PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL ACTIVITY OF MALVAVICUS CONZATTI LEAVES

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ABSTRACT
The present study deals with the preliminary phytochemical analysis and anti-microbial activity of leaf methanol extract of Malvavicus conzatti. The preliminary phytochemical analysis showed various primary and secondary metabolites present in the extract. The leaf methanol extract was tested against Gram positive and Gram negative bacteria by Cup plate method at 100 to 400µg/ml concentrations. The result of phytochemical analysis showed methanol extract consists of Flavonoids, Tannins, Steroids and Phenolic compounds. The leaf methanol extract possess significant anti-microbial activity against the gram positive strains viz., Staphylococcus aureus, Bacillus subtilis, and gram negative strains viz., Salmonella typhi, Pseudomonas aeruginosa. The minimum inhibitory concentration (MIC) of the extracts ranges from 100 to 400µg/ml. Therefore, the result obtained suggests that the methanol extract exhibited significant anti-microbial activity.

KEYWORDS: Malvavicus conzatti. Anti-bacterial, Flavonoids, Tannins.

INTRODUCTION
Herbal medicines play a pivotal role in treating various ailments and their phytochemicals were isolated for establishing the mechanistic pathways for curing the diseases. Secondary metabolites present in plants have multiple roles to be treated as therapeutics. In recent years plants based secondary metabolites has been used as ingredients in Ayurvedic and traditional formulations. Medicinal plants represent a rich source of anti-microbial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Microbial resistance toward antimicrobial agents is increasing day by day due to the inappropriate and regular use of existing antimicrobials. Earlier studies reported that around 70-80% of pathogens found in hospitals were resistant for minimal one antibiotic leading to increased mortality due to nosocomial infections. These conditions lead to discovering new novel molecules to treat microbial infections. Due to increasing resistance to antimicrobials, many researchers now focused on indispensable phytoconstituents isolated from medicinal plants. It is pivotal to develop new anti-microbial drugs of herbal origin in the pipeline to substitute the older ones. The present study deals with Malvavicus conzatti flowering plant belonging to Malvaceae. The leaves are used for treating wounds, cuts and boils. Flower are used for anti-spermaticogenic activity, fruit were used to make jelly or syrup. Both the fruit and flowers are used to make herbal teas. The present study evaluates the Phytochemical analysis and Anti-microbial activity of Malvavicus conzatti leaves.

MATERIALS AND METHODS
Plant Collection and Preparation
The fresh leaves of Malvavicus conzatti were collected from Tirupathi (Chittoor) in the month of August 2016 and authenticated by Dr. Madhava chetty, Department of Botany, Sri Padmavathi University, Tirupathi. A voucher specimen was deposited in the School of Pharmacy, Anurag Group of institutions, Venkatapur, Ghatkeshar. The plant material was washed with water to remove soil, mud, debris and other adhering materials and dried thoroughly in air under shade at room temperature. Coarse prepared powdered drug was passed through sieve no.40 and stored in air tight container.

Preparation of plant extract
Freshly collected leaves were shade dried at room temperature and coarsely powdered. The powdered material was successively extracted with petroleum ether, ethyl acetate and methanol by Soxhlet extraction. The crude extracts were evaporated to dryness.

Preliminary Phytochemical screening
The extracts were analyzed for preliminary phytochemical screening for the presence of various primary and secondary metabolites.
Anti-bacterial activity

Test organisms

Totally, four bacterial strains were used for the study, which include the gram positive strains viz., Staphylococcus aureus, Bacillus subtilis and gram negative strains viz., Salmonella typhi, Pseudomonas aeruginosa. The test bacteria were seeded into sterile nutrient broth tubes and incubated over night at 37°C to obtain broth cultures. Anti-bacterial activity of leaf extracts was evaluated by agar well diffusion assay. Using sterile swabs, the broth cultures of test bacteria were inoculated all over the surface of sterile nutrient agar plates. Using a 100 g/ml sterile cork borer, wells were punched in the plates. The wells were filled with test extracts, standard antibiotics Streptomycin, control sample (DMSO) and the plates were incubated for 4hr at 37°C, zone of inhibition was measured.

Sample preparation

Anti-microbial activity of the extracts was tested at various concentrations ranging from 100 µg/ml to 400 µg/ml. The methanol extract was weighed and dissolved in DMSO to prepare stock solution and the stock solution was used to get desired concentrations of the text extracts.

Antimicrobial assay

The methanol extracts were tested for their effect against the growth of pathogenic bacteria by disc diffusion method. The leaf methanol extract at four different concentrations viz., 100 µg/ml, 200 µg/ml, 300 µg/ml and 400 µg/ml were employed for antimicrobial activity. The antibiotic discs, Streptomycin (10µg/ml) served as positive control for bacteria. The bacteria tested were inoculated into nutrient agar. After the incubation period of 24 hours at a temperature of 37°C, three or four colonies isolated from these media were inoculated on 4ml of nutrient broth and incubated for 2 hrs at 37°C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing agar medium were streaked separately with these microbial suspensions of bacteria. Sterile filter paper discs impregnated with 100, 200, 300 and 400 µg/ml extracts and control discs were placed over the culture plates. After equilibrium at 4°C, the plates were incubated overnight at 37°C and the diameter of any resulting zones of inhibition was measured. Triplicates were maintained for all the experiments.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

The result of phytochemical screening of extracts revealed the presence of phenolic compounds, flavonoids, tannins, alkaloids and steroids. Particularly, methanol extract exhibited good sources of different classes of compounds compared to other extracts like petroleum ether and ethyl acetate extracts.

Table 1: Preliminary phytochemical screening of Malvavicus conzotti leaf extract.

<table>
<thead>
<tr>
<th>Phyto Constitutions</th>
<th>Petroleum ether extract</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phyllons</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+: Present, - : Absent

Antibacterial activity

To carry out the Anti-bacterial activity, methanol extract was selected because methanol extract showed good source of different classes of compounds compared other extracts like petroleum ether and ethyl acetate. The antibacterial properties of methanol extracts of M. conzotti leave in vitro were showed in the Table (2). The plants extract exhibited a considerable level of inhibition against the entire test organism compared to the standard drug. The methanol extract was found active against tested gram positive bacteria; S. aureus, B. subtilis and gram negative bacteria; S. typhi, P. aeruginosa, the zone of inhibition is high in case of gram positive as comparative gram negative bacteria. This could be explaining the anti-bacterial activities are related to their chemical composition of the crude extract. In this study, the potential of M. conzotti to inhibit Gram-positive and Gram-negative bacteria was evaluated by agar well diffusion method. The presence of an inhibition zone around the well is an indication of anti-bacterial potential of extract. The extract exhibited inhibitory activity against test bacteria in a dose dependent manner. Overall, the extract showed marked inhibition of Gram-positive and Gram-negative bacteria. When compared to Gram-negative bacteria, Gram-positive bacteria show effective inhibitory activity.
Table 2: Anti-bacterial activity of leaf methanol extract of Malvavics conzatti.

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Strytomycin (10 µg/ml)</th>
<th>Methanol extract of M. conzatti Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone of inhibition</td>
<td>Zone of inhibition</td>
</tr>
<tr>
<td>S. aureus</td>
<td>15±0.42</td>
<td>07.20±0.2</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>14±0.2</td>
<td>08.40±0.3</td>
</tr>
<tr>
<td>S. typhi</td>
<td>12.5±0.8</td>
<td>06.20±0.2</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>12.8±0.56</td>
<td>07.00±0.2</td>
</tr>
</tbody>
</table>

ZI were expressed as mean ± standard deviation of three replicates. Low activity (1-6mm), moderate activity (7-10 mm), high activity (11-10 mm), high activity (11-15 mm).

ZI-Zone of inhibition

CONCLUSIONS
The result of preliminary phytochemical screening suggest that the leaf methanol extract show good sources of beneficial phytochemical compounds like Flavonoids, Phenolic compounds, Steroids, Alkaloids, Tannins, Carbohydrates and Amino acids and the result of anti-bacterial activity showed that the extract exhibited inhibitory activity against gram positive bacteria; S. aureus, B. subtilis and gram negative bacteria; S. typhi, P. aeruginosa, in a dose dependent manner. Overall, all extracts showed marked inhibition of gram-positive bacteria than gram-negative bacteria and anti-bacterial activity which may be attributed to its higher phenolic and flavonoids content.

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REFERENCES