Screening of antibacterial potential of leaves and leaf derived callus extracts of Solanum trilobatum L. an important medicinal plant

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ABSTRACT

The aim of present investigation was deals with the antibacterial activity of leaves and leaf derived callus extracts (ethyl acetate, ethanol, chloroform, and acetone) from the S. trilobatum L were tested against nine human pathogenic bacteria using agar well diffusion technique. A better inhibition zone was recorded in chloroform extract than other solvents of both extracts. Based on the results, it clearly indicates that most of the extracts were effective against S. aureus, S. typhi, S. dysentriae, S. sonii, C. diptheriae and S. boydii. The other solvent extracts (such as ethanol, acetone and ethyl acetate) showed moderate to least inhibitory effect against these organisms. The results from our findings indicated that S. trilobatum is one of the potential medicinal plant used for therapeutic purpose. Among the two extracts tested, callus extracts was found to be superior to field grown plants.

Key words: Solanum trilobatum L, Field plant, Callus, Pathogens, Antibacterial activity.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an enormous number of modern drugs have been isolated from natural sources especially from plants; many of these isolations were based on the uses of the agents in traditional medicine. This plant based, traditional medicine system continues to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (1). The World Health Organization (WHO) has also recommended the evaluation of plants for effectiveness against human diseases and for the development of safe modern drugs (2).

According to the literature cited the callus has the potential to show secondary metabolite activity and can often be compared with the field plant (3). The success of this strategy, however, depends on the validation of micro propagated plants through pharmacological screening on their suitability for use in traditional medicine.

Solanum trilobatum Linn (Family: Solanaceae), a thorny creeper with bluish white flower, widely distributed throughout India and has long been used in Siddha system of medicines to treat various diseases (4). It has been widely used as an expectorant and in the treatment of respiratory diseases including bronchial asthma (5), febrile infections, and tuberculosis (6). The methanolic extract of S. trilobatum has been shown to possess antioxidant activity (7) and hepatoprotective activity (8). Sobatum, the partially purified petroleum ether extract of S. trilobatum has been reported to be very effective in protecting UV induced damage, radiation-induced toxicity and inducing tumour reduction in mice (9,10). Solasodine and sobatum isolated from S. trilobatum plant has been shown to possess anti inflammatory activity (11). The methanolic extract has been reported to be very effective in protecting Panaeus monodon post larvae from bacterial attack (12) and the acetone extract has been shown to possess ovidical activity against Culex quinquesfasciatus and Culex tritaeniorhynchus (13); and oviposition deterrent and skin repellent activity against Anopheles stephensi (14).

In view of this medicinal and biological importance of Solanum trilobatum, the present investigation was undertaken to screen the antibacterial properties of different solvent extracts of micro propagated (callus) and outdoor grown Solanum trilobatum were tested against selected human pathogenic microorganisms.

MATERIALS AND METHODS

Plant material and sterilization

The fresh and healthy aerial parts of S. trilobatum were collected from the garden and nomenclature was identified by Dr. D. Natarajan, Assistant Professor, Department of Biotechnology, Periyar University, Salem, Tamil Nadu. The explants (leaves) were excised aseptically with sterile scissors and washed with running tap water (for 2 – 5 min) followed by treatment with 5% (v/v) BaviStin fungicide (for 2 min) and rinsed thoroughly with sterile distilled water, then surface sterilized initially with 0.25 % (v/v) Sodium hypochlorite (for 5 min) and rinsed thoroughly with sterile distilled water (2 – 3 times). Explants were surface disinfected with 0.1% HgCl2 (w/v) solution (for 3 – 10 min) and rinsed thoroughly with sterile distilled water (3 - 5 times), and trimmed with sterile blade and wet explants are dried in No.1 whatman filter paper before inoculation (15).

Induction of callus

Explants were inoculated for callus in MS media supplemented with 2 – 3% sucrose as carbon source and 0.7% agar (HI Media) along with various concentrations of auxins and cytokinins (2, 4 – D (0.1 – 0.3 mg L–1), NAA (0.1 – 0.5 mg L–1) respectively. The pH of the media was adjusted to 5.8 prior to autoclaving for 15 min at 121°C. Cultures were maintained under cool-white fluorescent light at 24 ± 2°C with 16 h photoperiod. The different kinds of callus mass was obtained at various concentration of NAA, 2, 4 – D, and combination of NAA + 2, 4 D. The 1.5 months old callus tissues (MS medium containing 2, 4 – D + NAA 1.0 + 1.0 mg L–1) of in vitro leaf explant (S. trilobatum) were extracted and used in this study. Simultaneously, the field grown (1.5 months old) S. trilobatum leaves were selected for in vitro screening of antimicrobial properties.

Preparation of Extracts

The known amount of field grown and callus mass samples (1.0 g) were collected, dried and subjected to cold extraction by using different solvents like ethanol, chloroform, acetone and ethyl acetate (each 10 ml) at room temperature for 24 – 48 hrs in from the various solvents. The extracts was evaporated and yielded black paste and stored at room temperature for further use.
Micro organism used
Nine pathogenic organisms viz. three Gram positive (Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus), and six Gram negative (Salmonella typhi, Shigella boydii, Shigella dysenteriae, Shigella sonnei, Cornyebacterium diptheriae, and Klebsiella pneumoniae), bacterial strains were used to determine the antibacterial activity of the crude extracts.

Antimicrobial assay tests
The screening of antibacterial activity of both leaf and callus tissue was done by using agar well diffusion method with few modifications. The Muller Hinton agar media was poured into sterilized petriplate and allowed to solidify. The young bacterial cultures were spread on the top of each petriplate using sterilized L rod. After, the well (5mm diameter) was made aseptically over the top of plates using sterile borer (5mm diameter). The each well is filled with two concentration of plant and callus extracts (25 & 50 µl/well). The antibiotic Amoxicillin (50 µl/well) is served as positive and DMSO (50 µl/well) act as negative control. These plates were incubated for 24 - 48 hours. After incubation period, the zone of inhibition around the each well was measured.

RESULTS

In vitro Growth of callus
The leaf explants of S. trilobatum were inoculated in MS medium supplemented with different concentrations of 2, 4-D, NAA alone and its combination for callus regeneration. Greenish brown hard, pale yellow green callus was obtained within two weeks of inoculation of explants at 0.3 mg L\(^{-1}\)2, 4 – D and 0.5 mg L\(^{-1}\) NAA and combination of 2, 4 – D + NAA (1.0 + 1.0 mg L\(^{-1}\)) yield morphogenic pale yellow brown calli. Whereas, other combinations of 2, 4 – D and NAA yielded slightly friable and brownish callus (Table 1; Figure 1).

<table>
<thead>
<tr>
<th>Hormone Name</th>
<th>Concentration (mg L(^{-1}))</th>
<th>Response of callus mass</th>
<th>Callus appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>0.1</td>
<td>-</td>
<td>No callus mass</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>++</td>
<td>Yellow white hard</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>+++</td>
<td>Yellow, greenish hard</td>
</tr>
<tr>
<td>2, 4 – D</td>
<td>0.1</td>
<td>+</td>
<td>Pale brown hard</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>+</td>
<td>Brown hard</td>
</tr>
<tr>
<td>2, 4 – D + NAA</td>
<td>1.00 + 1.00</td>
<td>+++</td>
<td>Greenish Brown hard</td>
</tr>
</tbody>
</table>

*, ++, +++ indicates slight, moderate and considerable callusing, - indicates no response

Antimicrobial activity
The results of antibacterial activity of different solvent extracts from the leaves of field grown and callus of S. trilobatum was tested against Gram-positive and Gram-negative bacterial pathogens were presented in table 2. All the extracts exhibited different degrees of antibacterial activity against most of the tested organisms. The results indicated that all the organisms were found to be more susceptible from lower to higher concentrations of extracts. The results clearly indicates that most of the extracts were effective against S. aureus, S. typhi, S. dysentriae, S. sonii, C. diptheriae and S. boydii. Among the two extracts tested, Callus tissue extracts were found to be more active when compared to field grown plants.

The chloroform extracts from callus of S. trilobatum inhibited the growth of several Gram-negative bacteria i.e. S. dysentriae (18mm, 20mm), S. typhi (16mm, 20mm), C. diptheriae (13mm, 15mm) and S. aureus (12mm, 14mm) respectively. Solvent extracts of S. trilobatum leaves at two different concentrations exhibited mild to moderate inhibition over the growth of tested bacterial pathogens. The solvent leaf extract of S. trilobatum was found to be more effective against the all tested microorganisms with the inhibition zone ranging from 22 to 8 mm. It was found that chloroform leaf extract exhibited highest degree of activity against S. dysentriae (each 16mm), S. typhi (each 16mm), S. Sonii (each 22mm). The callus extracts prepared from acetone had broad spectrum activity against all the tested organisms with better inhibitory effect ranging from 13 to 8 mm. The ethanol leaf extracts showed moderate inhibitory activity against the tested bacterial pathogens except S. dysentriae. Similarly, the extracts of acetone and ethyl acetate have shown considerable inhibitory activity against the selected pathogenic organisms. Besides, all extracts were inactive against the one gram positive organism i.e. L. acidophilus. Hence, all the leaf and leaf derived callus extracts have been expressed better antibacterial property against more than one microorganisms tested.

DISCUSSION
The present study was carried out to evaluate the efficacy of S. trilobatum L. (Field grown plants and callus) leaf extracts against nine human pathogenic bacterial strains. Our results revealed that chloroform, acetone and ethanolic leaf extract of (callus and field grown) S. trilobatum L. possess considerable growth inhibitory effect against tested microorganisms. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health.

The chloroform callus extract showed higher inhibitory activity against many pathogenic organisms and other solvents (such as ethanol, acetone and ethyl acetate) showed...
moderate to least inhibitory effect against same organisms. The zone of inhibition (mm) of the callus extracts showed comparatively higher than the field leaf extracts of same solvents against same micro organisms. The antibacterial activity of leaf and fruit extracts of S. trilobatum was tested against various pathogens like S. aureus, E. coli, P. aeruginosa and K. pneumonia using extracts of ethanol, acetone and ethyl acetate extracts and showed better inhibitory activity (19). The extracts of leaves of S. trilobatum have shown significant activity against two tested organisms (S. aureus (21mm) and P. aeruginosa (20mm)). The results of our findings showed callus chloroform extract expressed moderate to better antibacterial activity against S. aureus (12 mm, 14 mm), S. sonii (22mm), S. typhi and S. dysentriae (16mm). In fine, the leaf and leaf derived callus extracts were more susceptible to gram negative organisms. Various workers have already shown that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria (19, 20). These differences may be attributed to fact that the cell wall in Gram positive bacteria is a single layer, whereas the Gram negative cell wall is multilayered structure (21).

Our results are comparable to the results of the antibacterial assay of S. trilobatum L. were tested against the Gram negative bacterial strain, Bacillus subtilis, was more susceptible and the gram negative strain Staphylococccus epidermidis was least susceptible (23). A study on the extracts of different solvents of Petunia leaf and callus against E. coli, P. aeruginosa, B. subtilis, S. aureus and reporting that callus chloroform extract possessing high antibacterial potential against the selected pathogens (23).

Other researchers contributed that antibacterial effect of callus and natural plant extracts of Perumna serratifolia L, Bacopa monnieri L and Alocyphus cobbe L plants extracts against selected human pathogens like B. subtilis, E. faecalis, E. coli, K. pneumoniae and fungal cultures using various solvents (such as chloroform, acetone, methanol, ethanol and water) was showed enhanced antimicrobial activity (25-26). The antifungal activity of Rovolufia tetraphylla and Physalis minima leaf and callus extracts (using chloroform, petroleum ether, methanol, absolute alcohol, benzene) were tested against pathogenic fungi and bacteria reported the chloroform extracts of both plants were found more effective against bacterial and fungal pathogens (15). Cytological and antimicrobial activity of embryogenic callus induced from leaf cultures of Tinospora cordifolia (Willd.) Miers (25).

Based on our findings the callus extracts of S. trilobatum showed higher inhibitory against several gram negative organisms and suggested that the use of this plant material (in vitro parts) are recommended for various pharmaceuticals and derivatives of drugs in future.

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REFERENCES


