# PHYTOCHEMICAL ANALYSIS OF SOME *Crotalaria* SPECIES GROWING IN SAMARU-ZARIA, NIGERIA FOR THE PRESENCE OF PYRROLIZIDINE ALKALOIDS

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**Abstract:** A Phytochemical analysis of four species from the genus *Crotalaria* (known as *Bi-rana* or *Biya-rana* in Hausa) was carried out to determine the presence of toxic pyrrolizidine alkaloids (PAs) – chemical compounds known to have medicinal as well as toxic properties to human and veterinary animals. The analysis was conducted on the leaves and aerial parts of *C. lachnosema, C. microcarpa, C. naragutensis,* and *C. retusa* (Family: Fabaceae) by extracting the alkaloids with 1% $H_2SO_4$ in 50 % aqueous ethanol. General alkaloid tests were carried out on the extracts as well as specific PA test using Ehrlich reagent [4-dimethylaminobenzaldehyde (5 g) dissolved in a mixture of acetic acid (60 ml), water (30 ml), and 60% perchloric acid (10 ml)]. Thin layer chromatographic analysis was conducted by extracting the PAs with dichloromethane and developed on silica gel plate using hexane-ethyl acetate (1:1). The plate was treated with Ehrlich spray reagent (1 g p-dimethylaminobenzaldehyde in 100 ml ethanol + 15 ml concentrated hydrochloric acid) heated at $95^0$C for 15 min. to reveal the presence of PA as purple and blue spots in all the extracts. The results of the study suggest the presence of potentially hepatotoxic PAs in the leaves and aerial parts of the four plants samples.

**Keywords:** *Crotalaria* species, fabaceae, hepato-toxicity, pyrrolizidine alkaloids

## Introduction

Acute and chronic liver diseases caused by pyrrolizidine alkaloids (PAs) toxicity have been known and were endemic in the Central Asian Republics of the former USSR, in early 1930s with several outbreaks occurring and the cause was discovered to be the seeds of *Heliotropium* species which contaminated the staple food crops. PA have been reported to cause liver disease in both Human and veterinary animals, and consumption of plant containing them is of etiological significance in chronic liver diseases and cirrhosis (Bras *et al*., 1961). In humans, another way of coming in contact with PAs may be by taking honey collected by bees that visited PA-containing plant or by consuming milk or eggs produced by PA-contaminated animals. The plant Families *Boraginaceae*, *Asteraceae* (tribes *Senecioneae* and *Eupatorieae*), *Ochidaceae* and *Fabaceae* (mainly the Genus *Crotalaria*) have been reported as the major sources of PA constituting more than 95% of all the reported cases (Dharmananda, 2001; Rosemann, 2006).Almost all animal species tested were found to be susceptible to the toxic nature of pyrrolizidine alkaloids including Human beings (WHO, 1989). Grazing animals are noted to be more susceptible to PA poisoning due to their feeding on food contaminated with the toxic weeds and large-scale outbreaks have been reported from different part of the world including the temperate and cold climates (Kone and Kande, 2012).

Following the ingestion of herbal infusions for treatment of certain ailments or the accidental contamination of food, the PAs find their way to the liver causing veno-occulusive disease – a disease characterized by the dominant occlusive lesion of the centrilobular veins of the liver lobule. Many cases progress to cirrhosis with even reports of single episode of acute disease leading to cirrhosis, in spite of the fact that the patient has been remove from the source of the toxin and given symptomatic treatment. Mortality can be high due to hepatic failure in acute phase or due to hematemesis resulting from ruptured oesophageal varices caused by cirrhosis (Dharmananda, 2001; Kone and Kande, 2012; WHO, 1989).

Plants of the genus *Crotalaria* are of great importance medicinally, agriculturally and economically. On the other hand presence of PA in these plants poses a great toxic potential especially where they are taken as traditional remedies for ailments and/or the plants are found growing unrestrained in the region (Bildfell, 2016; Nuhu *et al.*, 2009a; WHO, 1989). We report the phytochemical analysis (with special emphasis on the presence of the toxic pyrrolizidine alkaloids) on the leaves and aerial parts of some *Crotalaria* species found growing unrestrained around Samaru-Zaria.

## Materials and Methods

### *Collection, identification and preparation of the samples*

Fresh samples of *Crotalaria lachnosema, C. microcarpa, C. naragutensis* and *C. retusa* were collected from Samaru-Zaria in Sabon Gari Local Government Area of Kaduna State, Nigeria. Samples were identified by the Taxonomist at the Herbarium unit of the Department of Botany Faculty of Life Sciences, Ahmadu Bello University, Zaria, by comparing with existing specimens with voucher numbers: 900, 114, 798, 872 and 292, respectively. The leaves and aerial parts of each sample were separated and allowed to dry under shade. The dried samples were powdered and each powdered sample was kept at room temperature in separate container until needed.

### *General chemical tests for alkaloids*

### *Extraction*

Five gram (5 g) of the drug was boiled with 50 ml of 1% $H_2SO_4$ in 50% aqueous ethanol. It was cooled, filtered, concentrated to about half its original volume; it was then transferred into a separating funnel. Concentrated ammonia solution was added until the solution was alkaline to litmus, and an equal volume of chloroform was added. The mixture was shaken gently and allowed to separate. The lower chloroform layer was run off into another separating funnel and extracted with 20 ml dilute $H_2SO_4$. Each extract was divided into 5 portions and used for the following tests (Evans, 2009; Kar, 2007).

### Test for Alkaloids

- **Mayer's test:** To the first portion of the filtrate, 1 ml of Mayer's reagent was added drop by drop. A cream coloured precipitate indicates the presence of alkaloid.
- **Dragendoff's test:** To the second portion of the filtrate, 1 ml Dragendoff's reagent was added drop by drop. A reddish-brown coloured precipitate indicates the presence of alkaloid.

- **Wagner's test:** To the third portion of the filtrate, 1 ml Wagner's reagent was added drop by drop. A reddish-brown coloured precipitate indicates the presence of alkaloid.
- **Hager's test:** To the fourth portion of the filtrate, 1 ml of saturated picric acid solution was added drop by drop. A yellow coloured precipitate indicates the presence of alkaloid.
- **Tannic acid test:** To the fifth portion of the filtrate 1 ml of freshly prepared 10% tannic acid solution was added. A brown coloured precipitate indicates the presences of alkaloids

***Specific tests for pyrrolizidine alkaloid***

Plant material (1 g) was extracted by grinding it with ascorbic acid (5%) and a small amount of purified sand. The solution was filtered and divided into two equal portions ("test" and "blank"). An aqueous solution (0.2 ml) of sodium nitroprusside (5%) containing sodium hydroxide was added to the "test" sample. Both portions were heated for approximately one min at $75^0$C; then Ehrlich reagent was added and heating continued for another one min. The Ehrlich reagent contains 4-dimethylaminobenzaldehyde (5 g) dissolved in a mixture of acetic acid (60 ml), water (30 ml), and 60% perchloric acid (10 ml). A magenta colour in the "test" compared with the "blank" indicates the presence of unsaturated pyrrolizidine alkaloid N-oxide. The intensity of the colour in the "test" compared with the "blank" can give a rough idea of the amount of alkaloids present (Mattock and Jukes, 1987).

***Thin layer chromatographic (TLC) analysis***

**Extraction:** Five gram of the powdered sample was extracted by moistening it with 5 ml of 10% solution of sodium hydroxide. It was then extracted with 50 ml of dichloromethane with stirring for 24 h. It was filtered and the filtrate evaporated to dryness and lyophilized. Samples of 10 mg extract were dissolved in 10 ml of dichloromethane and methanol in the ratio 1: 9 (v/v) (Kone and Kande, 2012).

**TLC of the extracts:** The extracts were chromatograph on pre-coated silica gel plate (0.25 mm Merck, Germany), using hexane-ethyl acetate (1:1) mobile phase. The plates were then air-dried, sprayed with Ehrlich spray reagent (1 g p-dimethylaminobenzaldehyde in 100 ml ethanol + 15 ml concentrated hydrochloric acid) heated at $95^0$C for 15 min. to reveal the presence of pyrrolizidine alkaloids as purple and blue spots (Kone and Kande, 2012).

**Results and Discussion**

The general tests for alkaloids in the plant extracts revealed that all the extracts contained alkaloids by giving various precipitates in at least four out of the five (except the leaves of *C. microcarpa* that gave three) tests when treated with the alkaloids reagents (Table 1). The specific test for pyrrolizidine alkaloids in the plants extracts when treated with Ehrlich's reagent gave a magenta colour in the 'test' sample and no colour change in the 'blank' sample (Plate I and Table 2). Tables 3 and 4 showed the number of spots, their colour (both in daylight and after spray) and also the $R_f$-values for the samples of the four *Crotalaria* species analyzed in the study. *C. lachnosema* leaves extract gave 3 purple spots whereas the aerial parts gave only one purple spot. The leaves of *C. microcarpa* gave 4 blue spots while aerial parts gave 2 blue spots. Both leaves and aerial parts extracts of *C. naragutensis* and *C. retusa* gave 2 spots (blue and purple each).

The present study revealed thatall the plant extracts gave precipitate when treated with the five general alkaloid reagents except in the case of *C. retusa*where both extracts didn't give the cream coloured precipitate with Mayer's reagent. Nonetheless, the extracts gave the required precipitates with the other reagents. The qualitative tests conducted are generally regarded as evidence for the presence of alkaloids (Evans, 2009); in all the plants extracts, a necessary step in establishing the presence of toxic alkaloids in plant samples (Nuhu *et al.*, 2009b). The specific test for PAs also as carried out in this study using Ehrlich reagent as described by Mattocks and Jukes (1987), a magenta colour was observed in the 'test' sample in all the extracts indicating the presence of PAs in both the leaves and aerial parts of the samples. The TLC method of Mattocks for the detection of PAs has been described as the most useful qualitative method for the potentially toxic pyrrolizidine alkaloids due it simplicity, ease of use and less time consumption (Kone and Kande, 2012; Nuhu *et al.*, 2009b).

**Table 1: General tests for the presence of alkaloids in the four *Crotalaria* species**

| Specie/ Reagent | Dragendoff | Wagner | Mayer | Hager | Tannic acid |
|---|---|---|---|---|---|
| ***C. lachnosema*** | | | | | |
| Leaves | Present | Present | Present | Present | Present |
| Aerial parts | Present | Absent | Present | Present | Present |
| ***C. microcarpa*** | | | | | |
| Leaves | Absent | Present | Absent | Present | Present |
| Aerial parts | Present | Absent | Present | Present | Present |
| ***C. naragutensis*** | | | | | |
| Leaves | Absent | Present | Present | Present | Present |
| Aerial parts | Present | Present | Present | Absent | Present |
| ***C. retusa*** | | | | | |
| Leaves | Absent | Present | Absent | Present | Present |
| Aerial parts | Present | Present | Absent | Present | Present |

**Table 2: Specific test for pyrrolizidine alkaloids in the leaves and aerial parts of the four *Crotalaria* species**

| Sample | Colour Observed | | Inference |
|---|---|---|---|
| | Leaves | Aerial Parts | |
| *C. lachnosema* | Magenta | Magenta | Present |
| *C. microcarpa* | Magenta | Magenta | Present |
| *C. naragutensis* | Magenta | Magenta | Present |
| *C. retusa* | Magenta | Magenta | Present |

**Table 3: TLC analysis of the leaves extracts of the four *Crotalaria* species**

| Specie | No. of spots | Colour of spot | | $R_f$-value |
|---|---|---|---|---|
| | | Daylight | After spray* | |
| *C. lachnosema* | 3 | Yellow | Purple | 0.78 |
| | | Green | Purple | 0.83 |
| | | Green | Purple | 0.91 |
| *C. microcarpa* | 4 | Yellow | Blue | 0.69 |
| | | Green | Blue | 0.75 |
| | | Green | Blue | 0.82 |
| | | Yellow | Blue | 0.91 |
| *C. naragutensis* | 2 | Yellow | Blue | 0.83 |
| | | Green | Purple | 0.91 |
| *C. retusa* | 2 | Yellow | Blue | 0.79 |
| | | Green | Purple | 0.86 |

*The plate was air-dried, sprayed with Ehrlich reagent and heated at $95^o$C for 15 min

Traditional medicine as practice in Africa has the potential of providing treatment to many illnesses. However, the use of toxic plants such as the *Crotalaria* species as reported by Kone and Kande, (2012) Nuhu *et al.*, (2009a) gave a serious cause for concern especially where the plants are found growing without any restrain.

**Table 4: TLC analysis of the aerial parts of the four *Crotalaria* species**

| Specie | No. of spots | Colour of spot | | $R_f$-value |
|---|---|---|---|---|
| | | Daylight | After Spray* | |
| *C. lachnosema* | 1 | Yellow | Purple | 0.83 |
| *C. microcarpa* | 2 | Colourless | Blue | 0.69 |
| | | Colourless | Blue | 0.88 |
| *C. naragutensis* | 2 | Yellow | Blue | 0.83 |
| | | Yellow | Purple | 0.91 |
| *C. retusa* | 2 | Yellow | Blue | 0.79 |
| | | Green | Purple | 0.86 |

*The plate was air-dried, sprayed with Ehrlich reagent and heated at 95°C for 15 min

**A** **B**

**Plate I:** Colour change from the 'blank' (test tube A) and the 'test' sample (test tube B) for the specific PA test using Ehrlich reagent

**Conclusion**

Phytochemical screening tests on the four *Crotalaria* species showed that they all contain alkaloids in both the leaves and aerial parts extracts and the specific test for pyrrolizidine alkaloid using Ehrlich's confirm the alkaloids to be pyrrolizidine type. The TLC analysis of *C lachnosema* showed three purple spots in the leaves extract and one in the aerial parts; *C. microcarpa* showed four blue spots in the leaves and 2 in the aerial; whereas *C. naragutensis* and *C. retusa* showed two spots each in both leaves and aerial parts extracts.

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