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# Antioxidant and Antimicrobial activity of *Croton caudatus* Geisel

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In this study different extracts of the leaves of *Croton caudatus* Geisel was evaluated for their antioxidant and antibacterial activity against human pathogenic gram +ve and gram –ve bacteria and antifungal activity against both human and plant pathogens. All the extracts exhibited varied levels of antibacterial and antifungal activity. The ethanolic extract shows antibacterial effect against all the tested bacteria with a diameter zone ranges from 8-12 mm. The ethanol extract of *Croton caudatus* leaves presented a good activity against *Staphylococcus aureus* and *Pseudomonas putida* with 12 mm inhibition zone diameter whereas methanolic extract presented a good activity against *Candida albicans*. Chloroformic and ethanolic extracts of *Croton caudatus* exhibited inhibition zone diameter of 10 and 12 mm, respectively against *Microphomina phaseolina*. The results obtained in this study appears to confirm the antioxidant and antimicrobial potential of *Croton caudatus* leaves and thereby may be useful in the treatment of diseases.

**Key Words: *Croton caudatus* Geisel, Antioxidant, Antibacterial, Antifungal activity.**

## INTRODUCTION

The use of plant extracts to treat infection is an age-old practise in large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases. Further acquaintance with different ethnic groups has contributed to the development of research on natural products to the increase in knowledge about the close relationship between the chemical content of plants and its biological properties against the pathogenic bacterial and fungal isolates. For these reasons, medicinal plants are important for the study of their traditional uses and proof through scientific method.

*Croton caudatus* Geisel, locally known as *Ranlung- damdawi* is a plant mainly grown in Saikot forest of Churachandpur District of Manipur which belongs to Euphorbiaceae family and are often used in treating worm infested cattle's like cow, pig, dog, buffalo etc. by the Hmar people, a local tribe of north-east India. It is also used for the treatment of piles, sinus, cold *etc*. This plant finds extensive application in relieving pain from sprains[1].

Many workers worked on the carcinogenic properties of Croton species. Several species are known for its purgative oil and believed to be antirheumatic. Active compounds like crotocaudin, isocrotocaudin, teucvidin, taraxerone and taraxerol are responsible for its carcinogenic activities[2,3].

Based on the fact that there is no scientific research reporting on the antioxidant and antimicrobial activity of this plant. Thus, we decided to take this opportunity to screen for both its potential antioxidant and antimicrobial activity. This work will be the first report on the antioxidant and antimicrobial properties of *Croton caudatus* Geisel and also validations of the local indigenous people's knowledge on the uses of this plant for the treatment of worm infection in their cattle.

## EXPERIMENTAL

The leaves of *Croton caudatus* Geisel were collected in the month of May, 2008 from the Saikot forest area of Churanchandpur District of Manipur at an altitude of 818 m above the sea level. The specimen has been conserved in the IBSD germplasm and the collected plant species was deposited (IBSD/M-193) in the herbarium of IBSD, Imphal, India.

**Test microorganisms:** Antimicrobial activity was assessed on a total of 14 microbial species including 7 bacteria, one yeast and 6 moulds. The bacterial strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India as Microbial Type culture Collection (MTTC) and the fungal pathogens were obtained from IARI, New Delhi. The pathogens used were Candida albicans ITCC-3179, *Rhizoctonia solani* ITCC-6491, *Aspergillus flavus* ITCC-1973, *Aspergillus fumigates* ITCC-6050, *Aspergillus niger* ITCC-2146 and *Microphomina phaseolina* ITCC-5519 while the bacterial

cultures include *Pseudomonas auriginosa* MTCC-2581, *Streptococcus mutans* (RIMS), *Staphylococcus aureus* (RIMS), *Escherichia coli* MTCC-1687, *Klebsealla pneumonia* ( RIMS), *Proteus vulgaris* (RIMS) and *Pseudomonas putida* MTCC-2759.

**Extract preparation:** The leaves of *Croton caudatus* Geisel were shade dried and made into the powder form with the help of Waring blender. The powdered material were extracted with different solvents starting from lower polarity to higher polarity *i.e.*, petroleum ether, chloroform, methanol, ethanol and water using cold extraction in shaker for 24 h at room temperature. Each extract was filtered using Whattman No. 1 filter paper and concentrated under reduced pressure to dryness below 40 ºC using Buchi vacuum evaporator. The dried extracts thus obtained were directly used for the determination of antioxidant and antimicrobial activities.

**Chemicals:** 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). L-Ascorbic acid, nutrient agar, potato dextrose agar, chloramphenicol, amphotericin were purchased from HiMedia. Chloroform, ethanol, methanol and petroleum ether were purchased from Merck Chemicals, Mumbai, India.

**Evaluation of antioxidant activity**

**DPPH radical scavenging activity:** The DPPH free radical scavenging was assessed according to Okada and Okada method[4] 0.1 mM DPPH (Sigma Aldrich) radical solution in ethanol was prepared. Different volumes of plant extract in water and methanol *i.e.*, 100, 200, 400, 500, 600, 800, 900 and 1000 µL were taken and the volume was made uniformly to 1000 µL using water and methanol and then 5 mL of the DPPH solution was added. Ascorbic acid was used as a positive control at 1 mg/mL. After incubation for 0.5 h in the dark, the absorbance was measured at 517 nm on UV-visible spectrometer Shimadzu, UV-1700. All determinations were performed in triplicate. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. The radical scavenging activity, expressed as percentage of inhibition were calculated using the equation:

Percentage of DPPH Inhibition

$$= \frac{[(\text{Control absorbance} - \text{samle absorbance})]}{\text{Control absorbance}} \times 100$$

**Determination of antimicrobial activity:** The antibacterial and antifungal activity were determined using the methanolic, ethanolic and chloroformic extracts of *Croton caudatus* Geisel leaves by means of disc diffusion assay[5,6] with little modifications. Twenty microliters of 16 days old bacterial cell suspensions containing $10^8$ CFU/mL cells were evenly spread on the sterile solidified nutrient agar (NA) plates (Hi-Media) and for fungal culture 20 µL of 72 h old spore suspensions containing $10^7$ CFU/mL cells were spread uniformly on sterile PDA plates with the help of sterile spreader. Once the plates had been aseptically dried, 6 mm sterile disc deposited with 20 µL of the crude plant extracts were placed on the agar plate. The plates innoculated with bacteria were incubated at 37 ºC for 24 h and for fungal cultures the plates were incubated at 30 ºC for 48-72 h and the growth inhibition zone diameter (IZD) was measured. Each tests were performed in triplicate.

## RESULTS AND DISCUSSION

**Antioxidant activity and total phenol content:** The antioxidant potential is inversely proportional to $IC_{50}$ value, which were calculated from the linear regression of the percentage antioxidant activity *versus* extracts concentrations (Fig. 1). The antioxidant activities of different crude extracts of *Croton caudatus* Geisel can be attributed to the presence of some components that have antioxidant activity. The plant extract nevertheless consist of various constituents. Therefore, determination of the components responsible for activity is very difficult. Several studies have reported the relationship between phenolic content and antioxidant activity. Some authors have found a correlation between the phenolic content and antioxidant activity, while others found no such relationship. Velioglu *et al.*[7] reported a strong relationship between the total phenolic content and antioxidant activity in certain plant products. There is a need to characterize phenolic compounds present within each plant extract to assign different antioxidant activities, to ascertain whether the phenolic structure affects antioxidant activity and also to determine whether synergism definitely occurs among certain phenolic compounds[8,9]. Chaterjee *et al.*[2], have reported the presence of di- and tri- terpenoids like teucvidin, taraxerone, taraxerol, taraxeryl acetate and sitosterol. The same author also reported the presence of isocrotocaudin from this plant[3].

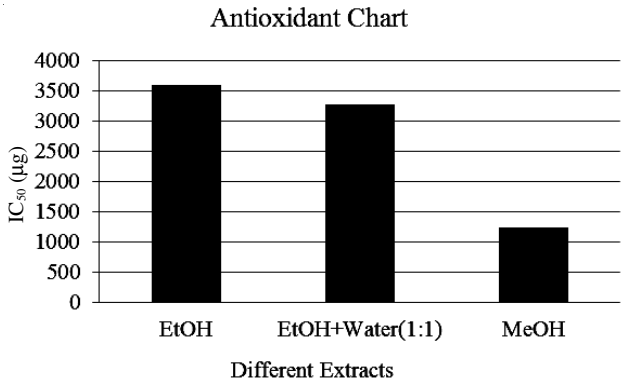


Fig. 1. Antioxidant activity of different crude extracts of *Croton caudatus* using (DPPH) method

**Antimicrobial activity:** The antibacterial and antifungal activity of the *Croton caudatus* Geisel extracts were determined (Tables 1 and 2). The antibacterial activity exhibited by methanol extract ranges from 7.97-10.00 mm with the highest activity of 10.03 mm against *Staphylococcus aureus* whereas chloroform extract shows strong activity against *Pseudomonas putida* with an inhibition zone diameter of 12.03 mm. Ethanol extract was found to be the most effective by giving an inhibition zone diamter ranges from 7.9-12.12 mm with highest activity against *Staphylococcus aureus* and *Pseudomonas putida* whose result is almost compatible with the results exhibited by chloramphenicol.

The antifungal activity was tested against the human and plant fungal pathogens. Methanolic extract shows highest activity of 10.13 mm (inhibition zone diamter) which is comparable with the known antibiotic (Amphopterecin) whereas the chloroform and ethanol extracts of *Croton caudatus* exhibited

TABLE-1
ANTIBACTERIAL ACTIVITY OF FOUR DIFFERENT EXTRACTS FROM *Croton caudatus* AS INHIBITION ZONES (mm)

| Bacterial strains | Origin | MeOH | Chloroform | Ethanol | Chloramphenicol |
|---|---|---|---|---|---|
| | | Inhibition zone diameter (mm) | | | |
| *Klebsiella pnumoneae* | RIMS | 8.0 ± 0.12 | 9.0 ± 0.12 | 8.1 ± 0.15 | 12.07 ± 0.13 |
| *Proteus vulgaris* | RIMS | 7.97 ± 0.2 | 0 ± 0 | 8.1 ± 0.06 | 14.03 ± 0.09 |
| *Staphylococcus aureus* | RIMS | 10.03 ± 0.08 | 0 ± 0 | 12.12 ± 0.17 | 12.07 ± 0.13 |
| *Escherichia coli* | MTCC-1687 | 8.1 ± 0.05 | 0 ± 0 | 8.1 ± 0.06 | 14.7 ± 3.5 |
| *Streptococcus mutans* | RIMS | 0 ± 0 | 7.43 ± 0.12 | 7.9 ± 0.12 | 8.67 ± 0.43 |
| *Pseudomonas putida* | MTCC-2759 | 10.0 ± 0.12 | 12.03 ± 0.09 | 12.1 ± 0.08 | 15.13 ± 0.07 |
| *Pseudomonas auriginosa* | MTCC-2581 | 8.0 ± 0.12 | 0 ± 0 | 10.13 ± 0.09 | 15.87 ± 0.07 |

RIMS-Regional Institute of Medical Science Hospital, Manipur. Methanolic, chloroformic, ethanolic extracts taken (20 µg/disc), chloramphenicol (20 µg/disc) used as standard.

TABLE-2
ANTIFUNGAL ACTIVITY OF FOUR DIFFERENT EXTRACTS FROM *Croton caudatus* AS INHIBITION ZONES (mm)

| Fungal strains | Origin | MeOH | Chloroform | Ethanol | Amphoptericin |
|---|---|---|---|---|---|
| | | Inhibition zone diameter (mm) | | | |
| *Aspergillus flavus* | ITCC-1973 | 0 ± 0 | 7.13 ± 0.13 | 7.13 ± 0.07 | 11.13 ± 0.07 |
| *A. fumigatus* | ITCC- 6050 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 8.93 ± 0.13 |
| *Candida albicans* | ITCC-3179 | 10.13 ± 0.07 | 8.13 ± 0.07 | 8.4 ± 0.2 | 10.07 ± 0.03 |
| *Rhizoctonia solani* | ITCC-6491 | 0 ± 0 | 7.13 ± 0.07 | 0 ± 0 | 0 ± 0 |
| *Macrophomina phaseolina* | ITCC-5519 | 0 ± 0 | 10.17 ± 0.09 | 12.13 ± 0.07 | 12.0 ± 0.12 |
| *Aspergillus niger* | ITCC-2146 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 9.0 ± 0.12 |

Methanolic, chloroformic, ethanolic extracts taken (20 µg/disc). Amphotericin-B (20 µg/disc) was used as standard.

strongest activity against one of the common soil borne plant pathogen *Macrophomina phaseolina* with an inhibition zone diameter of 10.17 and 12.13 mm, respectively.

## Conclusions

To conclude, the ethanolic extracts of the plant were found to possess higher antioxidant activity as compared to other extracts. The results achieved using these assay provides simple data making it possible to classify extracts according to their antioxidant and antimicrobial activity. The therapeutic value of the plant extracts may be partly due to their antioxidant activity. Further studies on the absorption and the effects of phytochemicals present in the plant extracts on antioxidant status in animal models are needed to evaluate their potential benefits.

The antibacterial efficacy of the ethanol fraction was concentration dependant against all the tested strains. In case of antifungal activity, the petroleum ether and chloroform extract shows moderate activity and little activity was observed in methanolic extract.

## REFERENCES


1. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, p. 82 (1956).
2. A. Chatterjee and A. Banerjee, *Tetrahedron*, **33**, 2407 (1977).
3. A. Chatterjee, A. Baneerjee and F. Bohlmann, *Phytochemistry*, **17**, 1777 (1978).
4. Y. Okada and M. Okada, *J. Agric. Food Chem*., **46**, 401 (1998).
5. K. Guven, F. Yucel and F. Centitas, *Pharm. Biol.*, **44**, 79 (2006).
6. National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standards NCCLS, Publication M2-A5, Villanova, PA, USA (1993).
7. Y.S. Velioglu, G. Mazza, L. Gao and B.D. Oomah, *J. Agric. Food Chem*., **46**, 4113 (1998).
8. H.E. Miller, *J. Am. Oil Chem. Soc*., **48**, 91 (1971).
9. G. Yen and P.D. Duh, *J. Am. Oil Chem. Soc*., **72**, 1065 (1995).