Antibacterial screening of the stem bark and leaf extracts of *Litsea glutinosa* (Lour.) C.B. Rob – an ethnomedicinally important tree of the Western Ghats

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**ABSTRACT**

**Introduction:** *Litsea glutinosa* (Lour.) C.B.Rob., is an aromatic medicinal tree belongs to the family Lauraceae and rarely distributed in the Western Ghats. Ethnomedicinally, the bark is used by the traditional practitioners as a demulcent, emollient and in the treatment of diarrhea and dysentery, and to facilitate wound healing process. The leaf paste is applied to relieve respiratory diseases; cough and also used as a demulcent and an emollient. **Methods:** The petroleum ether, chloroform and ethanol extracts of stem bark and leaf were screened for potential antibacterial activity using agar well diffusion method against 8 clinical strains isolated from infectious sources belonging to gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Salmonella paratyphi* and *Proteus* sp. Minimum Inhibitory Concentration (MIC) of the three sequential extracts of the leaf and stem bark was carried out against clinical isolated strains using two fold agar dilution method. **Results:** Ethanol extract of stem bark exhibited significant antibacterial activity with 2.5mg/ml MIC against *Bacillus subtilis* (19.20 ± 1.52), *Escherichia coli* (16.40 ± 0.55) and *Staphylococcus aureus* (15.20 ± 0.84) indicating the potent drug for the treatment of diarrhea and dysentery. Among the tree sequential extracts of the leaf the ethanol extract showed potent antibacterial activity against *Klebsiella pneumoniae* (16.40 ± 0.80) and may acting as a suitable drug for respiratory disorders. **Conclusion:** The obtained results justify the ethnomedicinal claims of *Litsea glutinosa*.

**Key words:** agar-well diffusion, clinical isolates, Lauraceae, Minimum inhibitory concentration, two fold agar dilution.

**INTRODUCTION**

In recent years there has been a rising interest in the discovery of new antimicrobial compounds, due to alarming increase in the rate of infections with multi-drug resistant microorganisms.[1] The increased prevalence of antibiotic resistance bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control bacterial diseases.[3] In the present scenario due to emergence of multiple drug resistance to human pathogenic bacteria and fungi, especially the antibiotic penicillins, cephalosporins and chloromphenical types involve the enzymatic inactivation of the antibiotic by hydrolysis or by the formation of an active derivative.[3]

Infectious diseases are the world’s leading cause of premature deaths, killing almost 50,000 people every day.[4] In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world.[5] The pathogenic microbes like *Escherichia*, *Klebsiella*, *Proteus*, *Staphylococcus*, *Bacillus*, *Salmonella* and *Pseudomonas* species are implicated to cause several infections in human, as they are found in multiple environmental habitats.[6,7] Natural products especially, those used in ethnomedicine provide a major source of innovative therapeutic agents for various conditions including infectious diseases.[6] Recently, herbal medicines have increasingly been used to treat many difficult diseases including several infections. Ethnobotanical reports suggested that the herbal extracts and their constituents have been increasingly used to treat many infectious diseases.[9,10] and
plants are the sleeping giants of pharmaceutical industry and provide natural source of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally.

_Litsea glutinosa_ is an aromatic evergreen medium-sized tree belongs to the family Lauraceae and found to be very rare medicinal plant in the Western Ghats. The traditional practitioners residing in the vicinity of forests of the Bhadra Wild Life Sanctuary of the Western Ghats are using the stem bark and leaf extracts as a demulcent and mild astringent for diarrhea and dysentery; the bark paste is applied to facilitate wound healing process. Leaf juice used to relieve respiratory disorders, roots are used for poulticing sprains and bruises, and in wounds and the berries yield oil which is used by some tribal practitioners in the treatment of rheumatism. The leaf juice for the treatment of the spontaneous and excessive flow of semen in young boys.

Phytochemical screening of the stem bark revealed the presence of tannin, β-sitosterol, and actinodaphnine. Boldine, norboldine, laurotetanine, n-methyllaurotetanine, _n_-methylactinodaphnine, quercetin, sebiferine, litseferine, Boldine, norboldine, laurotetanine, n-methyllaurotetanine, _n_-methylactinodaphnine, quercetin, sebiferine, litseferine etc., and two aporphine alkaloids, namely litseglutinine A and B.

So in the view of the above, the determination of potential antibacterial activity of _L. glutinosa_ extracts could be more informative for the future use in clinical treatment as natural antimicrobial agent. In the present study the antibacterial activities of the sequential extracts of the leaf and stem bark was screened against 8 pathogenic clinical isolates of bacteria.

**MATERIALS AND METHODS**

**Plant material and extraction**

The leaves and stem bark of _L. glutinosa_ were collected in October, 2010 from the Bhadra wild life sanctuary of the Western Ghats, Karnataka, India, and identified by Tariq Husain, Head and Scientist, Biodiversity and Angiosperm Taxonomy, National Botanical Research Institute, Lucknow, India and the voucher specimen (No. 97294) is deposited. Plant materials were cleaned with deionized water and dried at shade for a week, grounded mechanically and the powder was stored in air tight container. One kg of the powdered materials of each were refluxed in Soxhlet apparatus for 48 hrs in batches of 250 g each and sequentially extracted with the solvent petroleum ether, chloroform and ethanol. The solvent was evaporated and concentrated in vacuum under reduced pressure using a rotary flash evaporator (Buchi, Flawil, Switzerland), allowing the solvent to completely evaporate on a water bath then finally vacuum dried. 400 mg of crude extracts of petroleum ether, chloroform and methanol were reconstituted with 10% Dimethyl sulphoxide (DMSO). The standard antibacterial drug ciprofloxacin (BioChemika, ≥98.0% (HPLC) (Fluka) was also tested at 1 mg/ml concentration.

**Test microorganisms**

The bacterial strains used for screening antimicrobial activity were collected from different infectious status of patients who had not taken any antibacterial drugs for at least two weeks with the help of an authorized physician, in the district health center of Shivamogga, Karnataka State, India (Table 1). The clinical isolates were identified following a standard method (Cowan _et al._ 1993). The bacterial suspensions were diluted in 10⁻¹ to 10⁻⁸ phosphate buffered saline. Samples were homogenized and then loaded in six aliquots of 20 μl each onto nutrient agar plates (agar, 15 g/L, beef extract 1 g/L, peptone 5 g/L, NaCl 5 g/L, yeast extract 2 g/L; diameter 55 mm, final pH 7.0 ± 0.2).

The 8 clinically isolated bacterial cultures of both gram-positive and gram-negative bacterial strains used for screening are: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi* and *Proteus sp.* The working cultures were prepared by inoculating a loopful of each test microorganism in 3 ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 24 hours. The suspension was diluted with sterile distilled water to obtain approximately 10⁶ CFU/ml.

**Determination of minimum inhibitory concentrations (MICs)**

The agar dilution susceptibility test was performed based on modified method of NCCCLS and CLSI to determine the MIC. The Extracts dissolved in sterilized DMSO (400 mg/ml concentration) were taken as standard stock. A series of two fold dilutions of each extract in the final concentration of 40, 20, 10, 5 and 2.5 mg/ml were prepared in nutrient agar. After solidification, the plates were spotted with 100 μl of overnight grown bacterial cultures and provide novel or lead compounds that may be employed in controlling some infections globally.

### Table 1: Profile of the clinical strains used for antibacterial activity

<table>
<thead>
<tr>
<th>Clinical strains</th>
<th>Clinical condition</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Food poisoning</td>
<td>Stool</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Pneumonia</td>
<td>Sputum</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Burns</td>
<td>Mucus</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Food poisoning</td>
<td>Blood</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em></td>
<td>Hydronephrosis</td>
<td>Urine</td>
</tr>
<tr>
<td><em>Proteus sp.</em></td>
<td>UTI</td>
<td>Urine</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Foes poisoning</td>
<td>Stool</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Foes poisoning</td>
<td>Stool</td>
</tr>
</tbody>
</table>
approximately containing $1 \times 10^8$ CFU/ml. The test was carried out in triplicates. The plates were incubated overnight at 37°C. After 18 – 24 hours, the MIC was determined.

**Screening for antibacterial activity**

A sensitive radial diffusion technique was used for the assessment of antibacterial activity of the test samples. Sterilized nutrient agar medium was poured into sterilized Petri dishes. Nutrient broth containing 200 μl of test strain ($1 \times 10^6$ CFU/ml) of 24 h-incubated cultures of the clinical isolates were spread separately on the agar medium. Wells were created using a stainless steel sterilized cork borer (6.0 mm) under aseptic conditions. 100 μl of the plant extract at a concentration of 80 mg/ml were loaded into wells. 10% DMSO was used as negative control and the fluoroquinolone antibiotic Ciprofloxacin (BioChemika, ≥98.0% (HPLC) (Fluka)) was used as the standard.

**Statistical analysis**

The experiments were carried out in triplicates and the mean values of zone of inhibition were evaluated after 24 hrs of incubation. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard deviation of zone of inhibition values between the extracts against bacterial culture.

**RESULTS AND DISCUSSION**

The Soxhlet extraction of 1000 g of leaf powder yielded 2.72% w/v of petroleum ether, 1.4% w/v g of chloroform and 13.62% w/v of ethanol extract respectively. And the yield of stem bark extracts were 0.46% w/v of petroleum ether, 0.76% w/v g of chloroform and 23% w/v of ethanol extract respectively.

The MIC values of 6 extracts ranged from 2.5 to 20 mg/ml [Table 2]. Petroleum ether extract of leaf showed maximum activity with MIC 10 mg/ml for *Salmonella typhi*, and petroleum ether extract of stem bark extract showed 5 mg/ml MIC for *Bacillus subtilis*. Chloroform extract of leaf showed 5 mg/ml MIC for *Bacillus subtilis*, and chloroform extract of stem bark extract showed 2.5 mg/ml MIC for *Bacillus subtilis* and *Klebsiella pneumoniae*. And ethanol extract of ethanol exhibited 2.5 mg/ml MIC for *Klebsiella pneumoniae*. In general, all extracts showed less than 40 mg/ml MIC for the tested bacterial pathogens. Interestingly, ethanol extract of stem bark extract had higher activity in *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella typhi* even at a low concentration of 2.5 mg/ml.

The antibacterial activity of the organic solvent extracts showed varying magnitudes of inhibition patterns with standard positive control depending on the susceptibility of the tested microorganism. The mean inhibitory zone of six solvent extracts against 8 bacterial species is summarized in Table 3.

The analysis of petroleum ether extract of leaf against clinical bacterial pathogens showed a significant level of inhibition against *Salmonella typhi* (5.75 ± 0.50), and petroleum ether extract of stem bark showed high activity against *Bacillus subtilis* (11.80 ± 0.84). On the other hand, chloroform extract of leaf showed high activity against *Bacillus subtilis* (13.20 ± 0.84), and chloroform extract of stem bark showed significant activity against *Staphylococcus aureus* (13.60 ± 0.89), *Bacillus subtilis* (13.20 ± 0.84) and *Klebsiella pneumoniae* (12.0 ± 1.0). Interestingly, ethanol extract of leaf exhibits maximum inhibitory activity against *Klebsiella pneumoniae* (16.40 ± 0.80) in comparison to other extracts, and ethanol extract of stem bark exhibited significant high inhibitory zones against *Bacillus subtilis* (19.20 ± 1.52), *Escherichia coli* (16.40 ± 0.55), *Staphylococcus aureus* (15.20 ± 0.84), *Klebsiella pneumoniae* (14.80 ± 1.30) and *Salmonella typhi* (13.80 ± 0.45) in comparison to other extracts. It is understandable that ethanol extract of stem bark is more potent showing a higher degree of antimicrobial activity to tested clinical bacterial pathogens in comparison to other extracts. In addition, moderate effects were seen in petroleum ether and chloroform extracts of stem bark, and chloroform extract of leaf against *Klebsiella pneumoniae*.

**Table 2: Minimum inhibitory concentration (MIC) of various solvent extracts L. glutinosa against microorganisms**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Plant extracts</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem bark</td>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.E</td>
<td>C.E</td>
<td>E.E</td>
<td>P.E</td>
<td>C.E</td>
<td>E.E</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10</td>
<td>2.5</td>
<td>2.5</td>
<td>*</td>
<td>*</td>
<td>2.5</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td>20</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>20</td>
<td>10</td>
<td>2.5</td>
<td>*</td>
<td>*</td>
<td>5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>20</td>
<td>*</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>10</td>
<td>*</td>
<td>2.5</td>
<td>*</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>10</td>
<td>10</td>
<td>2.5</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Salmonella paratyphi</td>
<td>*</td>
<td>10</td>
<td>5</td>
<td>*</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>20</td>
<td>20</td>
<td>5</td>
<td>*</td>
<td>20</td>
<td>5</td>
</tr>
</tbody>
</table>

*No inhibition observed upto: 40 mg/ml.*
Extracts of *L. glutinosa* leaves exhibited high inhibitory potency against clinical pathogens. The phytochemical analysis showed the presence of effective biological compounds like alkaloids, flavonoids, tannins, and saponins. These derivatives could be potential alternatives to the traditional chemical control of clinical pathogenic bacteria. Fractionation and characterization of these active compounds will be the future work to investigate.

**CONCLUSION**

**REFERENCES**


Pradeepa, et al.: Antibacterial screening of the stem bark and leaf extracts of Litsea glutinosa (Lour.) C.B. Rob.


