Microbiological Screening of some Ethnobotanically important plant sp. for their antibacterial activity against Human Pathogenic organisms (i.e. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *pseudomonas* sp.)

8.1 : INTRODUCTION:

India is endowed with a wealth of medicinal plants, which have been a valuable source of natural products for maintaining human health. Plants produce a diverse range of bioactive molecules, making them rich source of various types of medicines. Medicinal plants are an important source of therapeutic remedies for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th Century (Zaika, 1975).

Natural antimicrobials have been often derived from plants, microorganisms or animal tissues. Microbes are closely associated with the health and welfare of people. Some are beneficial, while others are detrimental. Due to the increasing therapeutic problem in the treatment of infectious diseases, the search for new drugs from natural sources becomes imperative, because most rampant killer diseases in developing countries are of microbiological origin (Gundidza and Gaza, 1993).

Natural products from plants may offer new agents for antibiotic use. A special feature of higher plants is their ability to produce a large number of organic chemicals of high structural diversity of the so-called secondary metabolites (Evans et al., 1986). Such metabolites are divided into three different categories based on their mechanism of function viz., bacteriostatic, antimicrobial and chemotherapeutic (Castello et al., 2002). The antibacterial and antifungal studies of the plant extracts and pure compounds mostly carried out by the agar well diffusion method (Bauer and Kirby, 1966).

In this way, several studies on antibacterial and antifungal substances from plants have been conducted by a number of researchers (Barnabas and Nagarajan, 1988; Aday et al., 1989; Krishnakumar et al., 1997; Sattar et al., 2004; Ehsan et al., 2009; Wang et al., 2010). Medicinal plants occupy a distinct role in the life of people since ancient times (Latha and Pari, 2003). Species of *Andrographis* Wallich ex Nees
(Acanthaceae) are used in the Indian systems of medicines such as Ayurvedha, Homeopathy, Naturopathy.

It is now increasingly realized that if health facility is to be proved 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. But the investigation on folklore medicine leading to the discovery of new drugs remains incomplete, if the information are not processed clinically, pharmacologically in the laboratory.


In Addition to these a number of research workers are engaged in studying the antifungal activity 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 & Srivastava (1974).
Statistical records available indicate that about 2% 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 microorganisms and out of which 20% of the extracts is prominently active against fungus. Gilliver (1947) studies the extracts from 1915 species of flowering plants on *Venturia inequalis* and indicated that about 23% 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*.

Extracts of plant sp. like *Datura metel*, *Zingiber officinale*, *Allium sativum* and weed such as *Parthenium hysterophorous*, *Spiranthus indicus* were tied and reported effective against human and plant pathogens (Saxena et al., 1986). Considering the above mentioned facts in the present work an attempt has been made for the preliminary biological screening of the few selected ethnomedicinal plant sp. in relation to their antibacterial activity.

The aim of the present study was to investigate antibacterial properties of various leaf extracts of *Croton caudatus* Geiseler, *Scoparia dulcis* L. *Passiflora edulis* Sims. and *Clerodendrum glendulosum* Celeb. Ethyl acetate extracts of this plant were evaluated against some the bacterial strains i.e *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp. and *Klebsiella pneumoniae*.

**Human Pathogenic microorganisms**:

*Staphylococcus aureus*: They are gram positive spherical cocci approximately 1um in dia, arranged characteristically in grape like clusters. It grows on blood agar culture medium (PI.-100).

Pathogenesis: Staphylococal diseases may be classified as cutaneous and deep infections, food poisoning, skin diseases, urinary tract infection, respiratory infection, tonsilitis, pharyngitis, sinusitis etc.

*Pseudomonas* sp: It is slender, gram negative bacteria. It is a strict aerobe and grows well on ordinary media like nutrient broth and nutrient agar. The optimum
temperature for growth is $37^0 \text{C}$, but growth occurs at a wide range of temperature between $5^0 \text{C} - 42^0 \text{C}$ (Pl.-101).

Pathogenesis: The common infections caused by it are:

Urinary tract infection, acute menigitis, wound and burn infections, chronic otitis, eye infection and infantile diarrhea.

**Escherichia coli**: It is a gram negative bacteria measuring 1-3um and 0.4-0.7 um. It is an aerobe and facultative anaerobe and grows on ordinary culture medium at optimum temperature of $37^0 \text{C}$ in 18 -24 hrs. On MacConkey medium colonies are pink due to lactose fermentation (Pl.-103).

Pathogenesis: *E.coli* is the common organism responsible for urinary tract infection, diarrhoeal diseases etc.

**Klebsiella pneumoniae**: They are gram negative capsulated, non sporing, non-motile bacteria that grows well on ordinary media at optimum temperature of $37^0 \text{C}$ in 18-24 hrs. On Macconkey’s agar media the colonies appear large, mucoid and pink to red in colour (Pl.-102).

Pathogenesis: It is responsible for severe bronchopneumonia, urinary tract infections, septicaemia, meningitis and rarely diarrhea.
8.2: MATERIALS AND METHODS:

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

Preparation of extracts

The solvents used was Ethyl acetate. The powdered leaves (15 g) were taken and the extract was dissolved in 20 ml of ethyl acetate solvent. The extracts so obtained were then filtered through cotton plug and kept separately.

Microorganisms used

In this investigation four bacteria (Staphylococcus aureus, Escherichia coli, Pseudomonas Sp. and Klebsiella pneumoniae) were used. All the cultures were obtained in pure form from the strain of Deptt. of Microbiology, Silchar Medical College, Silchar.

3. Preparation of media:

a) Sterilization of glasswares etc.:

Microorganisms are responsible for contamination and infection. They are present all around. The aims of sterilization is to remove or destroy any microorganisms from materials or from its surfaces. Sterilization is a process by which an article, surface or medium is made free of all microorganisms either in vegetative or spore form. Properly cleaned and dried glasswares were sterilised by keeping them in hot air oven at 160 °C. The test tubes, conical flask etc. were plugged by cotton wool before heating. Forceps and inoculating loops were sterilised by holding in the flame until it became red hot (inside the laminar air flow). Disposable petridishes were used for the culture of microorganisms and to see the antibacterial activity.

b) Preparation of nutrient agar media:

Nutrient agar media (Hi-media Pvt. Ltd.) was used for this work. Nutrient agar (28 gms) was added to 100 ml of distilled water in a conical flask. The flask was then plugged with cotton wool and sterilised by autoclaving at 15 lbs pressure (121 °C)
for 15 minutes. The sterilised medium was then poured to petridishes and allowed to cool. Then the petridishes were kept in incubator for few hours.

c) Preparation of blood agar media:

Blood agar media was used for the culture of *Staphylococcus* sp. Blood agar base (42.5 gms) was added to 100 ml of distilled water in a conical flask. The flask was then sterilised by autoclaving at 15 lbs pressure (121\(^0\)c) for 15 minutes. Cooled at 40-50\(^0\)c and aseptically 7% sterile sheep’s blood was mixed well before pouring.

d) Preparation of peptone water:

Suspended 25.0 gms of peptone in 100 ml distilled water in a conical flask. The flask was then sterilised by autoclaving at 15 lbs (121\(^0\)c) for 15 minutes. Dispensed in test tubes and entered microorganisms in peptone water kept in incubator for few hours.

e) Preparation of Mac. conkey agar media:

Mac. conkey agar media was used for the culture of bacteria (*Escherichia coli*, *Pseudomonas* sp. and *Klebsiella pneumoniae*) Mac. conkey agar (28 gms) was added to 1000 ml of distilled water in a conical flask. The flask was then sterilised by autoclaving at 15 lbs pressure (121\(^0\)c) for 15 minutes. The sterilised medium was then poured to petridishes and allowed to cool. Then the petridishes were kept in an incubator for few hours.

4. Isolation of test organisms:

Test organisms like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas* sp. were maintained by pure culture method.

5. Known antibiotic discs activity against test organisms:

The inoculum of *Escherichia coli*, *Pseudomonas* sp. and *Klebsiella pneumoniae* *Staphylococcus aureus* were transferred to different freshly prepared petridishes containing nutrient agar media by peptone water and the prepared paper discs were placed on them separately and incubated at 37\(^0\) C for 48 hrs. formation of inhibition zone due to each disc were recorded. The results obtained are given in tabulated form. (Table 1). Known antibiotic discs viz: Streptomycin (S\(^{10}\)), Chloramphenicol (C\(^{10}\)), Norfloxacin (NX) and Tetracyclin (T) were placed on them.
seperately and incubated at 37°C for 48 hrs. Formation of inhibition zone due to each disc were recorded. The results obtained are given below in tabular form. (Table.1).

6. Application of filter paper disc containing plant extracts against test organisms:

Similar procedure was followed for the antibacterial activity of some known antibiotic discs viz: Streptomysin (S), Chloramphenicol (C), Norflaxacin (NX) and Tetracyclin (T). They were tested against the test organisms. After 48 hrs. formation of inhibition zone due to each disc were recorded. The results obtained are given in tabular form. (Table. 2).

Antibacterial screening

The agar well diffusion method was employed for the determination of antimicrobial activity of the leaf extracts. The petriplates containing 20 ml of Nutrient Agar medium were seeded with 24 h culture of the microorganisms. The wells (5 mm in diameter) were cut from the agar and the extract solution (5 mg/ml) was delivered into them. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zones were measured in millimeters (mm). Each experiment was repeated three times, and the average values were calculated.
8.3: RESULT AND DISCUSSION ON THE MICROBIOLOGICAL SCREENING

The results obtained are recorded in the table (1 and 2). And graphical representation of these data are given in the Fig. 1 and 2. From the data it can be seen that leaf extracts of Croton caudatus Geiseler formed average zone of inhibition 20.3 mm, 15.3 mm, 15.00 mm and 15.8 mm against Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus respectively.

The measured value of zone of inhibition proved that the 4 ethyl acetate leaf extracts showed good sensitivity against Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus. (P1.-95 to 98).

While whole plant extract of Scoparia dulcis L. formed average zone of inhibition at 6.0 mm, 6.33 mm, 7.33 mm and 7.70 mm against Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus respectively.

The extract of leaf of Passiflora edulis Sims. formed a zone of inhibition of 17.7 mm, 9.20 mm, 10.5 mm and 7.00 mm against Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus respectively.

Whereas, the leaf extract of Clerodendrum glandulosum Cceleb. formed zone of inhibition 6.33 mm, 7.70 mm, 6.00 mm and 7.00 mm against Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus respectively.

On the other hand from known antibiotic, it is observed that diameter of the zone of inhibition formed by Streptomycin is an average of 16.7 mm, 19.33 mm, 11.0 mm and 16.0 mm against Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus respectively. Similarly against the same test organisms the diameter of average zone of inhibition formed in case of Chloramphenicol has been 14.0 mm, 21.7 mm, 17.0 mm and 15.7 mm respectively.

Norfloxacin formed an average zone of inhibition of 22.7 mm, 18.0 mm, 25.33 mm and 9.0 mm against Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus respectively. Whereas, Tetracycllin formed a zone of inhibition of 7.33 mm, 22.7 mm, 15.33 mm and 9.33 mm against
Escherichia coli, Pseudomonas sp. Klebsiella pneumoniae and Staphylococcus aureus respectively. (P1.-91 to 94).

Table 1: Diameter in zone of inhibition formed by disc containing different plant extracts against test organism.

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Test organisms</th>
<th>Diameter in zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>Croton caudatus Geiseler. 6.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scoparia dulcis L. 17.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Passiflora foetida L. 6.33</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas sp</em></td>
<td>CleTodendrum glandulesium 7.00</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumonia</em></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Staphylococcus aureus</em></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Diameter in zone of inhibition formed by different leaf extracts against known human pathogenic microorganisms.
Table 2: Diameter in zone of inhibition formed by known antibiotic discs against test organisms.

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Test organisms</th>
<th>Diameter in zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Streptomycin (S°)</td>
</tr>
<tr>
<td>1</td>
<td>E. coli</td>
<td>16.00</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas sp</td>
<td>16.00</td>
</tr>
<tr>
<td>3</td>
<td>Klebsella sp</td>
<td>11.00</td>
</tr>
<tr>
<td>4</td>
<td>Staphylococcus sp</td>
<td>19.33</td>
</tr>
</tbody>
</table>

Fig.2: Diameter in zone of inhibition formed by known antibiotics against test organisms.
Plate 91: Diameter in zone of inhibition formed by known antibiotic discs against *Staphylococcus aureus*.

Plate 92: Diameter in zone of inhibition formed by known antibiotic discs against *Klebsiella pneumoniae*.

Plate 93: Diameter in zone of inhibition formed by known antibiotic discs against *Escherichia coli*.

Plate 94: Diameter in zone of inhibition formed by known antibiotic discs against *Pseudomonas* sp.

Plate 95: Diameter in zone of inhibition formed by disc containing plant extracts against *Escherichia coli*.

Plate 96: Diameter in zone of inhibition formed by disc containing plant extracts against *Staphylococcus aureus*.

Plate 97: Diameter in zone of inhibition formed by disc containing plant extracts against *Klebsiella pneumoniae*.

Plate 98: Diameter in zone of inhibition formed by disc containing plant extracts against *Pseudomonas* sp.

Plate 99: Investigator busy in preparing blood agar media.
Medicinal plants are of great importance to the health of individuals and the society. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body (Stephan et al. 2009). Medicinal plants also represent a rich source of antimicrobial agents. The use of plants as antibacterial agents is gradually gaining attention probably due to the high cost, unavailability and resistance of the allopathic drugs (Fransworth & Morris, 1976). In this regard, present investigation has been carried out on the screening of some ethnobotanical plant species against some known human pathogenic microorganisms. For this purpose 4 ethnobotanically important plant species were taken. These are: Croton caudatus Geisler, which is used to cure Cancer; Passiflora edulis Sims, used in diabetes, Clerodendrum glandulosum Ccled. for curing high blood pressure and Scoparia dulcis L. to cure diabetes. The antibacterial activities of leaf extracts of these 4 plant species were tested using some Gram positive bacteria (Staphylococcus aureus) and Gram negative bacteria (Escherichia coli, Klebsiella pneumoniae and Pseudomonas sp.) by disc diffusion method. The disc diffusion method for antibacterial activity showed significant reduction in the growth in terms of zone of inhibition around the disc. These activities may be due to the presence of various active principles in the leaf extracts. The result of the microbial screening support the ethnomedicinal use of these plant species. These plant species seem to have potential to be used as the source for the new antibacterial drugs. However, further work is needed to isolate and identify the bioactive(s) principle present in them. in order to develop new antibacterial and antifungal drugs.

From the above results, all the plant extracts showed the significant indication about the activities against human pathogenic organisms. So, all these plants are required to chemically analyse for human welfare. Therefore, the result of the investigation thus confirms the authenticity of the claim given by the tribal people.

Microbiological screening of Croton caudatus Geiseler, Passiflora edulis Sims, Scoparia dulcis L and Clerodendrum glandulosum Celeb. against some human
pathogenic organisms (i.e E. coli, Pseudomonas sp., Klebseilla pneumoniae and Staphylococcus aureus) confirms the authenticity of the claims given by the tribal people. The plant extract of the above mentioned 4 species showed the significant indication about the activities against human pathogenic organisms. Therefore, all these plants may be taken up for chemical analysis for human welfare. Thingbaijan et al. (2011) worked on the antimicrobial activity of leaf extracts of Eurya japonica Thunb. and Ficus auriculata Laur. against the same human pathogenic organisms (i.e E. coli, Pseudomonas sp., Klebseilla pneumoniae and Staphylococcus aureus) and recorded significant indication about the antibacterial activities.

Similar works are also reported by Mahesh & Satish (2008). They reported the antimicrobial activity of leaf extracts of Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer and Ziziphus mauritiana against Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus and Xanthomonas axonopodis pv. malvacearum and antifungal activity against Aspergillus flavus, Dreschlera turcica and Fusarium verticillioides. Singh et al. (2009). They reported the significant antimicrobial activities of stem bark of Balanites roxburghii Planch against E. coli, Salmonella typhi, Klebseilla pneumoniae and Staphylococcus aureus. Dutta Choudhury et al. (2009) worked on isolation, characterization and bio activity screening of compounds from Clerodendrum viscosum Vent. against some test organisms. Lalitha et al. (2010) worked on antimicrobial activity and phytochemical screening of the petroleum ether, acetone and ethanolic extracts of the aerial roots of Pothos aurea and also done