Antimicrobial activity of Salix tetrasperma Roxb. (Salicaceae)

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Abstract
Salix tetrasperma Roxb. belongs to the family Salicaceae. The plant is traditionally used and is reported to exhibit several bioactivities. The present study was carried out to investigate antimicrobial activity of leaf extract of S. tetrasperma. Extraction of shade dried and powdered leaf material was carried out by maceration process. Antibacterial activity was evaluated by agar well diffusion method against 4 Gram positive and 4 Gram negative bacteria. Antifungal activity was determined against 4 seed-borne fungi by poisoned food technique. The extract exhibited concentration dependent activity against test bacteria. Among Gram positive and Gram negative bacteria, S. epidermidis and P. aeruginosa displayed highest susceptibility to extract respectively. Inhibition of test fungi by extract was concentration dependent. Among fungi, marked and least susceptibility to extract was shown by Rhizopus sp. and Cladosporium sp. respectively. In suitable form, the leaf of S. tetrasperma can be used to treat bacterial infections and to manage seed-borne fungal diseases of plants.

Keywords: Salix tetrasperma, Maceration, Agar well diffusion, Poisoned food technique

1. Introduction
Antibiotic resistance is a global problem and is a serious and widespread problem in developing countries. Emergence of resistant pathogens is mainly due to indiscriminate use of antibiotics and the resistant pathogens are of serious concern in both hospitals and the community. Bacteria such as Staphylococcus aureus, Mycobacterium tuberculosis, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa are few among the antibiotic resistant bacteria. Antibiotic resistant bacteria are known limit the efficacy of current drugs and cause high mortality each year due to failure of treatment. This situation triggered immense research on searching for alternatives for disease therapy. Natural products, in particular botanicals, offer as an important alternative strategy for therapy against infectious diseases. Extracts and purified compounds from higher plants have shown to exhibit potent inhibitory activity against a wide range of pathogenic bacteria including antibiotic resistant bacteria [1-7].

Seed is an important input in the production of several crops. Seeds are considered as passive carriers of several pathogenic microorganisms. Fungi such as species of Aspergillus, Helminthosporium, Cladosporium, Penicillium, Rhizopus, Fusarium, Curvularia, Alternaria and Cercospora are associated with seeds. Many seed-borne fungi are known to cause reduction in seed quality, seed abortion, seed rot, reduction of germination ability and seedling damage. Besides, some seed-borne fungi such as Aspergillus and Penicillium are known to elaborate certain toxins (mycotoxins) which cause severe health hazards on consumption. Fungal diseases of plants are one of the major constraints in successful crop production and are known to cause severe yield loss. The unrestrained use of synthetic fungicides for the control of phytopathogenic fungi and diseases of crop plants resulted in serious threat to human health as well as environment. Extensive use of fungicide is leading to disturbed biodiversity (due to effects on non-target organisms) and development of resistance in the plant pathogens. Hence there is immense scope for searching alternative strategies for diseases control. The use of natural products such as plants and biological control agents appears to be the best alternatives for chemical agents [8-11].

The genus Salix comprises about several species mainly distributed in the temperate regions of the world and in higher altitudes of the tropics. Salix tetrasperma Roxb., belonging to the family Salicaceae, is a small sized tree characterized by glabrous to silky pubescent twigs and simple, shiny, elliptic-lanceolate leaves with acute apex and characteristic margin. Bark is steel grey in color, farrowed and scaly in appearance. Flowers are unisexual, sessile and are in catkin. Fruit is a 2-valved capsule, dry and dehiscent. The plant is commonly known as Indian willow and often grows along water bodies. The plant is found distributed in various parts of...
Karnataka such as Bangalore, Belgaum, Chikmagalur, Shimoga, Mysore, Kodagu, Hassan and Uttara Kannada [12, 13]. The plant is used as fuel wood, and in making furniture, roof, fence and agricultural tools. The twigs are made to make baskets [14-16]. Various parts of *S. tetrasperma* are used traditionally for the treatment of ailments or diseases such as pain, fever, rheumatism, epilepsy, venereal diseases, swellings, piles and bladder stone [17-21]. Several bioactivities such as analgesic [13], anti-inflammatory [13], antipyretic [22], antioxidant [23, 24], antimicrobial [24, 25], hypoglycaemic [26], laxative [27], diuretic [27], cytotoxic [28], insecticidal [24] and CNS activity [29]. The present study was carried out to investigate antibacterial and antifungal activity of solvent extract obtained from leaves of *S. tetrasperma*.

2. Materials and Methods
2.1. Media and chemicals
Three culture media viz. Nutrient agar, Nutrient broth and Potato dextrose agar and chemicals viz. methanol, Streptomycin and Dimethyl sulfoxide (DMSO) were purchased from HiMedia laboratories, Mumbai, India.

2.2. Plant material and extraction
The plant *S. tetrasperma* was collected at Siddarahanli, near Matturu, Shimogga, Karnataka during January 2017. The plant was authenticated by Prof. D. Rudrappa, Department of Botany, S.R.N.M.N College of Applied Sciences, Shimogga. The leaves were separated, washed, dried under shade and powdered. Extraction of leaf powder (10g) was carried out by maceration technique using methanol (100ml). The leaf material was left in methanol for two days in stoppered container and later filtered [30]. The leaf extract (LE) obtained was used for antimicrobial studies.

2.3. Test bacteria and inoculum preparation
Four Gram positive bacteria (*Staphylococcus aureus* NCIM 5345, *Staphylococcus epidermidis* NCIM 2493, *Bacillus subtilis* NCIM 2063 and *Bacillus cereus* NCIM 2016) and four Gram negative bacteria (*Klebsiella pneumoniae* NCIM 2957, *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200 and *Salmonella typhimurium* NCIM 2501) were used. The test bacteria were maintained on nutrient agar slants under refrigeration condition. The test bacteria were seeded into sterile nutrient broth tubes and incubated overnight at 37 °C.

2.4. Antibacterial activity of LE of *S. tetrasperma*
Agar well diffusion method as employed by Raghavendra et al. [31] was used to evaluate antibacterial activity of two concentrations of LE (10mg/ml and 20mg/ml) of *S. tetrasperma*. The method involved swabbing of overnight growth broth cultures of test bacteria on the surface of sterile nutrient agar plates aseptically followed by punching wells of 8mm diameter using a sterile cork-borer. The wells were labeled and filled with 100µl of LE, reference antibiotic (Streptomycin, 1mg/ml of sterile distilled water) and DMSO. The plates were left undisturbed for 24 hours at 37°C followed by measuring the diameter of inhibition zones formed around the wells.

2.5. Test fungi
Four seed-borne fungi viz. *Cladosporium* sp., *Helminthosporium* sp., *Rhizopus* sp. and *Penicillium* sp. isolated previously from paddy seeds were used. The test fungi were maintained on potato dextrose agar slants in refrigerator.

2.6. Antifungal activity of LE of *S. tetrasperma*
Poisoned food technique was employed to evaluate antifungal potential of LE of *S. tetrasperma*. Potato dextrose agar was prepared, autoclaved, poisoned with LE (0.5 and 1.0mg/ml of medium) and poured into sterile plates. The control (without LE) and poisoned potato dextrose agar plates were inoculated aseptically with well sporulated (7 days old) cultures of test fungi and the plates were incubated at room temperature for 5 days. Later, the diameter of fungal colonies was measured using a ruler. Extent of inhibition of mycelial growth of fungi (%) was calculated using the formula:

\[
\text{Mycelial growth inhibition} \ (%) = \left( \frac{D_c - D_t}{D_c} \right) \times 100
\]

where Dc refers to colony diameter of fungi in control plates and Dt denotes diameter of fungal colonies in poisoned plates[30].

2.7. Statistical analysis
Antibacterial and antifungal experiments were carried out in triplicate. The results were presented as Mean ± Standard deviation (S.D).

3. Results and Discussion
3.1. Antibacterial activity of LE of *S. tetrasperma*
In the present study, the LE of *S. tetrasperma* was found to exhibit concentration dependent inhibitory activity against test bacteria as noticed by an increase in the diameter of zones of inhibition around wells on increasing in the concentration of extract. LE exhibited varied inhibitory activity against test bacteria. Marked and least inhibitory activity was observed against *S. epidermidis* and *E. coli* respectively. At 10mg/ml concentration, LE was not effective in inhibiting *E. coli*. Among Gram positive and Gram negative bacteria, *S. epidermidis* and *P. aeruginosa* displayed highest susceptibility to extract respectively. Reference antibiotic displayed higher inhibition of test bacteria when compared to LE. Overall, LE as well as reference antibiotic were more active against Gram positive bacteria when compared to Gram negative bacteria. In an earlier study, Islam et al. [24] also observed the inhibition of leaf extract of *S. tetrasperma* against Gram positive bacteria while most of Gram negative bacteria remain unaffected. In another study, a herbal formulation containing *S. tetrasperma* was shown to inhibit acne causing *Propionibacterium acnes* and *Staphylococcus epidermidis*. Abdel-Hameed et al. [32] evaluated antibacterial activity of leaves of *S. tetrasperma* and observed that the plant was not effective in exhibiting antibacterial activity at 1mg/ml concentration tested. The lower susceptibility of Gram negative bacteria to LE of *S. tetrasperma* and antibiotic could be attributed to the presence of an outer membrane which might have acted as an additional barrier for the entry of LE and antibiotic.

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Zone of inhibition in cm (Mean± S.D)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LE 10mg/ml</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1.03±0.00</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>2.10±0.10</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>1.73±0.05</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>1.60±0.00</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>1.33±0.05</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>1.50±0.10</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>1.30±0.00</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

3.2. Antifungal activity of LE of *S. tetrasperma*
A considerable reduction in mycelial growth of test fungi was
observed in plates poisoned with LE of *S. tetrasperma*. The LE displayed concentration dependent inhibition of test fungi (Table 2). Among fungi, marked and least susceptibility to extract was shown by *Rhizopus* sp. and *Cladosporium* sp. respectively. At extract concentration of 1mg/ml, an inhibition of >60% of all fungi was observed. At 0.5mg/ml concentration, *Cladosporium* sp., *Rhizopus* sp., *Helminthosporium* sp. and *Penicillium* sp. were inhibited to 25.79%, 39.70%, 27.20% and 33.33% respectively. At 1.0mg/ml concentration, *Cladosporium* sp., *Rhizopus* sp., *Helminthosporium* sp. and *Penicillium* sp. were inhibited to 61.13%, 70.14%, 62.79% and 63.63% respectively. In a similar study, Pushpavathi et al.[25] observed marked inhibitory activity of leaf extract of *S. tetrasperma* against seed-borne fungi isolated from sorghum seeds. The study of Abdel-Hameed et al.[33] showed no inhibitory activity of leaf extract of *S. tetrasperma* against *Candida albicans* and *Aspergillus niger*. In a study by Deepak et al.[34], *S. tetrasperma* failed to show inhibition of zoosporangium formation of *Sclerospora graminicola*, causal agent of downy mildew disease of pearl millet.

Table 2: Colony diameter of test fungi in control and poisoned plates

<table>
<thead>
<tr>
<th>Test fungi</th>
<th>Colony diameter in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>Cladosporium</em> sp.</td>
<td>2.83±0.05</td>
</tr>
<tr>
<td><em>Rhizopus</em> sp.</td>
<td>6.80±0.00</td>
</tr>
<tr>
<td><em>Penicillium</em> sp.</td>
<td>3.30±0.00</td>
</tr>
<tr>
<td><em>Helminthosporium</em> sp.</td>
<td>4.30±0.10</td>
</tr>
</tbody>
</table>

4. Conclusion

In the present study, the LE of *S. tetrasperma* exhibited concentration dependent inhibitory activity against bacteria and fungi. The plant appears to be a suitable candidate for developing herbal formulation and lead drugs for the treatment of infectious bacterial diseases and management of plant diseases caused by seed mycotoxins.

5. Acknowledgements

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6. References

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