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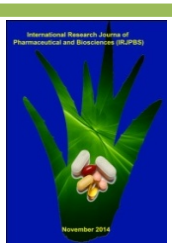

Microscopic evaluation and HPLC profiling of *Croton caudatus* Geiseler leaf

Longjam Shantabi[1], Ganesh Chandra Jagetia[1], Sh. Victoria Devi[2]and. H. Lalhlenmawia[2]

[1]Department of Zoology, Mizoram University, Tanhril 796004, Aizawl, India.

[2]Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Zemabawk 796017, Aizawl, India.

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***Corresponding author:**
longjam.shantabi@yahoo.com

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Abstract

Objective: The plants have provided medicine for healthcare since time immemorial and it is necessary to characterize the physical and physiochemical properties of a medicinal plant which is used regularly. The aim of the present investigation was to characterize the microscopic structure of *Croton caudatus* Geiseler leaf powder. **Methods:** Dried powder of the leaf was analyzed microscopically and HPLC profiling was carried out using standard protocols. **Results:** The detail microscopic analysis of powder revealed the presence of non-glandulartrichome, covering trichome, starch grain, calcium oxalate crystals and stone cells. Powdered drug, treated with different chemicals and its extracts with different solvent showed colour changes when illuminated with UV light. HPLC profiling showed the presence of various phytochemicals. **Conclusions:** The microscopic analysis and HPLC profile of the *Croton caudatus* Geiseler leaf is useful in standardization for quality, purity and for the correct sample identification.

Key words: *Croton caudatus*, microscopic, HPLC, covering trichomes.

INTRODUCTION

Ethnomedicine, encompasses the totality of health, knowledge, values, beliefs, skills and practices of members of a society including all the clinical and nonclinical activities that relate to human health [1]. As per WHO estimate, about 80% of the population in the developing countries depends mainly on plants or natural medicines for their health care needs [2,3].

Traditional medicine is defined by WHO as "including diverse health practices, approaches, knowledge and exercise applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent human illness". The indigenous traditional knowledge of medicinal plants of various ethnic communities, has been transmitted orally for centuries from one generation to the next is fast disappearing from the face of the earth due to the advent of modern medical practices and transformation of traditional culture. Therefore it is necessary to document the ethno medicinal information presently existing among the diverse communities before the traditional knowledge is completely lost out [4].

Croton caudatus Geiseler (belonging to family Euphorbiaceae) is a traditional medicinal plant. It is fairly widespread in South East Asia including Sri Lanka,Bhutan,Borneo,Burma, Indo-Myanmar region,Java,Laos,Malaysia,Nepal,Pakistan,Philippines, Singapore, Sumatra, Thailand and Vietnam.*Croton caudatus* Geiseler had long been known to Chin-Kuki inhabitants of Manipur and Mizoram.It is traditionallyused as a poultice for fever, sprains and treatment of liver diseases in various parts of Asia. Its roots are purgative.The whole plant is used for medicinal purposes and it has been found to be low in toxicity [5]. *Croton caudatus*is a traditional Dai Nationalistic medicine. The stems and leaves of *Croton caudatus*have been used for the treatment of malaria, ardent fever, convulsions, rheumatic arthritis, and numbness [6]. It is one of the constituents of Qi Wei KeTengZi Wan, which is a famous formula used by the Dai tribe of China for the treatment of pain and stomach diseases [7]. The leaves have been applied on festering wounds of injured cattles to ward off against maggots. *Croton caudatus* usually grows in peat swamp, deciduous and thick canopy evergreen forests. Sometimes,it grows near marginal areas along river or stream tracts. Its traditional uses for various medicinal purposes stimulated us to obtain an insight into its standardization of the plant, pharmacognostically for its utilization indifferent formulation. The present study dealt with the pharmacognostical characterizationalong with HPLC profiling for understanding the active components in the plant which may be helpful to develop the individual monograph.

MATERIALS AND METHODS

Experimental

The *Croton caudatus* was collected and its powder was analyzed microscopically. Ethanol extract of it was analyzed for the presence of various phytochemicals using HPLC.

Collection and extraction

Croton caudatus (CC) was identified by Professor Kumar Singh, a well known taxonomist of Manipur University and authenticated by Botanical survey of India, Shillong. The mature leaves of *Croton caudatus* were collected from Saikot, Churachandpur District of Manipur during the dry season. The cleaned and non-infected leaves were spread into the stainless steel trays and allowed to shade dry at room temperature in dark in clean and hygienic conditions to avoid entry of insects, animals, fungus, and extraneous terrestrial materials. The exhaust and free air circulation was allowed. The dried leaves were powdered in a grinder at room temperature. A sample of 100g of leaf powder was extracted sequentially with chloroform, ethanol and water in a Soxhlet apparatus. The extracts (CCE) were then evaporated to dryness under reduced pressure and stored at -80°C until further use.

CHEMICALS AND INSTRUMENTS

Glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. External features were recorded using Sony camera. Microphotographs were observed under a DM-2500 fluorescence microscope (Leica Microsystems, Wetzlar, Germany) equipped with 450–490 nm BP filter set with excitation at 453 nm using a 40 X N Plan objective. Microscopic structure was determined using standard protocol and solvents used for extraction includes viz. petroleumether, chloroform, ethanol and distilled water and reagents viz. Dilute nitric acid, dilute HCL, dilute sulphuric acid, 2% ferric chloride solution, 10% sodium hydroxide, 1M sodium hydroxide, 5% potassium hydroxide, phluroglucinol, ethanol, distilled water, ethyl acetate, chloroform, 1% potassium chloride, methanol (Hplc grade), glacial acetic acid, 10% ammonium hydroxide, Acetonitrile (Hplc grade), water (Hplc grade) ashless filter paper were used for the study .

MICROSCOPICAL INVESTIGATION

The powder was analysed according to the standard procedures [8] using various chemical reagents. The powder was stained with phluroglucinol reagent and were photographed and observed under a DM-2500 fluorescence microscope (Leica Microsystems, Wetzlar, Germany) equipped with 450–490 nm BP filter set with excitation at 453 nm using a 40 X N Plan objective.

High Performance Liquid Chromatography (HPLC)

Twenty grams of ethanol extract was dissolved in 20 ml ethanol and adsorbed onto silica gel (20 g) and loaded onto a column (150 g) prepared in 100% hexane. The column was eluted sequentially with hexane, graded mixtures of hexane: ethyl acetate in the ratio of 100:0, 99:1, 97:3, 95:5, 93:7, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40. All 28 fractions were collected after elution from the column and used for HPLC profiling.

Instrumentation

The high-Performance liquid chromatographic system consists Waters 515 HPLC pump , avalve type injector, Waters 2489 UV/ Visible Detector (Water, Singapore), Symmetry ® C_{18}(250×4.6 nm) column with a particle size of5µm.Other conditions: temperature – ambient, flow rate – 1 ml/min, injection volume – 5 µl, detection at 254nm. HPLC was done using different solvents system, Acetonitrile: water andwater: methanol (HPLC grade) in a gradient way of the collected fractions.

RESULT

Fluorescence Analysis

Crude drugs show their own characteristic fluorescence when exposed to ultra violet radiation and is dependent on its chemical constituents. This analysis is useful to identify adulterants duringcrude drug evaluation. The powder was subjected to fluorescence analysis as per the standard procedure[9], [10] asshown in Table 1.

Fluorescence Analysis of the extracts

The extracts were prepared as per their polarity in hot successive extraction technique.The colour changes were observed under UV light as shown in Table 2 and Fig 1-4.

Table 1: Fluorescence characteristics of leaf powder of *Croton caudatus.*

Treatment	Visible	UV (254 nm)
Powder	Light green	No change
Powder + dil HNO_3	Light Brown	Black
Powder + dilHCl	Mud Green	Brown
Powder +dil H_2SO_4	Mud Green	Grey
Powder + 2% $Fecl_3$	Dark Brown	Dark Black
Powder + 10% NaOH	Dark Green	Brown
Powder + 1N NaOH	Dark Green	Grey
Powder + 5% KOH	Dark Green	No colour
Powder + Pluroglucinol	Light Brown	Black
Powder + Ethanol	Dark Green	Grey
Powder + dist H_2O	Light Brown	Grey
Powder + Ethyl acetate	Yellowish Green	Dark Grey
Powder + Chloroform	Dark Green	Dark Grey
Powder + 1% KCl	Grey	Dark Brown
Powder + Methanol	Dark Green	Grey
Powder + Acetic acid	Reddish Brown	Grey
Powder + 10% NH_4OH	Mud Green	Dark Grey

Table 2: Fluorescence characteristics of leaf powderextracts of*Croton caudatus.*

Extract	Visible Short	UV (254 nm)
Chloroform	Dark Green	Black
Ethanol	Dark Green	Black
Aqueous	Dark brown	No change

Fig 1: Powder of *Croton caudatus*

Fig 2: Chloroform extract of *Croton caudatus*

Fig 3: Ethanolic extract of *Croton caudatus*

Fig 4: Aqueous extract of *Croton caudatus*

MICROSCOPICAL INVESTIGATION

The powder is dark green in colour, fine, odourless powder with bitter taste (Figure 1). The microscopic examination of the powder shows the presence of non-glandular trichome (Figure 5), covering trichome (Figure 6), starch grain (Figure 7), calcium oxalate crystals (Figure 8) and stone cells (Figure 9).

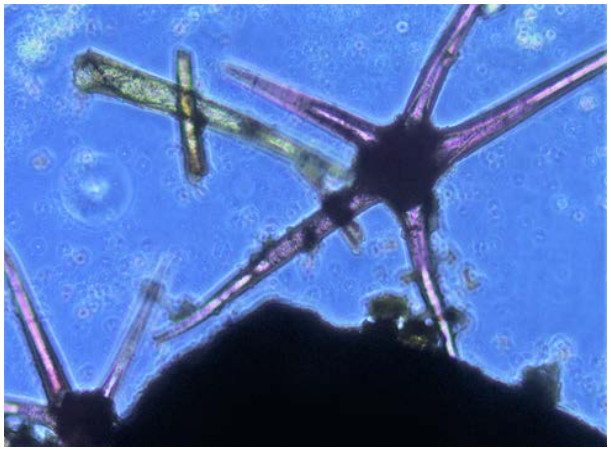


Fig 5: Non-glandular trichome

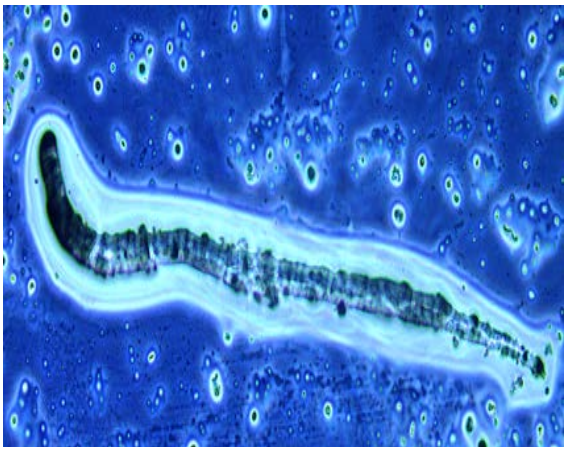


Fig 6: Covering trichome

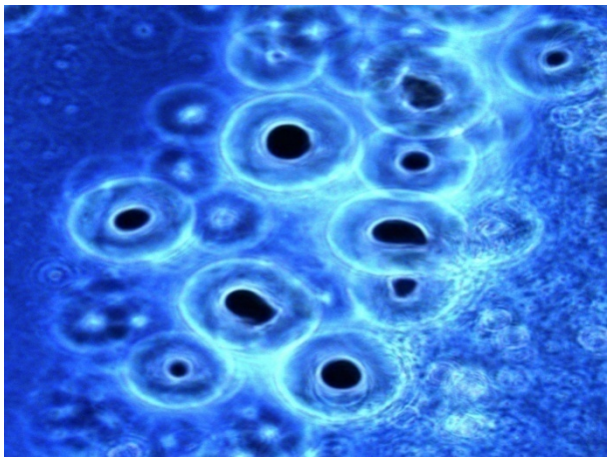


Fig 7: Starch grain

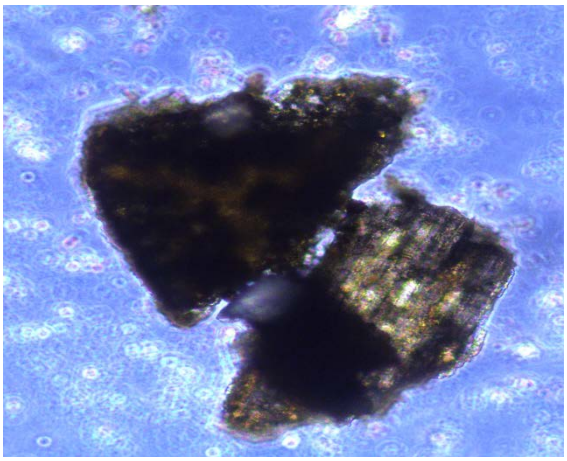


Fig 8: Calcium oxalate

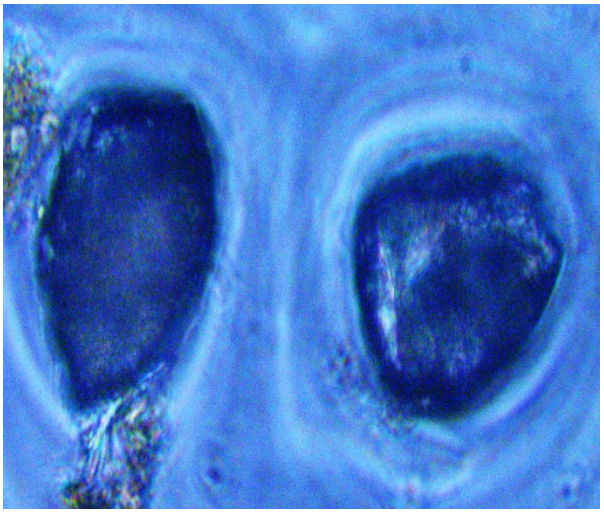
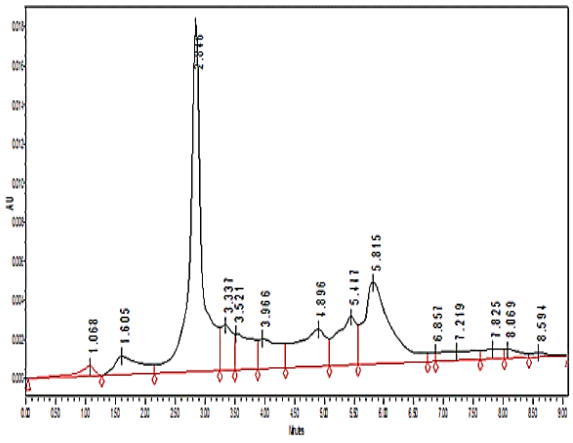


Fig 9: Stone cells

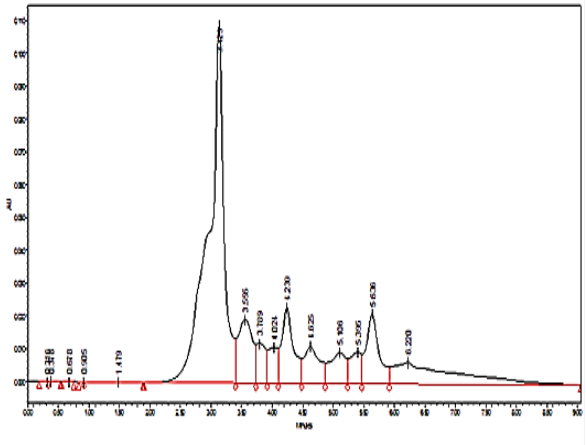
HPLC

The qualitative HPLC fingerprint profiles were selected at a wavelength of 254nm and flow rate- 1 ml/ min due to sharpness of the peaks and proper baseline. The HPLC profiles of Hexane and Ethyl acetate fraction of ethanolic extract of *Croton caudatus* after column chromatography usingsolvents Acetonitrile and Water (30:70) showed two prominent peaks at a retention time of 2.846 min and 5.815 min, three prominent peaks at a retention time 3.129 min, 4,239 min and 5.636 min using solvents Acetonitrile and Water (70:30)and displayed three prominent peaks with the retention time 3.334 min, 5.652 min and 5.994 minusing solvents Acetonitrile and Water (80:20). Lastly same fraction showed two prominent peaks at a retention time 5.575 min, and 8.008 min using Water and Methanol (90:10) respectively. HPLC profiling showed the presence of various phytochemicals (Figure 10a, 10b, 10c and 10d)

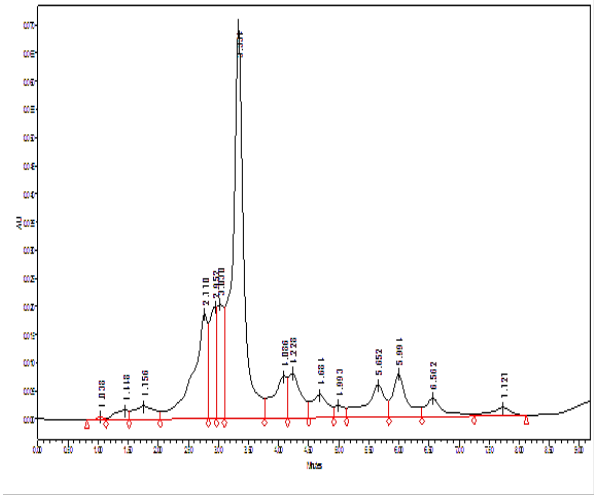
Figure 10: HPLC profiles of Hexane and Ethyl acetate fraction of ethanolic extract of *Croton caudatus*using different solvent systems.



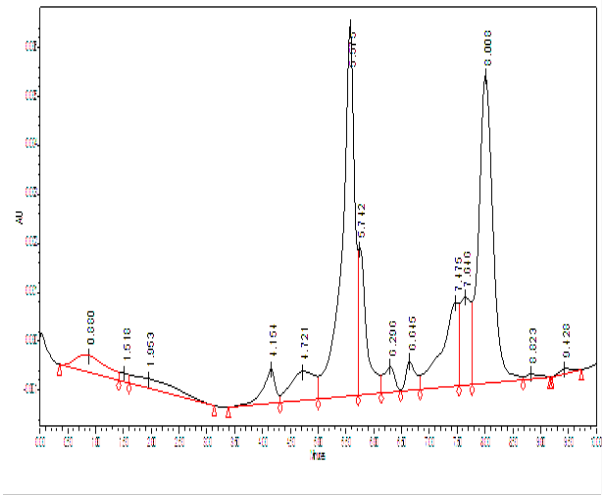
a) Acetonitrile and Water (30:70)



b) Acetonitrile and Water (70:30)



c) Acetonitrile and Water (80:20)



d) Water and Methanol (90:10)

DISCUSSION

It was found that the plant *Croton caudatus* Geiseler possesses several traditional and pharmacological uses and as a part of authentication of the sample, the microscopical and HPLC profiling of *Croton caudates* were studied. The microscopic evaluation of leaf of *Croton caudatus* andHPLC profiling of the extract have been carried out which would be of significant use in the identification of this drug.High performance liquid chromatography was examined in flow rate – 1 ml/min, injection volume – 5 µl, detection at 254nm which is particularly valuable for the preliminary separation and determination of plant constituents. This finding is useful to supplement the existing information with regard to identification and standardization of *Croton caudate* even in the powdered form of the leaf to distinguish it from drug and adulterant. The studies also suggest that the observed microscopical parameters are of great value in the quality control and formulation development.

Preliminary phytochemical screening and TLC profiling of various extracts of *Croton caudatus* revealed that it contains alkaloids, phytosterols, saponins, phlobatannins, cardiac glycosides, flavonoids, phenolics and terpenoids and the medicinal activity of this plant may be due to the concerted action of all these pharmacophores [11].Unicellular covering trichomes along withnon-grandular trichomes were the specific features in the powder microscopy.

CONCLUSION

The present study on Microscopic evaluation and HPLC profiling of *Croton caudatus* Geiseler leaf will provide useful information for its identity, purity and quality of the plant material for future reference.

AUTHORS` CONTRIBUTION

LS collected the *Croton caudatus* leaves, prepared powder and extracted sequentially with chloroform, ethanol and water in a Soxhlet apparatus and carried out the HPLC profiling and microscopic study. GCJ designed the experiment and prepared the final manuscript, SVD edited and helped with experimental designed and HLanalysed the HPLC profiling. All authors read and approved the final version of the manuscript.

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