COMPARATIVE ANTIMICROBIAL STUDY BETWEEN ANDROGRAPHIS PANICULATA AND AZADIRACHAT INDICA

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ABSTRACT
In vitro anti-microbial efficacy of dried seed extract of Azadirachta indica and dried leaves of Andrographis paniculata was assessed by agar cup plate method against four micro organisms; Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa and E. coli. Results of present investigation indicates that the methanolic extract of Andrographis paniculata and n-hexane extract of Azadirachta indica producing a significant (p< 0.05) antimicrobial activity. Out of two plants the leaves extract of Andrographis paniculata possess better antimicrobial properties than Azadirachta indica. Hence Andrographis paniculata will be the better choice for treatment of different pathologic conditions associated with above microbes. Andrographolide is the major active constituent of Andrographis paniculata which may be responsible for antimicrobial activity.

Keywords: Andrographis paniculata, Azadirachta indica, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli.

INTRODUCTION
Anti-microbials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, resins fatty acids gums which are capable of producing definite physiological action on body.[1]

Andrographis paniculata (A. paniculata) is in demand in terms of its medicinal properties. It has been used for centuries in Asia to treat gastro-intestinal tract and upper respiratory infections, fever, herpes, sore throat and a variety of other chronic and infectious diseases.[2] Azadirachta indica (A. indica) is in demand in terms of its medicinal properties. It has been used for centuries in Asia to treat leprosy, skin
problems, skin ulcers, intestine worms, anorexia, eye problems, epistaxis, biliousness and intestine worms\[3\]. The present paper reports comparison between in vitro antimicrobial efficacy of dried seed extract of $A. \text{indica}$ and dried leaves extract of $A. \text{paniculata}$ by pore plate method against four micro organisms; $\text{Proteus vulgaris}$, $\text{Staphylococcus aureus}$, $\text{Pseudomonas aeruginosa}$ and $\text{E.coli}$.

\section*{MATERIALS AND METHOD}

\subsection*{Collection of Plant Material}

The field established micro propagated plants of $A. \text{indica}$ seeds were collected from the local market of Berhampur Orissa (India) during the month of January, 2011. The leaf specimen was deposited in the P.G. Department of Botany, Berhampur university, Berhampur Odissa and $A. \text{paniculata}$ leaves were collected from the Natural Remedy Bangalore (India) during the month of January, 2011. It was authenticated by taxonomist Dr. M.K. Mishra, PhD, Prof. Botany, Berhampur University, Odisha.

\subsection*{Extraction Procedure of Plant Extract}

The seed part of the plant $A. \text{indica}$ was cut into pieces and was powdered by the help of grinder and passed through sieve 40 # mesh particle size and subjected to extraction with n-hexane by soxhlet apparatus for 15-20 cycles at temperature 50°C. After this, the maximum solvent is recovered by distillation and after this the content was concentrated by heating it in a water bath at 50°C.

Similarly the $A. \text{paniculata}$ leaves were chopped by the help of a grinder and passed through sieve 40 # mesh particle size and subjected to extraction with 99% methanol by soxhlet apparatus for 72 hours at temperature 70°C. After 72hrs the mantle was switched off and water flow was stopped. After cooling the plant material was removed by filtration through a cotton plug. The solvent of the extract was evaporated by using normal distillation. The concentrated mass was taken in a porcelain disc and evaporated in a water bath at 55°C. Then it was left at room temperature to get a dried mass of the extract. The extract was labeled and kept in freeze for further use.

\subsection*{Phytochemical estimation}

The $A. \text{indica}$ extracts were subjected to various chemical tests to determine presence of tannins, Pheobatannins, flavonoids, terpenoids and glycosides. Similarly the $A.$
paniculata extract was subjected to various biochemical tests to determine carbohydrate, protein and phenolics, flavonoid, saponin and alkaloid. TLC profile (Fig-1) was performed which indicating the presence of Andrographolide in A. paniculata extract.

**Microbial strains tested**

Four microbial strains were used from the Microbial Type Culture Collection (MTCC), Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India. They were gram negative *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and gram positive *Staphylococcus aureus*.

**Antimicrobial assay procedure**

Antimicrobial activity was determined by agar cup plate method. Petri plate containing 20 ml of nutrient agar medium (Ph 7.2-7.40) were seeded with a 24h culture of different bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*). Wells 8 mm diameter was cut into the different agar plates. Different concentration (100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml) of plant extracts i.e. A. indica and A. paniculata were tested by dissolving in 1% DMSO (Dimethyl Sulfoxide) \[^4\]. Tetracycline dish was taken as reference. The Petri plate with bacteria was incubated at 37± 2\(^0\) C for 24 hrs. The anti-microbial activity was based on the measurement of diameter of zone of inhibition.

**Statistical analysis**

Results were subjected to one-way ANOVA by the help of Data analysis software. \(p<0.05\) was considered as statically significant.
RESULT AND DISCUSSION

TABLE 1: ANTIMICROBIAL ACTIVITY OF N-HEXANE SEED EXTRACTS OF AZADIRACHTA INDICA

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Zone of inhibition in diameter(mm)</th>
<th>E Coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Staphylococcus aureus</th>
<th>Proteus vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (Tetracycline dish)</td>
<td>22±0.2*</td>
<td>23±0.2*</td>
<td>25±0.2*</td>
<td>24±0.2*</td>
<td></td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>8±0.38</td>
<td>7±0.5</td>
<td>6±0.67</td>
<td>6±0.44</td>
<td></td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>9±0.48</td>
<td>8±1.05</td>
<td>8±0.52</td>
<td>9±0.88</td>
<td></td>
</tr>
<tr>
<td>400 µg/ml</td>
<td>11±0.8</td>
<td>10±0.88*</td>
<td>12±0.85*</td>
<td>12±0.75*</td>
<td></td>
</tr>
<tr>
<td>800 µg/ml</td>
<td>12±0.82*</td>
<td>12±0.6*</td>
<td>13±0.44*</td>
<td>13±0.85</td>
<td></td>
</tr>
</tbody>
</table>

Values are in Mean±SEM. Where* p<0.05

TABLE 2: ANTIMICROBIAL ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF ANDROGRAPHIS PANICULATA

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Zone of inhibition in diameter(mm)</th>
<th>E. Coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Staphylococcus aureus</th>
<th>Proteus vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (Tetracycline dish)</td>
<td>22±0.2*</td>
<td>23±0.2*</td>
<td>25±0.2*</td>
<td>24±0.2*</td>
<td></td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>9±0.62*</td>
<td>10±0.44</td>
<td>10±0.6*</td>
<td>9±0.33*</td>
<td></td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>10±0.72</td>
<td>12±0.52</td>
<td>12±0.64*</td>
<td>11±.66</td>
<td></td>
</tr>
<tr>
<td>400 µg/ml</td>
<td>10±0.70*</td>
<td>13±0.54*</td>
<td>13±0.62*</td>
<td>11±0.58*</td>
<td></td>
</tr>
<tr>
<td>800 µg/ml</td>
<td>14±0.82*</td>
<td>14±0.66*</td>
<td>15±0.76*</td>
<td>14±0.72*</td>
<td></td>
</tr>
</tbody>
</table>

Values are in Mean±SEM. Where* p<0.05
Figure 1
The TLC profile indicating the Rf value 0.7 of Standard(S) i.e. pure Andrographolide and Test (T) i.e. Andrographis paniculata extract.

Figure 2
Comparative study of Zone of inhibition between leaf extract of A. paniculata and seed extract A. indica over Staphylococcus aureus at concentration 100, 200, 400 and 800 µg/ml respectively.
Figure 3
Photograph of Zone of inhibition std. tetracycline dish with comparison to control.

The antimicrobial activities of both A. paniculata (leaves) and A. indica extract (seeds) exhibited different degrees of antimicrobial activity against the test organisms. In A. indica, n-hexane used as a solvent and in A. paniculata, methanol used as solvent. The methanolic extract in the present study showed inhibitory effect against the entire gram positive, gram negative bacteria. The antimicrobial activities of the plant extracted in different solvents varied greatly because there are many factors influence the active compounds present in the plant. Amongst the gram positive and gram negative bacteria, gram positive bacterial strains were more susceptible to the extracts as compared to the gram negative bacteria. Generally gram positive bacteria are more resistant than gram negative bacteria [5, 6]. This could be due to several possible reasons; one is the presence of the double membrane surrounding each bacterial cells. Their outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why gram negative bacteria are generally more resistant than gram positive bacteria [7, 8]. Secondly the basis of their differences in susceptibility might be due to the cell wall composition of gram positive and gram negative bacteria. The experimental result indicates that ethanolic extract of A. paniculata and n-hexane extract of A. indica showing significance zone of inhibition (p<0.05) over all microorganisms that is Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa, E. Coli at concentration 100µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml (Table-1 and 2). Standard drug tetracycline exhibited marked zone of inhibition with comparison to control (Fig.3). In comparison study the extract of A. paniculata produced a better and remarkable antimicrobial activity than A. indica (Fig.2).
This activity may be due to presence of Andrographolide as a major active constituent. Hence it will be more choice for treatment of different infectious disorders associated with above microbes.

ACKNOWLEDGEMENTS

I am grateful to management of Roland Institute of pharmaceutical sciences, Berhampur, Orissa for providing the necessary facilities for study.

REFERENCE


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