EVALUATION OF ANTIMICROBIAL ACTIVITY OF CROTON ROXBURGHII BALAK. (EUPHORBIACEAE) LEAVES

Patel ED*1, Padiya RH2 and Acharya RN3

1Ph.D. Scholar, Dept. of Dravyaguna, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat – 361 008.
2Ph.D. Scholar, Dept. of Dravyaguna, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat – 361 008.
3Associate Professor, Dept. of Dravyaguna, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat – 361 008.

ABSTRACT
Antimicrobial activity of methanol extracts of Croton roxburghii Balak. (Euphorbiaceae) leaves, in different concentrations (5 μg/ml, 25 μg/ml, 50 μg/ml, 100 μg/ml, 250 μg/ml), was carried out as a part of search for new antimicrobial substance against important human pathogens. Antibacterial and antifungal activities were carried out against six human pathogens like, two Gram positive bacteria namely B. subtilis & S. aureus, two Gram negative bacteria namely E.coli & K.pneumoniae and two fungal strains namely A.flavus & C.albicans, by disc diffusion method following standard procedures. The zone of inhibition of the extract was compared with that of different standard drugs like Streptomycin, Cipfodoxime and Gentamycin, for antibacterial activity while Amphotericin, Fluconazole and Clotrimazole, for antifungal activity. The observed results (zone of inhibition) showed that methanol extract of Croton roxburghii leaves possess effective antibacterial and antifungal activities against all tested organisms comparable with that of standard drugs.

Keywords: Antibacterial activity, antifungal activity, Croton roxburghii leaves, methanol extract, microbial load.

INTRODUCTION
Due to increasing antibiotics resistance in microorganisms and synthetic antibiotics side effects, medicinal plants are now gaining popularity in the treatment of bacterial infections[1]. Antimicrobials of plant origin have an extremely large therapeutic potential. They are effective in the treatment of infectious diseases, while simultaneously all eviating many of the side effects that are often connected with synthetic antimicrobials[2]. Croton roxburghii Balak. has been traditionally used by tribal people for several medicinal purposes[3]. The leaves of the plant are large, green in colour, 5-10 by 2-4.5 inch size, oblong- lanceolate, crenate or serrate[4-11] and are reported for their application on snake bite, boils, wounds and to cure blindness & cataract[4]. Though the plant part is
used for the management of infectious conditions, but till yet, leaves are not reported for its antimicrobial activities. Hence, the present research was carried out to evaluate the antimicrobial activity of methanol extract of *Croton roxburghii* Balak. leaves against some human pathogenic bacteria and fungi.

**MATERIALS AND METHODS**

**Collection of plant materials**
The plant *Croton roxburghii* Balak. (Euphorbiaceae) was collected after identifying it from its natural habitat of Gandhamardan hill ranges, Bolangir, Odisha, India; after studying its morphological characters and comparing them with the characters mentioned in various floras[5-11]. The leaves were collected, washed properly under running water, to make them free from foreign matter like sand, soil etc. and dried under shade. Herbarium voucher No. 6047 was also prepared and submitted to Pharmacognosy museum of IPGT & RA, Jamnagar, for future reference.

**Preparation of sample**
For the analysis, shade dried leaves of *Croton roxburghii* plant were coarsely powdered to 60# and stored in airtight glass container.

**Extract preparation:** 1g of *Croton roxburghii* leaves powder was extracted with methanol by sonicating it for 10 min and then keeping it overnight. Next day after filtration, methanol evaporated, then by taking weight of residue, 5 different concentrations 5 µg/ml, 25 µg/ml, 50 µg/ml, 100µg/ml, 250µg/ml of the sample, were prepared. These are used for determination of antimicrobial activity.

**Culture conditions**
The antimicrobial efficacy of this plant material tested on 6 different strains, two Gram positive bacteria namely *Bacillus subtilis* (NCIM 2063) & *Staphylococcus aureus* (NCIM 2079), two Gram negative bacteria namely *Escherichia coli* NCIM 2065 and *Klebsiella pneumoniae* (NCIM 2719) and two fungal strains namely *Aspergillus flavus* (NCIM 1028) & *Candida albicans* (NCIM 3471). All cultures were obtained from NCL, Pune. 24 hour old cultures of all these organisms were inoculated in sterile broths and incubated till 0.5 Mcfarland standard turbidity obtained, and then used for assay[14].

**Determination of microbial load for plant material**
Microbial load of *Croton roxburghii* leaves powder was carried out by total viable aerobic count method\[12-13\].

To 500 mg, accurately weighed sample, 1-2 drops of Tween80 and a homogeneous suspension was prepared by slowly adding 5 ml of sterile buffered sodium chloride peptone (SBSCP) solution of pH 7.0. This suspension was diluted $10^{-1}$ onwards as required in sterile dilution blanks (SBSCP). One ml each from these aliquots was added to sterile melted and cooled top agar (Soyabean casein digest agar, for fungal count Potato dextrose agar medium used) tubes. These tubes were poured to sterile petri dishes and allowed to solidify. These plates were incubated at 30-35°C for 48 hours. The numbers of colonies were counted and the results were expressed in Cfu / g.

$$\text{Cfu / g} = \frac{\text{Number of average colonies}}{\text{Dilution} \times \text{Volume plated}}$$

**Antimicrobial assay**

The antimicrobial activity of methanol extracts of *Croton roxburghii* leaves was studied in different concentrations (5 μg/ml, 25 μg/ml, 50 μg/ml, 100 μg/ml, 250 μg/ml) against selected pathogenic bacterial and fungal strains. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition.

Sterile soybean casein digest agar (25 ml/plate) used for antibacterial activity and sterile sabouraud agar (25ml per plate) used for antifungal activity. Medium obtained from Himedia laboratories. Sterile 20 ml medium poured in sterile plates aseptically and let them solidified. Then inoculate 0.5 ml of culture in 5 ml sterile melted and cooled medium and poured them on solidified agar plates aseptically. After solidification made well with the help of cup borer and inoculate 0.3 ml of each sample in the well and for antibiotic discs there is no need to make wells and directly place disc on agar surface aseptically. For diffusion purpose plates were placed in refrigerator for 20-25 minutes. Then Incubate plates at 37°C for 24 hrs except sabouraud agar plates and plates containing *K. pneumoniae* organism, they incubated at 30°C for 24-48 hrs. After incubation, zone of inhibition was measured with Himedia antibiotic zone scale- c\[15\].
Pathogen study

Same extracts were used for antimicrobial activity assay, these extracts transferred to specialized mediums given below and incubated at their optimum temperature for growth, then after incubation plates were observed and concluded results\(^{[16]}\).

Selective differential mediums according pathogens:
- Pseudomonas aeruginosa – Citrimide agar
- Salmonella typhi – TSI agar slant, XLD agar
- Escherichia coli – EMB agar
- Staphylococcus aureus – Mannitol salt agar

RESULTS AND DISCUSSION

Croton roxburghii leaves coarse powder is greenish yellow in colour.

Microbial load:

The observations on the microbial load of Croton roxburghii leaves showed that the tasted samples, when collected from their natural sources, are either free or within prescribed limit of the microbes\(^{[17]}\). (Table 1)

**TABLE 1: MICROBIAL LOAD REPORT OF CROTON ROXBURGHII LEAVES**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample C. roxburghii leaves</th>
<th>Permissible Microbial contamination limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Viable Aerobic Count (Cfu/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Bacterial count</td>
<td>$7.8 \times 10^3$</td>
<td>$10^7/g$</td>
</tr>
<tr>
<td>b) Fungal count</td>
<td>10</td>
<td>$10^7/g$</td>
</tr>
<tr>
<td>Pathogens (per gram)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) S. aureus</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>b) E. coli</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>c) P. aeruginosa</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>d) S. typhi</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Antimicrobial activity:

The result shows that the extracts of Croton roxburghii leaves were found to be effective against all the microorganism tested. The inhibitory effect of C. roxburghii leaves methanol extracts ($5, 25, 50, 100, 250$ g/ml) showed ($14, 15, 16, 17, 20$mm) against E. coli; ($00, 12, 13, 16, 18$mm) against K. pneumoniae; ($12, 13, 14, 15, 16$mm) against S.
aureus; (00, 12, 13, 14, 16mm) against B. subtilis; (00, 00, 12, 13, 14mm) against S. flavus; (16, 17, 18, 19, 20mm) against C. albicans.

The antibacterial and antifungal activity of the methanol extracts of Croton roxburghii leaves, increased linearly with increase in concentration of extracts ( g/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, E. coli and S. aureus were more sensitive as compared to K. pneumoniae and S. aureus but E. coli and K. pneumoniae showed good results. For fungal activity C. albicans showed good result and more sensitive as compare to S. flavus. The growth inhibition zone measured ranged from 12-20 mm for all the sensitive bacteria, and ranged from11-22 mm for fungal strains. (Table 2, 3)

**TABLE 2: ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACTS OF CROTON ROXBURGHII LEAVES AND STANDARD DRUGS AGAINST GRAM +VE AND GRAM -VE ORGANISMS**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. subtilis (NCIM 2063)</td>
</tr>
<tr>
<td>C. roxburghii leaves methanol extract</td>
<td>5 µg/ml</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>25 µg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>100 µg/ml</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>250 µg/ml</td>
<td>16</td>
</tr>
<tr>
<td>Methanol (Control)</td>
<td>-</td>
<td>Nil</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10 µg</td>
<td>28</td>
</tr>
<tr>
<td>Cifpodoxime</td>
<td>10 µg</td>
<td>22</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10 µg</td>
<td>27</td>
</tr>
</tbody>
</table>

www.pharmasm.com  IC Value – 4.01  3968
Effect of *C. roxburghii* leaves methanol extract against Gram +ve and Gram –ve Strains

**Figure 1**

Effect of standard drug against Gram +ve and Gram -ve strains

**Figure 2**

| TABLE 3: ANTIFUNGAL ACTIVITY OF METHANOL EXTRACTS OF CROTON ROXBURGHII LEAVES AND STANDARD DRUGS |
|--------------------------------------------------|----------------------------------|-----------------|
| Sample                                           | Concentration                   | Zone of inhibition (mm) |
|                                                  |                                  | *S. flavus* (NCIM 1028) | *C. albicans* (NCIM 3471) |
| C. roxburghii leaves methanol extract            | 5 µg/ml                          | Nil              | 16 |
|                                                  | 25 µg/ml                         | Nil              | 17 |
|                                                  | 50 µg/ml                         | 12               | 18 |
|                                                  | 100 µg/ml                        | 13               | 19 |
|                                                  | 250 µg/ml                        | 14               | 20 |
| Methanol (Control)                               | -                                | Nil              | 11 |
| Amphoterin B 50 µg                               | 10 µg                            | 14               | 19 |
| Fluconazole 30 µg                                | 10 µg                            | 11               | 28 |
| Clotrimazole 10 µg                               | 10 µg                            | 24               | 30 |
Figure 3
Effect of *C. roxburghii* leaves methanol extract against fungal strains

Figure 4
Effect of standard drugs against fungal strains
Plate 1
Antimicrobial activity of *Croton roxburghii* leaf Meoh extract

B. subtilis 250
B. subtilis standard
B. subtilis Meoh control

S. aureus 250
S. aureus standard
S. aureus Meoh control

E. coli 250
E. coli standard
E. coli Meoh control

K. pneumoniae 250
K. pneumoniae standard
K. pneumoniae Meoh control
Plate 2
Antimicrobial activity of *Croton roxburghii* leaf Meoh extract

www.pharmasm.com

IC Value – 4.01

3972
CONCLUSION

This study concludes that the methanol extract of leaves of plant *Croton roxburghii* possess antibacterial and antifungal activities. However, more studies are needed to prove the crude extracts as the antimicrobial agents. The present results will form the foundation for selection of plant species for further exploration in the potential detection of new natural bioactive compounds.

ACKNOWLEDGEMENT

We express our thankfulness to Mr. Hota BN, Rtd. DFO, Govt. Of Odisha; Mr. Govind Baba, Traditional practitioner; Mr. Pareswar Pradhan, Pharmacognosy expert; Mr. Malaya Das, Forest Range Officer, Govt. of Odisha and other traditional healer who helped us during drug collection at Gandhamardan hills, Bolangir and Bargarh, Odisha. Authors are thankful to Dr Subrata De sir, Dr. Pankaj Nariya and Ms. Rinkal Rana, RMD Research Centre, Waghaldhara, Gujarat, for providing facilities for antimicrobial studies. Authors are also thankful to P. Sahu, Pharmacognosist and Mr. Dhala for their help in plant collection.

REFERENCE


For Correspondence:
Esha D. Patel
Email: esha.ayupharma@gmail.com