**In vitro** evaluation of antibacterial activity of crude extracts of *Ficus benghalensis* Linn., the banyan tree leaves

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The antibacterial activity of *Ficus benghalensis* Linn., the banyan tree, leaves was tested on bacterial pathogens of humans. Hexane, chloroform and methanol extracts were used for the study of antibacterial activity by agar well diffusion method. The methanol extract showed higher antibacterial activity in comparison to the other two solvents. Relatively higher minimum inhibitory concentration was obtained with methanol extract for *Klebsiella pneumoniae* and *Micrococcus luteus*; however, less inhibitory effect was noted for chloroform and hexane extracts. Gram-negative bacteria were more susceptible than Gram-positive. Present study validates the traditional use of banyan leaves in medicine.

**Keywords:** *Ficus benghalensis*, Banyan Tree, Antibacterial, Gram-negative bacteria, Agar-well diffusion.

**IPC code; Int. cl. (2011.01)—**A61K 36/00, A61P 31/04

**Introduction**

Existence and survival of human kind is impossible without plant kingdom, as plants are the primary producers and play important role in sustaining the life forms on earth. Search for newer drugs from plants has been on the rise, since many of the microorganisms are posing serious health problems. According to a recent estimate by WHO, more than 3.5 billion people in the developing world rely on plants as source of medicine for various ailments. Over 20,000 plant species have been reported to possess medicinal values and many of them are yet to be explored for their potential. Besides, many of the existing synthetic drugs cause various side effects. Therefore, drug development from plant-based compounds could be useful in meeting this demand for newer drugs with minimal effects.

*Ficus benghalensis* Linn. (Moraceae) is commonly known as Banyan tree, *Vata* or *Vada* in Ayurveda. The plant is a large evergreen tree distributed all over India from sub-Himalayan region to the deciduous forest of Deccan Peninsula. It is grown in gardens and road sides for shades. This tree is considered to be sacred in many places in India, particularly West Bengal. Some natural compounds, viz. glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentariacontan-5-one, β-sitosterol-α-D-glucose and meso-inositol have been isolated from the bark of this species. The fruit extracts exhibited antitumor activity in the potato disc bioassay. The leaves contain 9.63% crude protein, 6.84% crude fibres, 2.53% CaO, and 0.4% phosphorous. The latex contains caoytchoue (2.4%), resin, albumin, cerin, sugar, and malic acid. It is used in Ayurveda for the treatment of diarrhoea, dysentery, piles and as a hypoglycemic. The bark extracts exhibited anti-inflammatory activity and inhibits insulinase activity from the liver and kidney. It was also found to inhibit the lipid peroxidation. Various extracts of the plant were screened for its anti-allergic and anti-stress potential in asthma by milk-induced leucocytosis and milk induced eosinophilia. However, though there are diverse medicinal properties attributed to this plant, few studies have been carried out to shed light on its antibacterial activity. The present study was carried out to investigate antibacterial activity of its leaves on selected pathogenic bacteria.

**Materials and Methods**

The present study was done in the Department of Botany, Andhra University, Visakhapatnam. Leaves of *F. benghalensis* were collected from University premises during winter season.

**Test organisms**

Pure bacterial cultures (Table 1) were obtained from Microbial type culture collection, Institute of Microbial Technology (IMTECH), Chandigarh, India and were maintained in nutrient agar (Himedia).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Catalogue number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>MTCC B2274</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>MTCC B0439</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>MTCC B9637</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>MTCC B2405</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>MTCC B1538</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>MTCC B0426</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>MTCC B2488</td>
</tr>
</tbody>
</table>

| Table 1—List of tested bacterial strains and their catalogue numbers

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Extraction procedure
Leaves were washed thoroughly under running tap water, dried on paper towel, then shade dried and finally crushed to fine powder in mixture grinder. The dried powder of the leaves was allowed to Soxhlet for successive extraction with hexane, chloroform and methanol. The liquid extract was collected, filtered and evaporated to dryness using rotary evaporator (Heidolph, Heizbad, Laborota 4001, Germany 2002). A semisolid or dried crude extracts of leaves so obtained was re-suspended in an inert solvent, dimethyl sulphoxide (DMSO)

Antibacterial activity
The antibacterial susceptibility test was carried out using agar well diffusion method. Extracts from leaves were delivered into well form lower to higher concentration and plates were incubated at 37°C for 24 h. The presence of zone of inhibition was regular as the indicator of antimicrobial action was expressed in terms of average diameter of the zone of inhibition measured in millimeter. Each test was carried out in triplicate. DMSO and Chloramphenicol (10 µg/disc) were used as negative and positive controls, respectively and the values were compared with F. benghalensis leaf extracts.

Minimum inhibitory concentration (MIC) was determined by the broth dilution method. Different concentrations of leaf extract in hexane, chloroform and methanol (ranging from 0.01 to 5.00 mg/ml) were tested separately for each bacterium and inhibition zone of microbial growth in the plates containing test solutions was judged by comparison with blank control plates. MIC is defined as the lowest concentration of test samples that result in a complete inhibition of visible growth. Experiments were carried out in triplicate. The results of antibacterial activity were interpreted as sensitive (<14 mm), intermediate activity (14-17 mm) and susceptible (>17 mm).

Results and Discussion
The results of antibacterial activity and MIC values of solvent extracts of Ficus benghalensis leaves are summarized in Tables 2 and 3, respectively. Hexane extract showed less activity; chloroform extract showed moderate activity, while methanol extract showed the high antibacterial activity against all tested bacteria.

Hexane extract was found to be resistance against Klebsiella pneumoniae, Pseudomonas aeruginosa and Micrococcus luteus for low concentration, while Klebsiella pneumoniae showed intermediate activity to its high concentration. Escherichia coli, Proteus vulgaris, Bacillus subtilis and Enterococcus faecalis did not show any inhibition zones.

Chloroform extract was found effective against Klebsiella pneumoniae and Micrococcus luteus showed intermediate activity against Pseudomonas aeruginosa irrespective of its concentrations whereas Escherichia coli, Proteus vulgaris and Enterococcus

<table>
<thead>
<tr>
<th>Solvent Extract</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BS</td>
</tr>
<tr>
<td>Hexane</td>
<td></td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>12</td>
</tr>
<tr>
<td>300 mg/ml</td>
<td>14</td>
</tr>
<tr>
<td>Chloroform</td>
<td></td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>12</td>
</tr>
<tr>
<td>300 mg/ml</td>
<td>14</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>16</td>
</tr>
<tr>
<td>300 mg/ml</td>
<td>17</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
</tr>
<tr>
<td>10 µg</td>
<td>23</td>
</tr>
<tr>
<td>DMSO</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>11</td>
</tr>
</tbody>
</table>

= no activity
BS: Bacillus subtilis
EF: Enterococcus faecalis
EC: Escherichia coli
KP: Klebsiella pneumoniae
ML: Micrococcus luteus
PV: Proteus vulgaris
PA: Pseudomonas aeruginosa
faecalis were resistance to low concentration and evinced intermediate activity to high concentration. Bacillus subtilis did not show inhibition zone.

Methanol extract exhibited promising activity against all tested bacteria for both concentrations. It showed fairly high degree of sensitivity against Klebsiella pneumoniae followed by Micrococcus luteus. Compared with standard antibiotic, methanol extract showed the high inhibition zone values than ciprofloxacin (10µg). Hence it indicated that methanol extract of F. benghalensis leaves has broad spectrum of antibacterial activity against all tested bacteria. DMSO, a negative control, did not show zone of inhibition and indicated that it is not interfering with the formation of zone of inhibition.

From the MIC values, it is observed that methanol extract showed significant antimicrobial activity followed by chloroform and hexane extracts (Table 3). The MIC value of methanol extract was 0.01 mg/ml for Klebsiella pneumoniae while 0.1 mg/ml against Micrococcus luteus and 1.0 mg/ml for Enterococcus faecalis. The MIC value of chloroform extract for K. pneumoniae was 1.0 mg/ml, whereas for M. luteus it was 3.0mg/ml. The hexane extract exhibited the same value against all tested bacteria, it was >5.0 mg/ml.

Earlier, it was found that hydro-alcoholic bark extract of F. benghalensis is effective against Actinomyces viscosus. Aerial root aqueous and hexane extracts were found to show antimicrobial activity against Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli strains. The stem bark was reported to have antimicrobial activity but it varied as geography and environmental conditions changed. Methanol extract of bark exhibits good activity compared to chloroform and aqueous extracts against enterotoxigenic E. coli isolated from diarrheal patients. Aqueous extracts of its bark exhibited significant antibacterial activity against S. aureus, Pseudomonas aeruginosa and K. pneumoniae. In the present study hexane, chloroform and methanol extracts of leaves were found to be the most sensitive against K. pneumoniae and Micrococcus luteus strains.

Among the three extracts, the methanol extract exhibited promising results than chloroform and hexane extracts. Methanol proved as the most effective solvent for extracting broad spectrum of antimicrobial compounds from plants. Previous reports available in support of antimicrobial activity of plant extracts have said that this activity is due to the presence of saponins. Gram-negative bacteria were reported to be more resistant to antibiotics treatment. Hence, it is important to identify an effective plant drug having broad spectrum of inhibition against Gram-negative bacteria to overcome the ailments caused by the microorganisms.

**Conclusion**

The findings of this study have shown that F. benghalensis extracts are more active against Gram-negative bacteria than Gram-positive. The plant may be a future drug candidate to prove its efficacy as a preventive and therapeutic agent against these pathogenic bacteria.

**Acknowledgment**

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**References**


