Antimicrobial Activity of Leaf extract of *Andrographis paniculata* Wall

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ABSTRACT

Antimicrobial activity of leaf extract of *Andrographis paniculata* Wall., was studied using different solvent like chloroform, acetone, ethanol and water against bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungal strains *Aspergillus niger* and *Penicillium chrysogenum*. The antimicrobial activity was determined by disc diffusion method. Out of the four extract used, acetone and ethanol extracts were found to be highly active against *Staphylococcus aureus* and *Bacillus subtilis*. Highest in acetone (12 mm) and lowest in ethanol (10 mm). The MIC values were obtained by serial dilution method.

Key words: *Andrographis paniculata*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Aspergillus niger*

INTRODUCTION

*Andrographis paniculata* Wall., of the family Acanthaceae the much branched annual herb. The plant is distributed throughout the tropics. It is found in the plains of India in Assam, U.P., M.P., A.P, Tamil Nadu and Kerala. Traditional practitioners use this in hyperdipsia, wounds, ulcers, chronic fever, malarial and cough, bronchitis, skin diseases, leprosy, flatulence, colic, diarrhoea, dysentery, (Warrier et al. 1993). It is used to overcome *sannipata* type of fever, difficulty in breathing, hemopathy burning sensation, cough, skin diseases, fever, ulcer and worms. It is also useful in acidity and liver complaints (Aiyer and Kolammal 1962). The important preparations using the drug are Tiktagheta, Gorocandi gulika, Candanasava, Panchatiktam kasaya, etc. (Sivarajan et al. 1994). A preparation called “Alui” is prepared by mixing powdered cumin (*Cuminum cyminum*) and large cardamom (*Amomum subula tum*) in the juice of this plant and administered for the treatment of malaria (Thakur et al. 1989). It is known as “King of bitters”. It is the source of several diterpenoids of which the bitter water soluble lactone andrographolidic properties. As medicinal plants are gaining more importance in Pharmaceutical industries for the preparation of new phyto Medicines, this study was undertaken to check its properties as a drug (Sule et al. 2010).

MATERIALS AND METHODS

Extraction Procedure

The leaves of *Andrographis paniculata* Wall. Were collected from Dandeli, Uttara Kannada District, Karnataka State, Southern India. The leaves were dried under shade and made in to coarse powder using an electrical grinder. The powder was subjected for successive extraction with chloroform, acetone, ethanol and water using Soxhlet apparatus separately. The extracts were dried and dissolved in DMF (Dimethyl formamide) solution and screened for antimicrobial activity.

Preliminary Phytochemical Screening

The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The preliminary phytochemical analysis (Kokate 1993) was carried out by following procedures:

Test for Alkaloids

A small portion of the extract is stirred with few drops of 1% Hydrochloric acid and filtered. The filtrate is treated with Wagner’s reagent. Reddish brown precipitate indicates the presence of alkaloids.

Test for Sapohins

One ml of extract is diluted with 20ml of distilled water and shaken vigorously for 15 min formation of stable foam indicates the presence of saponin

Test for Tannins

Development of blue green color in the extract when treated with ferric chloride indicates the presence of tannins.
Test for Phenols
Phenol test Small quantity of extract is diluted with 5% ferric chloride solution. Development of intense color indicates the presence of phenols.

Test for Steroids and Triterpenes
Leibermann- Burchards test- The extract is treated with 50% sulphuric acid and a few drops of acetic anhydride are added. The development of reddish brown ring indicates the presence of steroids.

Salkowskis test- A few drops of chloroform and few drops of concentrated sulphuric acid was added to the extract. Appearance of yellow color in the lower portion indicates the presence of triterpenes

Test for Flavonoids
Ferric chloride test- The extract is treated with few drops of 5% ferric chloride. The appearance of blackish green color indicates the presence of flavonoids.

Antimicrobial assay:
The antimicrobial screening was done by using three bacterial strains like Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and fungal strains, Aspergillus niger and Penicillium chrysogenum. All the bacterial strains were obtained from Department of Microbiology BNDC, Dandeli. The remaining fungal strains were obtained from the P.G.Department of Botany (Microbiology laboratory), Karnatak University Dharwad.

The antimicrobial activity was determined by disc diffusion method (Bauer et al 1966). Three different concentrations of 25mg/ml, 50mg/ml and 100mg/ml respectively were prepared. Each sterile disc was loaded with 10µl of test extract and placed on the agar plates inoculated with respective micro organisms. The plates were kept for half an hour for pre incubation diffusion. Then the plates were kept for incubation at 37°C for 24 hrs for bacteria and 48 hrs for fungi. At the end of incubation zones around the discs were measured in mm using Hi Antibiotic Zone scale. The study was performed in triplicate. Streptomycin disc was used as standard for bacteria and Nystain disc for fungi.

Determination of Minimum concentration
The minimum inhibitory concentration was determined by serial dilution method (Rollins and Joseph 2000). Serial dilution of the extract was prepared in the test tubes containing peptone water as diluents. Fifty mg of the extract was dissolved in one ml of DMF which is further subjected for two fold dilution. Totally 10 test tubes were maintained. The final concentration of the extract was now one half of the original concentration in each test tube. Each bacterial isolate was inoculated at 37°C for 24hrs. The tubes were then examined for the presence of growth considering turbidity as criterion. The highest dilution in each series that did not show turbidity and thus no growth was considered to be the MIC of the organism.

RESULTS AND DISCUSSION
Table 1 contains the phytochemical analysis of the leaf extract of Andrographis paniculata which shows the presence of flavonoids and phenolic compounds. Table 2 gives the antimicrobial activity of Andrographis paniculata leaf extract and the zone of inhibition in comparison with the standard used. Acetone and alcohol extracts showed high activity against Bacillus subtilis and Staphylococcus aureus. The highest zone of inhibition in case of Acetone extract against Bacillus subtilis is of 16mm which is very much nearer to the standard zone of inhibition (18mm) and against Staphylococcus aureus the zone of inhibition was 15mm. Alcohol extract also showed good inhibitory activity against these strains and the zone of inhibition obtained were 13mm and 15mm respectively. Both of the extracts were inactive against rest of the strains used. The antimicrobial activity may be due to the presence of flavonoids and phenolic compounds present in the plant as secondary metabolites.

Table 1: Phytoconstituents of Andrographis paniculata leaf extract

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/Triterpenes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2: Zone of inhibition of different extracts of *Andrographis paniculata* against different pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 50 100</td>
<td>25 50 100</td>
<td>25 50 100</td>
<td>25 50 100</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Penicillum chrysogenum</em></td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Minimum Inhibitory Concentration (mg/ml) of Acetone and Ethanol of leaves of *Andrographis paniculata*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Acetone</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Table 3 shows the MIC values obtained against *Staphylococcus aureus* and *Bacillus subtilis*, which is same for both the strains (12.5mg/ml). A similar type of study made with leaf extract of *Morinda citrifolia* L. (Usha *et al* 2010). Showed that ethanol extract of was highly effective against *Staphylococcus aureus* and *Bacillus subtilis* apart from other strains. Similarly the acetone extract of *Cassia auriculata* flower also inhibited the growth of these two strains (Maneemegalai and Naveen 2010). However, the extract here was found to be a broad spectrum microbial inhibitor. The present study indicates that the phytochemicals of *Andrographis paniculata* has significant inhibition for a gram positive microbes, *Staphylococcus aureus* and *Bacillus subtilis*

**Conclusion**

The good antimicrobial activity of the *Andrographis paniculata* leaf extract against *Staphylococcus aureus* and *Bacillus subtilis* is an indication that the leaf extract is beneficial as a cure for skin diseases. The inhibiting nature of *Morinda citrifolia* and *Cassia auriculata* on the growth of *Staphylococcus aureus* and *Bacillus subtilis* suggests that instead of a single drug treatment multiple drug formulation would be more effective. With a wide spectrum of inhibition against both Gram-positive bacteria leaf extract of *Andrographis paniculata* is worthy of further investigation as a natural wide spectrum antibacterial agent in the treatment of infectious disease.

**LITERATURE CITED**


