Antimicrobial Screening of Wild Solanum Species against Human Respiratory Tract Infecting Biota by Bioautography Method

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ABSTRACT
In current world Natural products from plants are of keen interest for the discovery of antimicrobial compounds in the field of medicine. The present study was aimed to evaluate the antimicrobial activities against selected seven human respiratory tract infecting biota in three wild Solanum species namely Solanum indicum, Solanum trilobatum and Solanum xanthocarpum which are widely used in folk and traditional medicine to cure fever, whooping cough, bronchitis and common cold. The organic solvents such as Hexane, Acetone and Ethanol extract of leaves and fruits were used to find out the virulent activity of plant extracts on human pathogens. The standard disc diffusion assay was performed and the best five solvent extracts had chosen to perform TLC. Out of five solvent extracts three was chosen for direct bioautography study through agar overlay assay. All the extracts of the plants were tested against gram- positive and gram-negative microorganism to know there antimicrobial properties. Of these, leaf and fruit extracts of Solanum indicum, leaf of Solanum trilobatum exhibited maximum inhibition of the microbial growth than Solanum xanthocarpum extract.

Keywords: Antimicrobial, Solanum sp., Plant extract, disc diffusion assay, bioautography, agar overlay assay.

INTRODUCTION
Medicinal plants have been used for several years in daily life to treat various diseases all over the world. Impressive numbers of modern drugs have been isolated from natural source and many are based on their use in traditional medicine. About 80% of individuals from developed countries using traditional medicine, which has been seen as a valuable source of medicine with proven potential of treating infectious diseases³. Compared to the synthetic drug agents plant compounds have lesser side effects. According to world health organization, medicinal plant would be the source to obtain a variety of drugs. Therefore, importance should be given to investigate their properties, safety and efficiency² and to provide the impetus to the search for novel substances from various sources of medicinal plants³. Several medicinal plants have been screened for their antimicrobial and antiviral properties⁴. Since plants produced a diverse range of bioactive molecules they will play a dominant role in the maintenance of human health⁵. The number of multi drug resistant microbial strains and their susceptibility to antibiotic are continuously increasing. This increase has been attributed to indiscriminate use of broad spectrum of using antibiotics and immune suppressive agents. There is an urgent need for the development of indigenous alternative antimicrobial molecules for the effective treatments of some serious diseases in the light of growing cases of microbial resistance to the time honoured antibiotics⁶. Almost as soon as antibacterial drug were developed, bacteria responded by manifesting various forms of resistance⁷. In this the wild plants which are used in traditional medicine such as Solanum indicum, Solanum trilobatum, Solanum xanthocarpum was further studied in order to insights the antimicrobial properties of the metabolites of the plants. Which help in identification of potential compounds and development of drug against Respiratory tract infecting biota.

MATERIALS AND METHODS
Plant Material Collection
The plant samples like leaves and fruits of Solanum xanthocarpum, Solanum trilobatum and Solanum indicum were collected from Eastern Ghats of Krishnagiri District, Tamil Nadu. The plant was identified and confirmed by Dr. G. Jeya Jothi, Assistant Professor, Department of Plant Biology and Biotechnology, Loyola College, Chennai.

Extraction Method
Leaves and fruits of Solanum xanthocarpum, Solanum trilobatum and Solanum indicum were shade dried and subsequently powdered and packed in air tight containers. Powdered plant material (20g) was soaked in 100% solvents at room temperature for three days on a rotary shaker (120rpm). Sequential extraction was carried out starting from low polar solvent to highly polar solvents. All extract were filtered through Buchner funnel with Whatman’s filter Paper No.1 and the extracts were dried to remove the solvent and stored in brown bottles.

Culture of Microorganisms
The human respiratory tract infecting microorganisms were employed in the screening. Gram-positive and Gram-negative microorganisms used in this study were collected from Department of Plant Biology and Biotechnology

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Table 1: In vitro antimicrobial activity of crude extract from the leaves of Solanum trilobatum (Linn) Jack, Solanum indicum (Linn) Jack and Solanum xanthocarpum (Sbard& H. wendl) Jack by disc diffusion method.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Microorganisms</th>
<th>S. trilobatum</th>
<th>S. indicum</th>
<th>S. xanthocarpum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HX</td>
<td>AC</td>
<td>EA</td>
<td>HX</td>
</tr>
<tr>
<td>1</td>
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<td>10</td>
<td>NZ</td>
</tr>
<tr>
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<td>Klebsiella pneumonia</td>
<td>NZ</td>
<td>NZ</td>
<td>NZ</td>
</tr>
<tr>
<td>4</td>
<td>E. coli</td>
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<td>NZ</td>
<td>NZ</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
<td>13</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>Salmonella typhi</td>
<td>NZ</td>
<td>8</td>
<td>NZ</td>
</tr>
<tr>
<td>7</td>
<td>Salmonella paratyphi</td>
<td>NZ</td>
<td>8</td>
<td>NZ</td>
</tr>
</tbody>
</table>

NZ: No zone, HX: Hexane, AC: Acetone, EA: Ethanol, CN: Control Disc content: Crude extracts 3.0 mg/disc; Control: Erythromycin 15mg/disc

Table 2: In vitro antimicrobial activity of crude extract from the fruits of Solanum trilobatum (Linn) Jack, Solanum indicum (Linn) Jack and Solanum xanthocarpum (Sbard& H. wendl) Jack by disc diffusion method.

<table>
<thead>
<tr>
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<th>S. indicum</th>
<th>S. xanthocarpum</th>
</tr>
</thead>
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<tr>
<td></td>
<td>HX</td>
<td>AC</td>
<td>EA</td>
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</tr>
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<td>Streptococcus pneumonia</td>
<td>NZ</td>
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<td>2</td>
<td>Streptococcus pyogenes</td>
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<td>Klebsiella pneumonia</td>
<td>NZ</td>
<td>NZ</td>
<td>NZ</td>
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<td>4</td>
<td>E. coli</td>
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<td>Pseudomonas aeruginosa</td>
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<td>6</td>
<td>Salmonella typhi</td>
<td>10</td>
<td>NZ</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Salmonella paratyphi</td>
<td>NZ</td>
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</tr>
</tbody>
</table>

NZ: No zone, HX: Hexane, AC: Acetone, EA: Ethanol, CN: Control Disc content: Crude extracts 3.0 mg/disc; Control: Erythromycin 15mg/disc

Laboratory. Namely Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Salmonella paratyphi. The stock cultures were maintained in nutrient agar (NA) slant at 4°C and as the sub cultures. Working cultures were prepared by inoculating a loop full of each test microorganism in 3 ml of nutrient broth (NB). Broths were incubated at 37 °C for 24 hours. The suspension was diluted with sterile distilled water to obtain approximately 10⁶ CFU/ml.

Antimicrobial screening

The antimicrobial screening was carried out by standard disc diffusion method and the compounds with more activity are subjected to separation using thin layer chromatography. Then the activities of separated compounds in TCL plates are studied using direct bioautography method.

Media Preparation and Disc Diffusion Method

Kirby-Bauer method was followed for disc diffusion assay⁴. In vitro antimicrobial activity was screened by using Nutrient Agar (NA) obtained from Hi-media. The NA plates were prepared by pouring 20 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 to 10 minutes and 0.1% inoculums suspension swabbed uniformly and the inoculums were allowed to stand. The extract with 3mg/disc concentration was loaded on 5mm sterile discs. The loaded discs were placed on the surface of medium and compound was allowed to diffuse and the plates were kept for incubation at 37 °C for 24hrs. Erythromycin (5µg/disc) was used as control. After 24 hours, the inhibition zone formed around the disc was measured with transparent ruler in millimetre. These studies were performed in triplicate.

Thin Layer Chromatography

Five solvents were used for thin layer chromatography in different ratios. The TLC plates were cut into the size of 10x5 (height × breath). Totally five samples were prepared, which showed best activity in disc diffusion method was chosen to run TLC. Hexane, Chloroform, Ethyl acetate, Acetone and Methanol were used in the ratios of Hexane: chloroform→ 9:1, 7:3, 5:5; Ethyl acetate: Methanol→ 9:1, 7:3, 5:5; Hexane: Chloroform: Ethyl acetate→6: 3: 1, 7: 2: 1 and Ethyl acetate: Acetone: Methanol→6: 3: 1, 7: 2: 1.

Rf Value

The location of each spot on the plate was represented.
RESULTS

In current study antimicrobial properties were tested on three extracts via Agar overlay method. The bioactive molecule was separated by Thin Layer Chromatography method by employing organic solvents system. For this process four leaf extract of S. trilobatum, S. xanthocarpum and S. indicum and a fruit extract were chosen namely, S. trilobatum leaf (Hexane and Acetone extract) and S. indicum leaf (hexane and acetone) and fruit (ethanol). The RF value was calculated and the results were tabulated (Table 4). Among the solvent systems hexane: chloroform system showed good separation of compounds.

Agar overlay Bioautography

Table 3: Rf value of various extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of extract</th>
<th>Solvent and its ratio</th>
<th>Solvent</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AL₂</td>
<td>Hexane: Chloroform</td>
<td>7:3</td>
<td>0.34, 0.48, 0.55, 0.72, 0.85, 0.89</td>
</tr>
<tr>
<td>2</td>
<td>CL₂</td>
<td>Ethyl acetate: Methanol</td>
<td>8:2</td>
<td>0.13, 0.23, 0.31, 0.58, 0.83, 0.96</td>
</tr>
<tr>
<td>3</td>
<td>CF₃</td>
<td>Ethyl acetate: Methanol</td>
<td>6:3:1</td>
<td>0.30, 0.37, 0.50, 0.58, 0.67, 0.90, 0.96</td>
</tr>
</tbody>
</table>

All the three leaf extracts of S. trilobatum, S. xanthocarpum and S. indicum demonstrated antibacterial activity against all the microbes except Klebsiella pneumonia in S. trilobatum and E. coli in S. indicum. In S. trilobatum maximum activity of 15 mm was recorded in hexane & acetone extracts against Pseudomonas aeruginosa and in Streptococcus pyogenes(acetone). Lowest activity of 9 mm was observed in acetone extract against Salmonella typhi and Salmonella paratyphi respectively. In S. xanthocarpum maximum activity of 16 mm was recorded in Salmonella typhi (hexane and ethanol), Streptococcus pyogenes (hexane). No bacteria was resistance to this plant extracts. Lowest inhibitory activity of 8 mm was seen in ethanol extract against Salmonella paratyphi. In S.indicum maximum activity of 18 mm was recorded in acetone extract against Streptococcus pyogenes, 18 mm was recorded in ethanol extract against Salmonella typhi. Lowest inhibitory activity of 8mm was seen in acetone extract against Klebsiella pneumonia and Pseudomonas aeruginosa respectively.

Antimicrobial activity of S. trilobatum, S. xanthocarpum and S. indicum fruit extracts (table: 2)

All the three fruit extracts of S. trilobatum, S. xanthocarpum and S. indicum demonstrated antibacterial activity against all the microbes except Streptococcus pneumonia, Salmonella paratyphi and Klebsiella pneumonia in S. trilobatum, Klebsiella pneumonia in S. xanthocarpum and E. coli in S. indicum. In S. trilobatum maximum activity of 14 mm was recorded in ethanol extract against Streptococcus pyogenes and E. coli. Lowest inhibitory activity of 8 mm was seen in hexane extract against Pseudomonas aeruginosa. In S. xanthocarpum maximum activity of 15 mm was recorded in ethanol extract against Streptococcus pyogenes and Salmonella typhi. Lowest inhibitory activity of 8 mm was observed in hexane extract against E. coli. In S. indicum maximum activity of 20 mm was recorded in ethanol extract against Streptococcus pyogenes, Salmonella typhi and Pseudomonas aeruginosa. Lowest inhibitory activity of 8mm was seen in hexane extract against Streptococcus pneumoniae.

Thin Layer Chromatography

The bioactive molecule was separated by Thin Layer Chromatography method by employing organic solvents system. For this process four leaf extract of S. trilobatum, S. xanthocarpum and S. indicum and a fruit extract were chosen namely, S. trilobatum leaf (Hexane and Acetone extract) and S. indicum leaf (hexane and acetone) and fruit (ethanol). The RF value was calculated and the results were tabulated (Table 4). Among the solvent systems hexane: chloroform system showed good separation of compounds.

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Three samples were selected based on the separation of compounds for bioautography. And these were tested against three bacterial organisms *Streptococcus pyogenes*, *Streptococcus pneumonia* and *Pseudomonas aeruginosa*. The inhibition zone was observed against *Streptococcus pyogenes*.

**In-vitro** Antimicrobial activity of *Solanum trilobatum* (Linn) Jack fruit and leave extracts.


**In-vitro** Antimicrobial activity of *Solanum indium* (Linn)Jack fruit and leave extracts


**In-vitro** Antimicrobial activity of *Solanum xanthocarpum* (Schard& H. wendl) Jack fruit and leave extracts


Image showing separation through TLC and Agar overlay assay activity against *Streptococcus pyogenes*.
pyogenes against Solanum indicum leaf extract (CL2) by the compound at Retention factor of 0.67 with a zone of 6mm, followed by Solanum trilobatum leaf extract (AL2) by the compound at Rf value 0.80 showing a zone of 4mm. No inhibition zone was observed against Streptococcus pneumoniae and Pseudomonas aeruginosa.

DISCUSSION
The revival of interest on traditional medicines is mainly due to multidrug resistance in pathogens against modern synthetic drugs. Herbal medicines do not cause drug resistance against bacteria. The utilization of various indigenous medicinal plants is flourishing in different countries even today; with nearly 80% of rural population still depend on plant based medicines for primary health care10. In India, nearly 2500 species of herbal plants are used and 90% of them provide raw material for the herbal pharmaceuticals, which are collected from the wild habitats11. In the present study both leaves and fruit extracts of S. trilobatum, S. xanthocarpum and S. indicum showed antibacterial activities against almost all the tested organisms. Earlier investigation on antimicrobial activities of plant extract clearly showed that solvents like hexane, ethyl acetate, acetone, methanol and ethanol can extract active principles from plant parts2. In the present study, leaf extracts of S. trilobatum and S. xanthocarpum were more active than the fruit extracts; this might be due to the presence of maximum number of phytochemicals in leaves than fruits12. In S. indicum both the leaf and fruit extracts shows very good activities. Earlier study reported that ethanol and acetone extracts of plants had inhibited growth of many bacteria including E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae13. Same observation was reported by2. Ethanol extract of S. trilobatum leaves showed antibacterial activity against tested bacterial strain Klebsiella pneumoniae upto10 mm12 whereas in the present study there was no inhibition zone observed in the solvent extracts. In the present study the ethanol extract of Solanum indicum inhibits a maximum zone of 20Mm in Streptococcus pyogenes, in acetone extract inhibits a maximum zone of 16Mm against Streptococcus pyogenes. Agar overlay technique is a hybrid of the two other methods and works successfully with a range of microorganism. The acetone extract of S. indicum and S. trilobatum showed activity against Streptococcus pyogenes in this method.

CONCLUSION
Solvent extract of Solanum trilobatum, Solanum xanthocarpum and Solanum indicum exhibited antimicrobial activity and inhibiting the growth of gram positive and gram negative bacteria at different degrees indicating the presence of broad spectrum antibiotics. Bioautography revealed the presence of specific and selective antimicrobial compounds in the extracts which may or may not have broad range activity. Further research needs to be done to extract and identify the active compounds. The active compound needs to be isolated and studied for its toxicity and in vivo efficacy resulting in development of better antimicrobial drugs.

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The authors are grateful to Rev. Dr. Arockiasamy Xavier,Principal, Loyola College, and Dr. R. Ravindhran, Head of the Department of Plant Biology and Biotechnology, Loyola College, for their constant help and continuous encouragement.

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