CHAPTER - 4
4. BIOLOGICAL SCREENING OF ETHNOMEDICINAL CLAIMS:

4.1. INTRODUCTION

It is now increasingly realized that if health facility is to be provided to all particularly in the developing countries where majority section of the population live below poverty line, emphasis on traditional system of medicine should be given top priority. Therefore, renovation of old ideas of herbal medicine with modern techniques and technology is the need of the day. Ethno-medicobotanical investigation in this line of demand has emerged as a promising branch of research. An analysis of modern medicine reveals that approximately one fourth of all the medicines now in use are derived from some 2500 flowering plants. Some so called revolutionary drugs discovered within last four decades or so were known in some form or the other in folklore medicines. This has necessitated the need to study folklore about uses of medicinal plants and how best these can be utilized for better and inexpensive health care.

It is now admitted that ethnic groups are the source of all indigenous drugs. As they live in and around remote forest areas far away from the availability of modern medicine their primary needs for medicine are always managed by herbal drugs and hence they play a significant role in preserving them.
On the other hand any investigation on folklore medicine leading to the discovery of new drugs remains incomplete if the information are not processed in the laboratory clinically, pharmacologically and toxicologically. Considering the above facts an attempt has been made for biological screening of a few species as regards their antimicrobial activity.


In addition to these a number of research workers are engaged in studying the antifungal acitivity of some plant extract, so as to find out the active principles involved therein. Notable among them are Angel et al (1930); Little et al (1948); Baruah et al
(1963); Dixit and Srivastava (1974); Narain and Panda (1976); Misra and Dixit (1977a, b, c, d, 1978, 1980).

Statistical records available indicate that about two percent of the total higher plants has been screened for pesticidal properties. Sehgal (1961) reported that extracts of plants from 157 families are significantly active against microorganism and out of which 20 percent of the extracts is prominently active against fungus. Gilliver (1947) studied the extract from 1915 species of flowering plants on *Venturia inequalis* and indicated that about 23 percent of 113 families possesses antifungal property.

According to Osborne (1943) out of 2300 species of plants investigated belonging to 166 families only 63 genera showed toxicity against *Staphylococcus aureus* and *Escherichia coli*.

Antimicrobial activity of seeds of some ethnomedicinal plants was tested by Saxena and Vyas (1986) using test organisms - *Aspergillus faminatus*, *Trichophyton mentagrophytes*, *Candida albicans*, *Escherichia coli*, *Bacillus subtilus* and *Streptococcus faecalis*. They reported that out of 14 species the extract of only 8 exhibited activity against one or more test organism.

Others who have done commendable works in this field are - Kurup (1956), Bhatnagar *et al* (1961) and Gained *et al* (1964).
During present investigation it has been observed that out of 170 species, 27 species are used as haemostatic and antiseptic by the Reang Tribe of Assam. Amongst these 4 species viz; *Alpinia nigra* (Gaertn.) Burtt. (rhizome); *Curculigo capitulata* L. (tuberous root); *Oroxyllum indicum* (L) Vent. (bark) and *Tournefortia montana* Lour. var. *griffithii* (Cl.) Johnston (leaves) have been selected for biological screening as regards their antimicrobial activity to ensure Reang's claim.
4.2. MATERIALS AND METHODS:

Modified filter paper disc method as suggested by Vincent and Vincent (1944) was followed for carrying out antimicrobial activity test. Details of the work done in this connection are as follows:

1. Preparation of plant extract:

Since the tribe use aqueous extract of concerned plant parts for their antiseptic action, 20 g each of parts of respective plants under present investigation were crushed by adding 10 ml of water to each. The extracts so obtained were then filtered through cotton plug and kept separately.

2. Preparation of paper discs containing plant extract:

5 mm discs of whatman No-1 filter paper were prepared and immersed in each of the filtered plant extract and kept overnight. These were then taken out, dried at room temperature and finally sterilised by keeping under UV radiation for 1 h.

3. Preparation of media and isolation of test organism:

(a) Sterilization of glassware etc.:

Cleanly washed, perfectly dried glasswares were sterilized by keeping in hot air oven at 160°C for 1 h. Test tubes, conical flasks etc. were plugged with cotton wool before heating. Petridishes, Pipettes etc. were wrapped in paper before sterilization.
Innoculating loops and forceps were sterilized by holding in flame till they became red hot.

b. Preparation of media:

Nutrient agar medium (Hi-media Pvt. Ltd.) was used for this investigation. 1.49 g of nutrient agar was weighed out and dissolved in 500 ml distilled water taken in a conical flask. The flask was then plugged with cotton and sterilized by autoclaving at a pressure approximately 15 lb and 120°C for 15 minutes, sterilized medium was then transferred to petridishes and allowed to cool.

c. Isolation of soil bacteria:

Bacteria were cultured from soil using dilution plate technique. In this process 1 g of soil sample was suspended in 1000 ml of sterile water resulting in the dilution of 10^{-3}. For isolation of bacteria 10^{-5} dilution of soil sample was used. From 10^{-5} dilution 1 ml of soil solution was innoculated to the culture plate and incubated at 37°C for 24 h.

4. Identification and preparation of pure culture of test organism:
a. Identification of microbial colony:

Microscopic observations were made for identification of microbial colony from mixed culture following gram's staining method. Colonies having *Staphylococcus sp* and *Streptococcus sp* were marked for obtaining pure culture. Following characteristic features were taken into consideration for identification of *Staphylococcus sp* and *Streptococcus sp*.

**Staphylococcus:**

After 24 hours of incubation the colonies were large (2-3 mm diameter), circular, convex, smooth, shiny, opaque and easily emulsifiable. On agar slope confluent growth presented a characteristic oil paint appearance.

Individual cells of the bacterium were spherical, approximately 1μ in diameter, arranged characteristically in grape like clusters. They were non motile, non capsulated and gram positive.

**Streptococcus:**

Individual cocci are spherical or oval, 0.5 to 1.0μ in diameter, arranged in long chain. They were non-motile, non-sporing and gram positive.

b. Preparation of pure culture:
A loop made up of Nichrome resistance wire, 24.S.W.G. size, was charged with specimen to be cultured from specific colony. One loopful of the specimen was then transferred on to the surface of petriplates containing well dried medium on which it was spread over a small area at the periphery. The innoculum was then distributed thinly over the plate by streaking it with the loop in a series of parallel lines in different segment of the plate. The loop was flamed and cooled between two streaks. The plate were then incubated for proper growth of bacteria. Same procedure was repeated thrice for better isolation of test organisms.

5. Application of filter paper disc against test organisms:

From the pure cultures of *Staphylococcus sp.* and *Streptococcus sp.* obtained so, innoculum was transferred on to fresh plates with nutrient agar media and prepared paper disc were placed on them separately and incubated at 37 ± 1°C for 18 h. After 18 h zones of inhibition formed due to each disc content in each case were measured and recorded. (Table-6).

6. Activity of known antibiotic discs against test organisms:

Following same procedure antibacterial activity of some known antibiotics like Streptomycin, Gentamycin, Nalidixicacid, Cifrofloxacin, Tetracycline and Chloromphenicol were also tested against the same test organisms using standard
sensitivity discs prepared and marketed by Hi-Media Laboratories Pvt. Ltd., for comparative analysis. The results obtained were tabulated in Table-6.1.
Photo - 47: Sensitivity of different standard antibiotic discs against *Streptococcus sp.*
Photo - 40: Disc prepared with aqueous rhizome extract of *Alpinia nigra* (Gaertn.) Burtt. showing sensitivity against *Staphylococcus sp.*

Photo - 41: Disc prepared with aqueous extract of *Alpinia nigra* (Gaertn.) Burtt. (Rhizome) showing sensitivity against *Streptococcus sp.*
Photo - 42: Disc prepared with aqueous root extract of Curculigo capitulata Lour. showing sensitivity against Streptococcus sp.

Photo - 43: Disc containing aqueous leaf extract of Tournefortia montana Lour. Var. griffithi (Cl.) John. - showing sensitivity against Streptococcus sp.
Photo - 44: Disc containing aqueous leaf extract of *Tournefortia montana* Lour. var. *griffithii* (CL) John. - showing sensitivity against *Staphylococcus sp.*

Photo - 45: Disc containing aqueous bark extract of *Oroxylum indicum* (L.) Vent. - showing sensitivity against *Streptococcus sp.*
Photo - 46: Disc containing aqueous bark extract of *Oroxylum indicum* (L.) Vent. - showing sensitivity against *Staphylococcus* sp.
4.3. RESULTS AND DISCUSSION:

Data recorded in Table-6 reveal that rhizome extract of *Alpinia uigra* (Gaertn.) Burtt. formed average 7.67 mm and 6.67 mm zone of inhibition against *Streptococcus sp.* and *Staphylococcus sp.* respectively.

Extract of tuberous root of *Curculigo capitulata* L. formed average 7 mm zone of inhibition against *Streptococcus sp.* No inhibition zone by the plant was seen against *Staphylococcus sp.*

Bark extract of *Oroxylum indicum* (L.) Vent. showed average 7.33 mm zone of inhibition against both *Streptococcus sp.* and *Staphylococcus sp.* where as leaf extract of *Tournefortia montana* Lour. Var. *griffithi* (Cl.) Johnston. formed average 10 mm and 10.67 mm zone of inhibition against *Streptococcus sp.* and *Staphylococcus sp.* respectively.

As regards known antibiotics it was observed that zone of inhibition formed by *Streptomycin* is 11.33 mm and 11.67 mm against *Streptococcus sp.* and *Staphylococcus sp.* respectively. Average zone of inhibition formed against same test organisms in case of *Ceftamycin* is 15.67 mm, and 18 mm, in case of *Nalidixic acid* is 15.33 mm and 17 mm, in case of *Ciprofloxacin* is 20 mm in case of *Tetracycline* is 14 mm and 15.67 mm and in case of *Chloromphenicol* is 15 mm respectively.
Although sensitivity of the known antibiotics are higher than that of the plant parts in case of all the test organisms yet, inhibition zone formed by the plant extracts clearly indicate the presence of antibacterial substances. The result thus confirms the authenticity of the reported use of the plant by the Reang Tribe. Since the test organisms used in the present investigation cultured from soil and its pathogenicity was not studied, further work in this line is essential. Isolation and identification of active principles from the plant parts used may lead to discovery of new antiseptics.
Table - 6: Inhibition zone formed by discs with different plant extract against *Streptococcus sp.* and *Staphylococcus sp.*

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Parts used</th>
<th>Test organisms</th>
<th>Inhibition zone (mm)</th>
<th>Average zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alpinia nigra</em></td>
<td>Rhizomes</td>
<td><em>Streptococcus sp.</em></td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus sp.</em></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><em>Curculigo capitulata</em></td>
<td>Tuberous roots</td>
<td><em>Streptococcus sp.</em></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus sp.</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Oroxylum indicum</em></td>
<td>Bark</td>
<td><em>Streptococcus sp.</em></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus sp.</em></td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td><em>Tournefortia montana</em></td>
<td>Leaf</td>
<td><em>Streptococcus sp.</em></td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus sp.</em></td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

*R1, R2, R3 = Replication.*
<table>
<thead>
<tr>
<th>Disc contents</th>
<th>Test organisms</th>
<th>Inhibition zone (mm)</th>
<th>Average zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R1</td>
<td>R2</td>
</tr>
<tr>
<td>Streptomycin</td>
<td><em>Streptococcus sp.</em></td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>10 mcg/disc</td>
<td><em>Staphylococcus sp.</em></td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Gentamycin</td>
<td><em>Streptococcus sp.</em></td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>10 mcg/disc</td>
<td><em>Staphylococcus sp.</em></td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td><em>Streptococcus sp.</em></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>20 mcg/disc</td>
<td><em>Staphylococcus sp.</em></td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td><em>Streptococcus sp.</em></td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>10 mcg/disc</td>
<td><em>Staphylococcus sp.</em></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Tetracycline</td>
<td><em>Streptococcus sp.</em></td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>30 mcg/disc</td>
<td><em>Staphylococcus sp.</em></td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Chloromphenicol</td>
<td><em>Streptococcus sp.</em></td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>30 mcg/disc</td>
<td><em>Staphylococcus sp.</em></td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

R₁, R₂, R₃, = Replication.