CHAPTER 7

ANTIMICROBIAL ACTIVITY OF MUCUNA PRURIENS AND PHYLLANTHUS NIRURI ON SELECTED MICROORGANISMS
A MODERN ANTIBIOTIC ERA

More than 50 years have passed since the modern antibiotic era opened with the first clinical trial of penicillin in early 1941. In the intervening years medicinal practice has been transformed and the use of antibiotics has grown to enormous proportions. Most of the natural antibiotics have been isolated from soil microorganisms through intensive screening. In 1952, the bulk of the agents reported in the literature were derived from the streptomyces with most of the remainder coming from other bacteria and fungi. By 1985, the total number of new agents had increased to 220, but the percentage derived from the streptomyces had declined as had the number derived from other bacteria and fungi. One observed instead a dramatic increase in the use of rarer microorganisms [1]. The reason for this lie largely in the perception that the point of diminishing returns had been reached using classical methodology, and if newer agents were to be discovered, fishing in a different gene pool was more likely to prove useful.

In spite of recent developments of antibiotics and newer synthetic drugs, a vast majority of people depend on traditional medicines for their primary health care needs and it can safely presumed that a major part of traditional therapy involves the use of plant extracts or their active principle.

SCREENING OF ANTIMICROBIAL PLANTS FOR NEW PHARMACEUTICALS

Plants are the oldest source of pharmacologically active compounds, and have provided mankind with many medically useful compounds for centuries [2]. Today it is estimated that more than two thirds of the world’s population relies on plant derived drugs; some 7000 medicinal compounds used in the Western pharmacopoeia are derived from plants [3]. In the USA approximately 25% of all prescription drugs used contain one or more bioactive compounds derived from vascular plants [4,5]. Thus, phytochemical screening of plants species, especially of ethnopharmaceutical use, will provide valuable baseline information in the search for new pharmaceuticals. Yet fewer than 10% of the world’s plant species have been examined for the presence of bioactive compounds [6]. Hence screening of antimicrobial plants for new agents possess an enormous challenge and is important especially with the emergence of drug resistant disease strains.

During the past 10 years there has been a substantial resurgence of interest and pursuit of natural products discovery and development, both in the public and private
sectors. Explanation for this, possibly transient or at least cyclical revival might include: the increasingly sophisticated science that can be brought to bear on the discovery and development processes [7] and the very real threat of the disappearance of the biodiversity essential for such research. It has only been in the past two decades or so that interest in higher plant antimicrobial agents has been reawakened world wide, and the literature in this area is becoming substantial [1].

**Efficacy of Traditionally Used Plants**

The search for natural products to cure diseases represents an area of great interest in which plants have been the most important source. In South African traditional medicine, the use of plants is a widespread practice, and the persistence in the use of medicinal plants among people of urban and rural communities in South Africa could be considered as evidence of their efficacy [8]. Although there is an important local ethnobotanical bibliography describing the most frequently used plants in the treatment of conditions consistent with sepsis and other diseases, there are very few experimental studies, which validate the therapeutic properties of these plants.

**7.1. Materials and Methods**

**7.1.1. Test Compounds**

Methanol extracts of *Mucuna pruriens* (*MEMP*) seeds and *Phyllanthus niruri* (MEPN) at the concentration of 500µg and 750µg/ml.

**7.1.2. Bacterial Strains Employed**

Microorganisms (*Staphylococcus aureus* 8531, *Staphylococcus aureus* ML 174, *Staphylococcus aureus* ML 152, *Bacillus pumillus* 8241, *Bacillus cereus*, *Escherichia coli* 51, *Escherichia coli* 54B, *Vibrea cholera* 14035, *Vibrea cholera* 1353, and *Vibrea cholera* 226101) were obtained from the stock culture of Central Drugs Laboratory (CDL), Kolkata; Indian Institute of Chemical Biology (IICB), Kolkata and Mycology and Plant Pathology Laboratory, Calcutta University, Kolkata, India.

**7.1.3. Media**

**7.1.3.1. Liquid culture media**

Peptone water

Bacterial peptone - - - - 0.8% and

NaCl - - - - - - - - - - - - - 0.5%, pH 7.2 - 7.4.
Nutrient broth

Bacterial peptone - - - - - 1.0% and
Beef extract - - - - - - - - - 1.0%

Mueller-Hinton broth

Beef extract - - - - - - - - - 2.0%
Casein hydrolysate - - - - - 1.75% and
Starch - - - - - - - - - - 1.5%, pH 7.4.

Sabouraud’s dextrose broth

Dextrose - - - - - - - - - - 4.0% and
Peptone - - - - - - - - - - - 1.0%, pH 5.4.

7.1.3.2. Solid culture media

Peptone agar

Agar - - - - - - - - - - - - - - - 1.0%
bacteriological peptone - - 1.0% and
NaCl, pH 7.2—7.4.

This as used as a highly reproducible medium of low nutrient value for standardized
sensitivity result with respective to all Gram negative bacteria.

Nutrient agar

Agar - - - - - - - - - - - - - - - 2.0%
Beef extract - - - - - - - - - - 0.55%
Bacteriological peptone - - - - 1.0% and
NaCl - - - - - - - - - - - - - - - 0.05%, pH 7.2—7.4.

Mueller-Hinton agar

Beef extract - - - - - - - - - - 2.0%
Casein hydrolysate - - - - 1.75%
Starch - - - - - - - - - - - 1.5% and
Agar - - - - - - - - - - - - - - - 1.0%, pH 7.4, autoclaved at 121°C for 20 minutes.

Sabouraud’s dextrose agar

Dextrose - - - - - - - - - 4%
Peptone - - - - - - - - - - - 1% and
Agar - - - - - - - - - - - - - - - 1.5%, pH 5.4.
7.1.4. PREPARATION OF STANDARD SOLUTIONS

Stock solutions of 10mcg/ml (antibiotic Chloramphenicol) and 500µg/ml and 750µg/ml extract solutions were prepared for the antimicrobial studies by dissolving a suitable quantity in appropriate diluent. These were stored at 4°C, the solutions were used over a period not exceeding 3 months from the date of preparation.

7.1.5. PREPARATION OF DISCS CONTAINING EXTRACT OR ANTIBIOTIC

The discs were punched from the Whatman No. 1 filter paper and were 6.25mm in diameter. These were sterilized by heat at 160°C for an hour in batches on 100 in screw-capped Bijou bottles. The standard and test drug stock solutions were prepared and preserved as described above.

The final concentration of antibiotic to be present in the disc was 10µg/ml, two stock solutions having 500µg and 750µg/ml and a blank (without extract and/or antibiotic) were prepared. The following procedure was followed to prepare drug-impregnated discs; 1ml of stock solution containing 500µg/ml and 750µg/ml and an antibiotic (Chloramphenicol) were added to each bottle of the discs. Each disc absorbed 0.01ml of the solution, so that entire 1ml volume was absorbed thereby producing discs having 10µg/ml of the antibiotic. The same procedure was followed for the extracts. The maximum concentration of the extracts to be present in a disc were 200µg/ml and a stock solution containing 200µg/ml of extract added to each bottle of the discs. Each disc absorbed 0.01ml of the solution, so that the entire 1.0ml volume was absorbed thereby producing discs having 200µg of the extracts. The discs were used in the wet condition and were maintained at 4°C or below until needed to retain the potency for at least 3 months in the screw-capped bottles. The discs were allowed to warm at room temperature before use.

7.1.6. BACTERIAL INOCULUM

All organisms were grown over night on nutrient agar/Mueller-Hinton agar plates at 37°C and harvested during the stationary phase. The suspension was standardizing by diluting with saline or broth to a density visually equivalent to the McFarland standard 0.5% (a turbidity standard prepared by adding 0.5ml of a BaCl₂ solution to 99.5ml of 1% H₂SO₄) corresponding approximately to 5x10⁵cfu/ml. The inoculations of the plates were done
within 15 minutes of diluting the broth culture, so that the standardization was maintained throughout the study.

7.1.7. DETERMINATION OF ANTIMICROBIAL ACTIVITY

Agar cultures of the test microorganisms were prepared as described [9]. Three to five similar colonies were selected and transferred with loop into 5 ml broth. The broth cultures were incubated for 24 hours at 37°C. The MEMP and MEPN were dissolved in sterile water by magnetic stirrer. For screening, sterile, 6 mm diameter filter paper disc were impregnated with 500 µg/ml and 750 µg/ml of the MEMP and MEPN, respectively. Then the paper discs were placed in Mueller Hinton agar. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was to 10⁵ colony forming units (1 × 10⁵ cfu/ml). The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. In order to determine the antibacterial effect of the extracts, chloramphenicol (10 µg/ml) were used as positive control.

7.2. RESULTS

7.2.1. EFFECT OF MEMP AND MEPN ON ANTIMICROBIAL ACTIVITY

The data presented in Table 1 and 2 indicate that the methanol extract of Mucuna pruriens (MEMP) and Phyllanthus niruri (MEPN) inhibit the growth of some of the tested microorganisms (Gram positive and Gram negative) to various degrees. Both the extracts at a concentration of 500 µg/ml and 750 µg/ml exhibited significant (P < 0.05) antimicrobial effect against all the tested microorganisms. The MEMP and MEPN shown strong antimicrobial activity against Bacillus pumillus 8241, Escherichia. Coli 5B and Vibrae Cholera 1353 and 226101. However, their activity against Staphylococcus aureus ML 152 and Vibrae cholera 14035 was found to be significantly (P > 0.05) very less. The antimicrobial activity was compared with the standard Chloramphenicol at a concentration of 10 µg/ml.

7.2.2. STATISTICAL ANALYSIS

All treatments were performed in triplicate and each data point in the results is the mean of three replicate tests. All experiments were repeated at least once. The statistical
significance of a treatment effect was evaluated by student’s $t$-test and the values were expressed as mean ± SE. Probability limit was set at $P < 0.05$.

7.3. DISCUSSION

The antimicrobial activity of the *MEMP* and *MEPN* was studied by the disc diffusion method against various Gram positive and Gram negative microorganisms. Disc diffusion methods are used extensively to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of the substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. Because of the limited capacity of discs, holes or cylinders are preferably used [10]. *MEMP* and *MEPN* showed a broad spectrum of activity against all the bacterial strains at a concentration of 500µg/ml and 750µg/ml as shown in Table 1 and 2. Chloramphenicol (10µg/ml) was used as a positive control.

On the basis of the results it can be concluded that the methanol extract of *Mucuna pruriens* seeds and *Phyllanthus niruri* has significant antimicrobial activity.
Tab 1: Antimicrobial effect of methanol extract of *Mucuna pruriens* (MEMP) on selected microbial species

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>10% DMSO/ml</th>
<th>MEMP 500µg/ml</th>
<th>MEMP 750µg/ml</th>
<th>Chloramphenicol (10µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> 8531</td>
<td>6</td>
<td>8</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ML 174</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ML 152</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td><em>Bacillus pumilus</em> 8241</td>
<td>7</td>
<td>14</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 51</td>
<td>7</td>
<td>6</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 54B</td>
<td>6</td>
<td>11</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td><em>Vibrea cholera</em> 14035</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td><em>Vibrea cholera</em> 1353</td>
<td>6</td>
<td>15</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td><em>Vibrea cholera</em> 226101</td>
<td>6</td>
<td>19</td>
<td>23</td>
<td>26</td>
</tr>
</tbody>
</table>

6-9mm: less activity; 10-14mm: moderate activity; ≥15mm: strong activity.

All the values were the mean of three experiments.

The values given are the diameter of zone of inhibition (mm) including disc diameter of 6mm.
**Tab 2: Antimicrobial effect of methanol extract of *Phyllanthus niruri* (MEPN) on selected microbial species**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>10% DMSO/ml</th>
<th>MEPN 500µg/ml</th>
<th>MEPN 750µg/ml</th>
<th>Chloramphenicol (10µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> 8531</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ML 174</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ML 152</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td><em>Bacillus pumilus</em> 8241</td>
<td>7</td>
<td>7</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>6</td>
<td>10</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 51</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 54B</td>
<td>6</td>
<td>12</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td><em>Vibrea cholera</em> 14035</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td><em>Vibrea cholera</em> 1353</td>
<td>6</td>
<td>11</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td><em>Vibrea cholera</em> 226101</td>
<td>6</td>
<td>10</td>
<td>14</td>
<td>21</td>
</tr>
</tbody>
</table>

6-9mm: less activity; 10-14mm: moderate activity; ≥15mm: strong activity.

All the values were the mean of three experiments.

The values given are the diameter of zone of inhibition (mm) including disc diameter of 6mm.
Fig 1: Effect of methanol extract of *Mucuna pruriens* (*MEMP*) on selected microorganisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Diameter (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>20</td>
</tr>
<tr>
<td>Bacillus pumillus</td>
<td>15</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>10</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>5</td>
</tr>
</tbody>
</table>

Legend:
- Microorganism
- Staphylococcus aureus 8531
- Bacillus pumillus 8241
- Escherichia coli 54 B
- Vibrio cholera 226101
- Bacillus cereus
- Vibrio cholera 14035
- Escherichia coli 51
- Vibrio cholera 1353
Fig 2: Effect of methanol extract of *Phyllanthus niruri (MEPN)*
on selected microorganisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>10% DMSO/ml</th>
<th>MEPN 500 g/ml</th>
<th>MEPN 750 g/ml</th>
<th>Chloramphenicol 10 g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus 8531</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus ML 152</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus pumillus 8241</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli 51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli 54 B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrea cholera 1353</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrea cholera 226101</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
7.4. REFERENCES


