STUDIES ON ANTIMICROBIAL ACTIVITY AND ISOLATION OF
ANTIMICROBIAL PHYTOPHARMACEUTICALS FROM
SALACIA OBLONGA WALL.

Abstract

*Salacia oblonga* Wall. ex Wight & Arn. belonging to the family *celastraceae* is a large woody climber distributed in Sri Lanka and Southern India. The antimicrobial activity of *S. oblonga* was evaluated by extracting the aerial and root parts of plants in different solvents e.g., ethyl acetate, chloroform, hexane, petroleum ether, methanol, ethanol, and water alone and also in combination with HCl. The six Gram negative and five Gram positive pathogenic microorganisms were used for the present study. Gram Positive (+ve) *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Bacillus subtilis & Listeria monocytogenes* (MTCC 1143) and Gram negative (-ve) *Klebsiella pneumonia, Enterobacter aerogenes* (MTCC 111), *Enterobacter cloacae, Pseudomonas aeruginosa, Escherichia coli & Salmonella typhimurium* (MTCC 98).

Extracts of aerial and root parts of *S. oblonga* had exhibited the antimicrobial activity against the different pathogenic organisms. Except for the water, petroleum ether and hexane extracts, all the extracts had significant antimicrobial activity against most of the microorganisms.
tested in the agar well diffusion assay. Antimicrobial activity of *S. oblonga* aerial and root (neutral and acidic) parts extracts and the standard antibiotic amikacin was depicted as the zone of inhibition on agar-well diffusion method. The antimicrobial activity of the extracted aerial and root parts in acidic pH showed good activity compared with neutral pH. The zone of inhibition is high in ethyl acetate aerial acidic (21.67±0.54 mm) & root acidic (21.33±0.58 mm) extract with *K. pneumoniae*. The MICs and MBCs for the EtOAc aerial and root (neutral and acidic) extracts were equal or less to those of positive controls and were in the ranges 0.03 – 5.00 and 0.07– >5.00 mg/ml, respectively. Analysis of ethyl acetate, chloroform, methanol, ethanol, hexane, petroleum ether and water extracts of *S. oblonga* by GC-MS (Gas chromatography Mass spectrum) revealed the following compounds [58 (EtOAc), 78 (CHCl₃), 23 (Hexane), 27 (Petroleum ether), 88 (MeOH), 20 (EtOH) and 3 (Water)] identified in the aerial neutral & acidic and root neutral &acidic extracts by comparing their mass spectra (MS) and retention indices (RI) with Wiley library literature data and spectra database. The EtOAc root acidic extract was further divided into four different fractions by column chromatography. Further, the fourth fraction which is showing maximum antimicrobial activity was subjected to LC-MS. In LC-MS (Liquid chromatography mass spectrum) analysis three compounds were identified which are compound 1 γ-sitosterol (mol.
wt. 415.2), compound 2 β-amyrin (mol. wt. 425.3) and acetic acid, 4, 4, 6a, 6b, 8a, 11, 12, 14b-octamethyl-1, 2, 3, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14b-octadecahydropicen-3-yl ester (mol. wt. 467.3). The activity of these isolated compounds 1 and 2 has been confirmed in the earlier reports.

The present results in *Salacia oblonga* has brought about the possibility of consumption of plant extracts, which has provided scientific proof for the development of antibacterial products and the treatment of bacterial infections in the future. This study that has provided new scientific information about chemical profiling of its antimicrobial activity. Isolation of bioactive components can reveal the exact potential of *Salacia oblonga* to inhibit pathogenic bacteria and a broad spectrum antimicrobial support in developing herbal formulations in future.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CAN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>GCMS</td>
<td>Gas chromatography – Mass spectroscopy</td>
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<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HUS</td>
<td>Haemolytic-uremic syndrome</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>LCMS</td>
<td>Liquid chromatography – Mass spectroscopy</td>
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<tr>
<td>MeOH</td>
<td>Methanol</td>
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<tr>
<td>MBC</td>
<td>Minimum bactericidal concentration</td>
</tr>
<tr>
<td>MHA</td>
<td>Mueller-Hinton Agar</td>
</tr>
<tr>
<td>MHB</td>
<td>Mueller-Hinton broth</td>
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<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MTCC</td>
<td>Microbial Type Culture Collection</td>
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<tr>
<td>MRSA</td>
<td>Methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>NA</td>
<td>Nutrient agar</td>
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**NDM-1**  New Delhi metallo-beta-lactamase-1  
**RP-HPLC**  Reverse Phase High Performance Liquid Chromatography  
**SSSS**  Staphylococcal scalded skin syndrome  
**TSS**  Toxic shock syndrome  
**UTIs**  Urinary tract infections  
**UV-VIS**  Ultraviolet-Visible  

**SYMBOLS**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>l</td>
<td>Litre</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>m</td>
<td>metre</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
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<td>µl</td>
<td>Microlitre</td>
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<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>ºC</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
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