ANTIBACTERIAL ACTIVITY OF SIDA ACUTA BURM. F. AGAINST HUMAN PATHOGENS

ALKA JINDHAL*, PADMA KUMAR

Laboratory of Plant Tissue Culture and Secondary Metabolites, Department of Botany, University of Rajasthan, Jaipur-302055, India,
Email: jindal4@gmail.com

Received: 1 January 2012, Revised and Accepted: 7 February 2012

ABSTRACT

Alkaloids are well known for their antimicrobial properties. The present study was designed to evaluate the antibacterial activity of alkaloids of Sida acuta (Malvaceae) Burm. f. Alkaloids from different parts (root, stem, leaf and buds) of the plant were extracted and were screened for antibacterial activity by ‘Disc Diffusion Assay’ against two Gram negative bacteria (Escherichia coli and Proteus mirabilis) and one Gram positive bacteria (Staphylococcus aureus). Minimum inhibitory concentration, minimum bactericidal concentration and total activity of extracts against each sensitive pathogen have also been evaluated. Results of the present study indicate that all the tested alkaloid extracts of S. acuta have potent antibacterial activity against P. mirabilis and S. aureus whereas the extracts was found to be inactive against E. coli.

Keywords: Alkaloid, Sida acuta, Antibacterial activity, Disc diffusion assay, Total activity.

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs in microorganisms has gradually increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized frequently as therapeutic agents. This fact is the cause for concern, as numbers of patients are not responding positively towards existing antibiotics. Further emerging new multi-resistant bacterial strains, aggravate the problem.

The problem of microbial resistance is continuously growing hence the use of existing antimicrobial drugs in future is still uncertain. Therefore, immediate action is required to combat the problem, by encouraging research to develop new drugs; more so of herbal origin as synthetic drugs are known to cause side effects. The ultimate goal is to offer appropriate and efficient antimicrobial and present study is an effort in this direction.

Sida acuta Burm. f. a pantropical weed, native to Mexico and Central America but has spread throughout the tropics and sub tropics and frequently dominates pastures. All the plant parts exert various pharmacological properties includes antispasmodial, antimicrobial, antioxidant and cytotoxic activities. In Kenya, the whole plant is used to prepare “Bundugo”, a supplementary strength magically added to food to prepare “Bundugo”, a supplementary strength magically added to food poisoning in human beings. Hence in the present study screening for antibacterial potential of the alkaloid extracts of S. acuta has been undertaken.

MATERIALS AND METHODS

Collection and identification of plant material

Sida acuta Burm. f. was collected from different localities of Jaipur in the month of June, 2008 and was identified at Department of Botany, UOR. A voucher specimen (RUPL-20428) was also submitted to the Herbarium of Botany Department, UOR.

Extraction of alkaloids

Different parts of the plant (root, stem, leaves and buds) were separated and washed thoroughly; shade dried and finely powdered using a blender. Alkaloids were extracted with all the separated parts of the plant following the well established method of Harborne 13. Hundred grams of each finely powdered sample was extracted with 10% acetic acid in ethanol (final volume 500 ml) for 4 h. Extract were then concentrated to 1/6 of the original volume and made alkaline by NH4OH. Precipitates collected after centrifugation were washed with 1% NH4OH, filtered, dried in vacuum and weighed.

Test microorganisms

Bacterial strains of E.coli (MTCC 46), S. aureus (MTCC 87) and P. mirabilis (MTCC 1425) were procured from IMTECH, Chandigarh, India. These strains were grown and maintained on Muller Hinton agar medium (Beef extract 2.0 g; Peptone 17.5 g; Starch 1.5 g; Agar 17.0 g in 1000 ml of distilled water; Final pH 7.4 ± 0.2) at 37°C ± 4°C in the deep freezer until required for use.

Antibacterial activity of extracts

Disc diffusion assay (DDA)

Antibacterial screening of test extracts was carried out by the disc diffusion assay (DDA) method 14. Muller-Hinton agar media plates were seeded with the prepared culture suspensions (1×10^8 cfu/ml). Sterilized filter paper discs of 6 mm diameter (Whatman no. 1) were impregnated with 100 µl of extract of 10 mg/ml concentration to give a final concentration of 1 mg/disc, left to dry in vacuum to remove residual solvent, which might interfere with the determination. The extract discs were placed on the seeded media plates along with discs impregnated with of standard drug streptomycin in the same (1 mg/disc) concentration. These plates were kept at 4°C for 1 h for the diffusion of extracts into the media and thereafter were incubated at 37°C±2°C for 24 h. Zone of inhibitions (IZ) produced by the extracts around the discs were measured and the ‘Activity Indices’ (AI) was calculated by the well established formula. The experiment was performed three times to minimize the error and the mean values were recorded.

Activity Index (AI) = Inhibition zone of the sample
Inhibition zone of the standard

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

Extracts that showed positive results in the disc diffusion assay against test pathogens were further evaluated for minimum inhibitory concentration. The microbroth dilution method 15 was performed for the determination of MIC values. A series of solutions of extracts ranging from 1-10 mg/ml concentrations were prepared by re-suspending the extracts in acetone (Inactive against test pathogens). Thereafter test solutions were made by two fold serial dilution of stock solution and were added to MH broth media in wells of 96-wells microtiter plates. Hundred microlitre inoculum suspension of standard size [1×10^8 cfu/ml] was added to each well.
Cultures containing standard drug were used as positive control while culture suspensions were used as negative control. Each time two sets were prepared, one was kept for incubation while another was kept at 4°C for comparing the turbidity in the microtitre wells. MIC values were taken as the lowest concentration of the extracts in the well of the microtitre plate that showed no visible growth or turbidity after incubation. The turbidity of the wells in the microtitre plate was interpreted as the visible growth of microorganisms. Experiments were conducted three times and the mean values were recorded.

Minimum bactericidal concentration was carried out by subculturing 50 µl aliquots from each well. Least concentration of extract showing no visible growth on subculturing was taken as MBC value.

**Total Activity (TA)**

Total activity for each active extract was calculated by the well established formula\(^{16}\) TA value (ml/g) is the volume to which the extract can be diluted retaining the ability to inhibit the growth of microorganisms.

**Total Activity (TA) = Amount extracted from 1 g plant material (mg/g.d.w)**


### Table 1: Alkaloid content different parts of *S. acuta*.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Quantity (mg/g.d.w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>0.15</td>
</tr>
<tr>
<td>Stem</td>
<td>1.10</td>
</tr>
<tr>
<td>Leaf</td>
<td>3.35</td>
</tr>
<tr>
<td>Bud</td>
<td>7.00</td>
</tr>
</tbody>
</table>

### Table 2: Inhibition zone (IZ) and Activity index (AI) of alkaloids of *S. acuta*.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Test Microorganisms</th>
<th>MIC of the extract (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td></td>
<td>IZ (mm)</td>
<td>AI (mm)</td>
</tr>
<tr>
<td>Root</td>
<td>10</td>
<td>0.36</td>
</tr>
<tr>
<td>Stem</td>
<td>12</td>
<td>0.48</td>
</tr>
<tr>
<td>Leaf</td>
<td>14.8</td>
<td>0.59</td>
</tr>
<tr>
<td>Bud</td>
<td>15.5</td>
<td>0.62</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>20</td>
<td>25</td>
</tr>
</tbody>
</table>

### Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of alkaloids of *S. acuta*.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Test Microorganism</th>
<th>MIC of the extract (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td></td>
<td>(mg/ml)</td>
<td>(mg/ml)</td>
</tr>
<tr>
<td>Root</td>
<td>0.312</td>
<td>0.625</td>
</tr>
<tr>
<td>Stem</td>
<td>0.156</td>
<td>0.312</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.78</td>
<td>0.156</td>
</tr>
<tr>
<td>Bud</td>
<td>0.078</td>
<td>0.078</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The development of bacterial super resistant strains is resulting in failing of currently used antibiotic agents to end many bacterial infections. Hence a continuous research for getting new antimicrobial agents is the need of the present scenario, either by designing and synthesising new agents, chemically or through the search of new natural sources for antimicrobial agents. The present study is an effort to explore new sources of natural and potent antibacterial agent.

Several reports have shown the antimicrobial properties of plant extracts under laboratory conditions. In the present investigation *Sida acuta* has shown antibacterial potential against two out of three test pathogens which are the major causative organisms of various human diseases. Although the plant (*S. acuta*) has been studied previously for its antimicrobial activity but only restricted to the determination of IZ and that too without AI, MIC and MBC and TA evaluation. Hence could not explore for the preparation of antibiotics. However in the present study *S. acuta* has been evaluated for alkaloid extracts showed antibacterial potential against *S. aureus* and *P. mirabilis* while the extracts were found inactive against *E. coli*. The significant activity against *S. aureus* was shown by alkaloid extracts of buds (IZ-15.5 mm and AI-0.62), with same (0.078 mg/ml) values of MIC and MBC. Following by leaves (IZ-14.8 mm, AI-0.48, MIC-0.312 mg/ml and MBC-0.312 mg/ml), stem (IZ-12 mm, AI-0.48, MIC-0.316 mg/ml and MBC-0.312 mg/ml) and roots (IZ-9 mm, AI-0.36, MIC-0.625 mg/ml and MBC-1.25 mg/ml) whereas good activity against *P. mirabilis* was shown by alkaloid extract of leaves (IZ-12.6 mm, AI-0.52, MIC-0.156 mg/ml and MBC-0.312 mg/ml), followed by stem (IZ-10 mm, AI-0.1, MIC-0.312 mg/ml and MBC-0.625 mg/ml), roots (IZ-10 mm, AI-0.41) with same (0.625 mg/ml) values of MIC and MBC and buds (IZ-9 mm, AI-0.37, MIC-0.625 mg/ml and MBC-1.25 mg/ml). Table 2 and 3. Alkaloid extracts of buds and roots were found to be bactericidal in nature against *S. aureus* and *P. mirabilis*, respectively, showing same values of MIC and MBC. All the other active extracts were observed to be bacteriostatic in nature against *S. aureus* and *P. mirabilis*, where the MBC values were greater than MIC values. TA was also calculated and recorded (Table 4). TA was highest for alkaloid extracts of buds (89.74 ml/g) and leaf (21.47 ml/g) against *S. aureus* and *P. mirabilis*, respectively.

![Figure 1: Alkaloid content of different parts of *S. acuta*](image1.png)

![Figure 2: Inhibition zone of alkaloids of *S. acuta*.](image2.png)
extracts using various parameters viz. IZ, AI, MIC, MBC and TA. It is worth mentioning that most of the extracts exhibit very low MIC values (a desirable feature). Two active extracts were found to be bactericidal whereas remaining were found bacteriostatic in nature against S. aureus and P. mirabilis which indicates the significant potentiality of the tested extracts of S. acuta.

Acknowledgement

Authors are thankful to the Head of Botany Department, University of Rajasthan, Jaipur, India for providing all necessary facilities to carry out the work. Financial assistance provided by UGC is gratefully acknowledged.

REFERENCES