high concentration with LC$_{50}$ of 500ppm (µg/ml).

Ayo et al. (2007) reported that LC$_{50}$ values lower than 1000 µg/ml are considered bioactive. Therefore, the methanol extracts of *M. ferrea* leaves may have some significant biological activity.

The results of this present work may be of importance in the elucidation of the potential and medicinal uses of the extracts.
Conclusion

The antimicrobial and cytotoxic activity of Nahar leaves extracts, found in this study, may explain some of the traditional medicinal uses of the plants. These could also be of particular interest in relation to finding out its untapped efficacy and can also be a potential of chemically interesting and biologically important drug candidates.
Future challenges!!!

1. Characterization of the bioactive compounds of the leaves extracts

2. Detailed physico-chemical properties of the extract

3. Incorporation of the extract in water disinfection, food packaging materials and storage stability

4. And so on…
Jazakumullah Khairan for Your Audience
Wassalam Alaikum Warahmatullahi
Wabarakatuhu
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References


