EFFECT OF BIOACTIVE COMPOUNDS EXTRACTED FROM THE ROOT OF SOLANUM TORVUM SWARTZ. ON THE GROWTH OF BACTERIA OF IMPORTANCE TO HUMANS AND ANIMALS

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INTRODUCTION

Secondary metabolites, contained in medicinal plants, are bioactive compounds that are effective as chemotherapeutic agents. The therapeutic value of these metabolites has been exploited for the treatment of diverse infections. They have the added advantage that they do not develop microbial resistance even when used continuously, which is being the major problem associated with the use of antibiotics. The family Solanaceae, a widespread group of flowering plants, includes plants of countless medicinal potentialities. Many members of this family are also valued as ethnomedicine. Solanum torvum Swartz., an important medicinal plant belonging to this family, is used in the treatment of wound infections, coughs and sore throat.[1] Different parts of the plant are reported to possess antioxidant, antiplatelet aggregation, cardiovascular, analgesic, anti-inflammation, antiviral and antimicrobial activities.[2,3,4,5,6,7,8,9] Solanum torvum Sw., commonly known as Sundakai, is a spiny, tomentose, erect shrub, 2-3 m tall. Leaves of the plant are broadly oval to elliptical and have a shallow, indented edge and an acute to obtuse apex. Flowers are white and bell-shaped. They have lobed fruits which are seated on the calyx.[10] The fruits are edible, often eaten in their daily diet as vegetables by the common people and tribes of Bangladesh. It is also used as essential ingredient in Thai and Indian cuisine, while different parts of the plant are widely used in folk medicine.[11] It is commonly found and cultivated in the temperate and tropical regions of the world. It is distributed widely in Thailand where it is known as turkey berry or Thai eggplant.[12,13] The whole plant of S. torvum is used as a digestive, diuretic and sedative in ethnomedicine.[14] S. torvum has been reported to contain many phytochemicals which include alkaloids, saponins, sapogenins, flavonoids and glycosides.[14-17] Fruit and leaf decoctions are used to treat liver and spleen enlargement and coughing.[18] Leaves are used for haemostasis and anti-inflammation.[19] Leaves of S. torvum also possess antibacterial, antiviral and antifungal properties against human and plant pathogens. [4,7,21,22] For this reason, the current study aimed to investigate the antimicrobial efficacy of the extracts of root of S. torvum against nine common bacteria associated with humans.

MATERIALS AND METHODS

Collection of Sample

Root samples of S. torvum were collected from Thiruvalla, Pathanamthitta district, Kerala, India.
Bacterial Stains Used
Bacterial cultures used in this study were obtained from the culture collections of School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India. For the study nine bacterial cultures namely Staphylococcus aureus, E. coli, Klebsiella, Salmonella typhi, Salmonella paratyphi, Streptococcus, Bacillus, Pseudomonas and Proteus were used. The bacterial strains were maintained on Nutrient Agar (HiMedia, India) plates or slants and were stored at 4 °C before use.

Surface Cleaning and Sterilization of the Samples
In this study the root samples were surface sterilized following the modified procedure of Aneja. The samples were washed in running tap water for 10 minutes followed by detergent wash in 10 % Extran (Merck, India) for 10 minutes. The samples were then rinsed with distilled water and cut into small pieces in aseptic condition. The cut pieces were rinsed in 70 % ethanol for 30 seconds and washed again in distilled water till the ethanol smell completely disappeared. These were spread out in clean trays for oven drying.

Preparation of Extracts
A comparative assay of aqueous, ethanol, acetone and chloroform extracts of root of S. torvum was carried out in this study. The cleaned and cut root samples of S. torvum were oven dried at 60 °C, continuously, for 7 days. The dried samples were powdered using a clean grinder. The powder was stored in air sealed containers at room temperature before extraction. A fixed weight of 30 gm of powdered material was weighed out in aseptic condition and was extracted with the various solvents using the Soxhlet apparatus at a temperature of 60 °C for ethanol, acetone and chloroform and at 100 °C for water. The Soxhlet extraction was carried out continuously for 8 hrs. Each extract was concentrated by evaporation and made up to a final volume of 10 ml. The extracts were stored at room temperature, in sterile screw capped containers, till use.

Determination of Antimicrobial Activity
Preparation of Bacterial Suspension
Pure isolated colonies of the test bacteria were inoculated into 1 % peptone water and incubated at 37 °C for 48 h and were used as inoculum for lawn culture on Mueller Hinton Agar (HiMedia, India).

Sensitivity Discs
Sterile sensitivity discs of 6 mm diameter were prepared from Whatman No. 1 filter paper. The discs were sterilized by autoclaving and stored at room temperature till use. The discs were soaked in the extracts for 10 minutes and were used for disc diffusion assay.

Disc Diffusion Method
Mueller-Hinton Agar (MHA) was used as base medium for screening of antibacterial activity. Pure bacterial cultures of the test bacteria were used as inoculum on MHA. Using sterile cotton swab, 0.2 ml of 24 hr old culture was inoculated evenly on to the surface of MHA to make a lawn culture. For analyzing the antibacterial activity the discs carrying the extracts were impregnated on the seeded agar plate (2 discs per plate). Discs containing the respective solvents were used as controls. The experiment was performed in duplicates. The plates were incubated at 37 °C for 24 hrs and observed for zone of inhibition of growth around the discs. The antibacterial activity of the extracts was assayed by measuring the diameter of zone of inhibition around the wells to the nearest mm.

RESULTS AND DISCUSSION

Solanum torvum is a pharmacologically significant plant of Solanaceae family. The leaves, fruits, and stem of this plant have been mostly utilized by various ethnic groups for the treatment of ailments of different kinds. Though seldom reported, the roots of this plant have also been valued highly for its rich photochemical profile. The present study gives a comparative report of the antibacterial activity of aqueous, ethanolic, acetone and chloroform extracts of root of S. torvum against nine bacterial strains of importance to humans. It was observed that the different root extracts were ineffective against the growth of all Gram negative bacteria as indicated by the presence of confluent growth around the discs carrying the extracts except for E. coli. In this study E. coli was sensitive to the chloroform root extract of S. torvum (Table 1).

The aqueous and ethanol root extracts of S. torvum in this study exhibited strong antibacterial activity against S. aureus with a zone of growth inhibition of 10 and 20.5 mm, respectively (Table 1). Here the Bacillus sp. was also considerably inhibited by the ethanol root extract. Concordantly, Bari et al has reported strong antibacterial activity of methanolic root extract of S. torvum against B. cereus. The aqueous extract of this study inhibited the growth of Streptococcus also, though in a weak manner.

The acetone extract of root of S. torvum was neither effective against Gram positive nor against Gram negative bacteria in this study (Table 1). The aqueous extract, on the contrary, inhibited the growth of S. aureus alone; no other test bacteria exhibited growth inhibition with the extract. The growth of S. aureus and Streptococcus were inhibited by the chloroform extract of root of S. torvum while all the other bacteria showed resistance to the bioactive compounds in the extract (Table 1). Strong antibacterial activity has been recorded for leaf extract of S. torvum earlier. The phytochemical analysis of Balachandran et al has attributed the presence of methyl caffeate present in fruit extract of S. torvum as responsible for its antibacterial against Proteus vulgaris and Klebsiella pneumonia and antimycobacterial activity against Mycobacterium tuberculosis.
Table 1: In vitro antimicrobial activity of root extracts of Solanum torvum Sw. in different solvents.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Average diameter of zone of inhibition in different solvents (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella paratyphi</td>
<td>0</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>0</td>
</tr>
</tbody>
</table>

Sivapriya et al has reported the antibacterial activity of aqueous and ethanol extract of fruit coat of S. torvum against many pathogenic bacteria. Similar studies of Naimon et al have revealed strongest antibacterial activity for ethanolic leaf extract of S. torvum against B. cereus. Antibacterial effect of methanolic leaf extract of S. torvum has been reported against Xanthomonas campestris and Aeromonas hydrophila which affect plants and animals, respectively.

CONCLUSIONS

The studies on the antimicrobial profile of root of S. torvum are limited. The present study primarily aimed to investigate the antibacterial activity of root extracts S. torvum against nine bacterial strains. The results of this study reveal that except for E. coli, the Gram negative bacteria were not inhibited by the different root extracts and hence were seen resistant to the phytochemicals present in root of S. torvum. However S. aureus and Bacillus were strongly inhibited by the ethanol extract of root of S. torvum. The growth of Streptococcus was also inhibited by ethanol and chloroform extracts. From the results of this study we presume that the root extracts of S. torvum are strong inhibitors of Gram positive than Gram negative bacteria. The results of this antibacterial assay suggest the use of ethanolic extract of root of S. torvum against S. aureus and Bacillus. The study should be extended to more species of pathogenic bacteria to ascertain the therapeutic value of the phytochemical constituents present in the roots of S. torvum.

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