PHARMACOGNOSY EVALUATION AND TOXICITY STUDIES OF THREE CROTALARIA SPECIES (LEGUMINOSAE)

BY

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FEBRUARY 1999

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BY

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A THESIS SUBMITTED TO THE POST GRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACOGNOSY

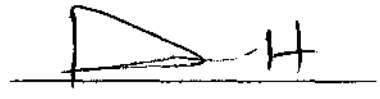
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FEBRUARY, 1999

DECLARATION

I hereby certify that the work reported in this thesis was carried out by me in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria, Nigeria. The works of other investigators are acknowledged and referred to accordingly.

I solemnly declare that no part of this thesis has been accepted in substance or concurrently submitted in candidature for any other degree.



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CERTIFICATION

This thesis entitled *PHARMACOGNOSTIC EVALUATION AND TOXICITY STUDIES OF THREE CROTALARIA SPECIES (LEGUMINOSAE)*, by Mrs Hadiza Nuhu meets the regulations governing the award of the degree of Doctor of Philosophy (Pharmacognosy) of Ahmadu Bello University, Zaria, Nigeria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I wish to dedicate this thesis to my brothers,

Alhaji Sani Nuhu Muhammad

and

Alhaji Salisu Nuhu Muhammad

May Allah shower His mercy on them, amin.

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ABSTRACT

The pharmacognostic evaluation of three species of the genus Crotalaria in Zaria is reported with special reference to their pyrrolizidine alkaloid contents and toxicity. The three species are Crotalaria microcarpa, Benth.; Crotalaria naraqutensis. Hutch.; and Crotalaria retusa, L.. Detailed study of their macroscopical and microscopica) features, physical constants, phytochemical and ethnomedical investigations in addition to toxicity studies were carried out on these species. Parameters for identification of these plants have been determined.

Alkaloids were detected in the aerial parts of all the three species except the leaves of C. naraqutensis. This confirms the classical toxonomical relationship between the species. Total alkaloid was determined by titrimetric method to be 0.17% and 0.06% for the leaves of C. retusa and C. microcarpa respectively. For the aerial parts, it was found to be 0.31% for C. retusa, 0.08% for C. microcarpa and 0.06% for C. naraqutensis. Other phytochemical constituents detected in the three Crotalaria species were flavonoids, tannins, carbohydrates and reducing sugars. Both anthraquinones and cardiac glycosides were detected in C. microcarpa only, cyanogenetic glycosides were detected in C naragutensis, while monosaccharides were detected in C. retusa only.

Calorimetric Mattocks tests revealed the presence of hepatotoxic pyrrolizidine alkaloid bases in the leaves and aerial parts of C. retusa only of the three species. This plant is therefore the only toxic species among the three investigated. The alkaloids of C. retusa were therefore tested for toxicity on rats and it proved to be toxic to all rats with marked microscopic lesions found principally in the liver, lungs, kidney and intestine. Toxicity pattern was typical of monocrotaline. The qualitative identification of these alkaloids by chromatography indicated that the toxic alkaloid, monocrotaline, was indeed present in C. retusa along with two other alkaloids. A total of four alkaloids

were also detected in C microcarpa and two in C naraqutensis aeriel parts. Extractive values of the Crotalaria species showed considerable differences especially for C. microcarpa while C. retusa and C. naraqutensis appear to be closely related. Alcohol soluble extractive value, acid insoluble ash, ash value and moisture content for C.microcarpa were found to be 15, 15, 20 and 5.6 respectively. While for C naraqutensis were determined to be 17.5, 7.5, 2.5 and 3. Values for C. retusa were 14, 12.5, 2 and 2.2.

Ethnomedical investigations of Crotalaria species was carried out in Zaria to determine available species, those utilised in ethnomedicine and for fodder and their recipes. Questionnaires were administered to herbalists, herdsmen, veterinary and medical doctors. The exercise revealed a general lack of awareness of the general toxicity of these species among the populace. Crotalaria poisoning was also found to occur in livestock. Suggestions are therefore presented for prevention. The study has also highlighted the importance of evaluating all Crotalaria species in order to distinguish toxic and non-toxic species of this genus and recommendations for further investigations have been made. Crotalaria species are indeed important species with significant pharmaceutical, medical, agricultural and economic implications.

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CHAPTER ONE



CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE SURVEY

1.1 Description of the Family Leguminosae

The Leguminosae is the second largest family of flowering plants (after the Compositae) (Trease and Evans, 1992). The family consists of 600 genera and 21,000 species. This family provides important drugs more than any other family of flowering plants does. Legumes are found in temperate regions, tropical regions, humid areas, arid savannah zones, highlands and lowlands; even aquatic legumes are known. They occur as shrubs, trees and herbaceous plants. They are worldwide important sources of protein. Most of them have the ability to fix atmospheric nitrogen in the soil with the help of nitrogen fixing bacteria present in their nodules. All leguminous species bear pods of different shapes and sizes. The pods split to release the seeds.

The family is divided into three subfamilies and each of the subfamilies is being considered by some workers [National Academy of Science Report, 1979] as a separate family. These are:

1. Caesalpiniodeae. (fam. *Caesalpiniaceae*)

This consists of about 2,800 species. They are mainly trees found in tropical savannah and forests of Africa, South America and Asia.

2. Mimosoideae (fam. *Mimosaceae*)

This subfamily consists of about 2,800 species. They are usually small trees and shrubs found in tropical and subtropical regions.

3. Papilionoideae (fam. *Faboideae or Papilionaceae*)

This consists of about 12,000 species. Many are herbs distributed worldwide. The Crotalaria species belong to this subfamily.

1.2 General Identity, Occurrence and Botanical Features of Crotalaria Species.

1.2.1 Botanical Identity.

Crotalaria species belong to the genus Crotalaria, family Leguminosae. The genus consists of about 600 species and Polhill [1982] divided it into 8 sections:

Section Crotalaria

Section Grandiflorae

Section Geniculatae

Section Dispermae

Section Schizostigma

Section Chrysocalycinae

Section Calycinae

Section Hedriocarpae

Each of the above sections is also divided into subsections.

1.2.2 Botanical Features and Occurrence

Trease and Evans [1992] reported 550 species of Crotalaria. Polhill [1982], on the other hand, reported about 600 species. The Crotalaria species occur in both the tropics and subtropics, particularly in the Southern hemisphere. They are most numerous in Eastern and Southern tropical Africa. The genus is the largest in tropical Africa and about 500 species have been recorded [Polhill 1982]. The species are commonly seen in open places from high mountains to semi-deserts. They appear to be variable, appearing in succession through the season. They are quite diverse and especially numerous, hence new reports of Crotalaria species especially in remote areas may be expected and this would increase the existing number.

The exact number of Crotalaria species found in Nigeria is not yet known. However, Polhill, [1982], in his studies of Crotalaria in Africa and Madagascar, noted that there might be between 10-15 species and that the species population is highest within Nigeria around Zaria, Kaduna, Bauchi and Jos areas. He noted the existence of least number of the species along riverine areas see (Figures 1 & 2).

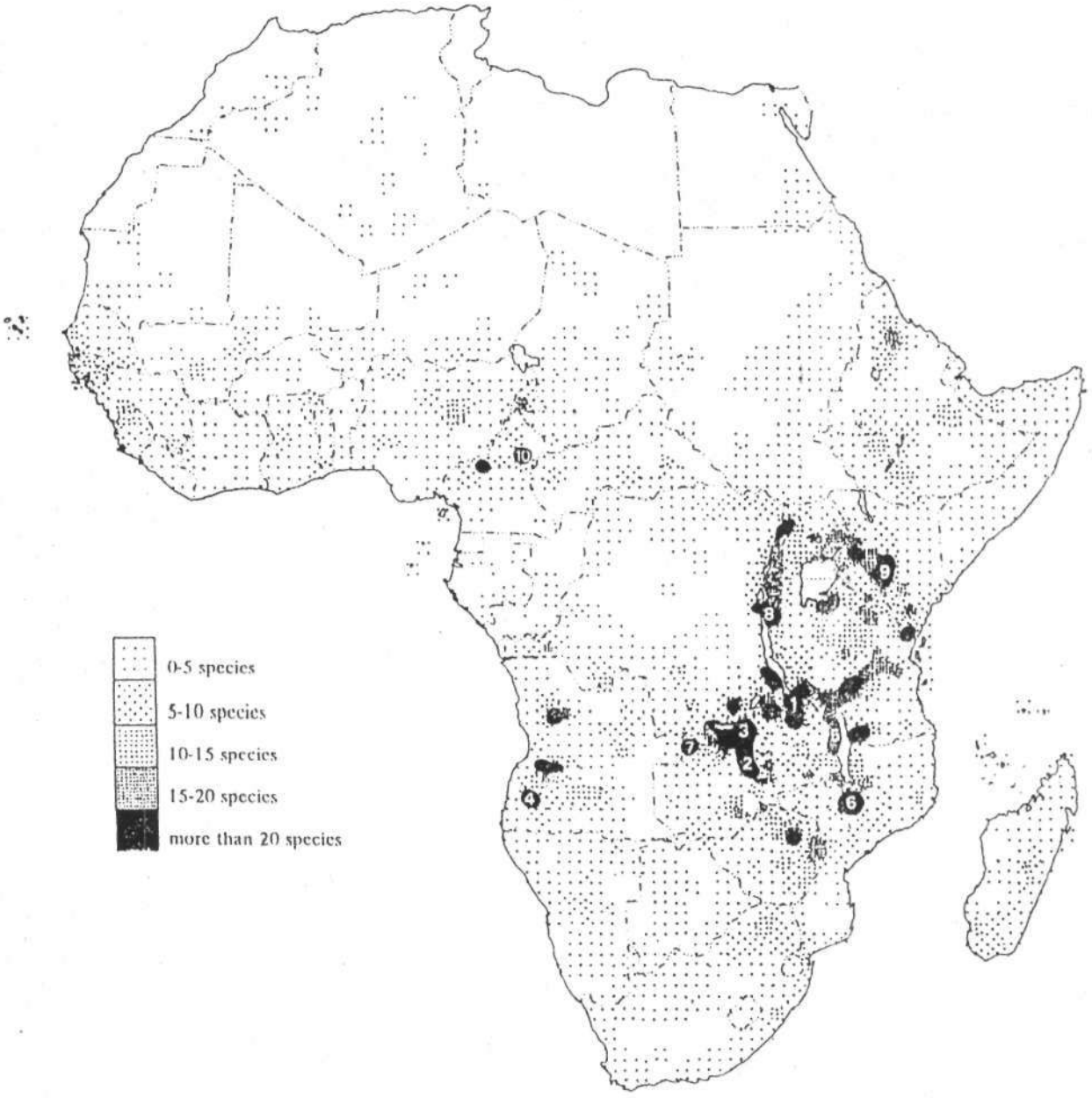


FIGURE 1 : SPECIES DENSITY OF CROTALARIA IN AFRICA.

. Highest figures: 1, Mbala 75 species; 2, Ndola 47; 3, Kundelungu 43; 4, Huila 39; 5, Nyika 35; 6, Shire Highlands 32; 7, Mwinilunga 31; 8, Usumbura 26; 9, Kenya Highlands 23; 10, Ngaoundéré 21.

(Adapted from Polhill, 1982)

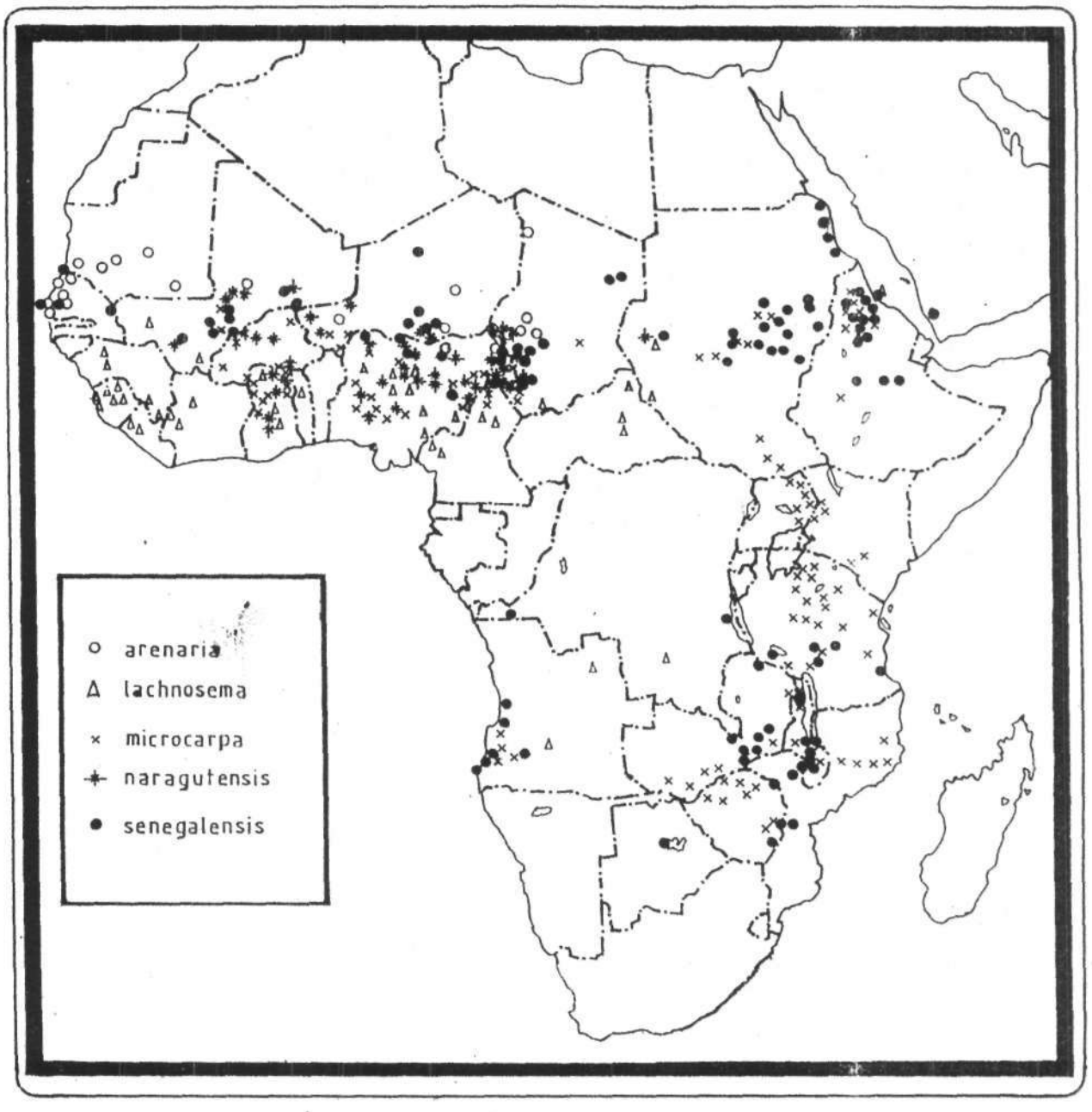


FIGURE 2: OCCURRENCE OF SOME CROTALARIA SPECIES IN PARTS OF AFRICA (Adapted from Polhill, 1982)

General Features

Crotalaria species are herbs or shrubs, mostly annuals though many are perennials (Polhill, 1982)..

Leaves:- Leaves are simple with one foliate or odd numbered (3 to 7) foliate, usually petiolate. Stipules are filiform to foliaceous or absent.

Flowers: usually yellow, less often white or blue, occur usually terminal (axillary) or in fewer cases when leaflets are sessile, flowers are solitary. Bract and paired bracteoles usually present, variably developed. Calyx usually with 5 subsequal lobes, sometimes lower 3 on a short lip or 2 - lip with long lobes, occasionally 4 lobes or lateral and upper lobe joined on either side. Standard, almost with 2 appendages at base inside, glabrous or hairy outside. Keel rounded to angled, generally produced into a well developed beek. Stamens all joined in a sheath open at base, anthers alternately long, basifixed and smaller dorsifixed. Style is curved or geniculate with thicker base - almost always with 1 to 2 lines of hairs on upper part. Stigma, small, rarely bilobed.

Fruit: Subsessile to long-stipitate usually inflated, usually dehiscent, sometimes tardily dehiscent, one or more seeds.

Seeds: usually oblique-cordiform to oblong-reniform. Members of the genus Crotalaria are closely similar to members of the genus Lebeckia Thumb (a genus of about 40 species) and Aspalathus L, Rafnia Thumb and Wiborgia Thumb. These species are, however, more restricted to the Cape and Southern Africa. Crotalaria species could easily be distinguished from these by the hairs on the

upper part of the style which has a thicker lower part with anthers clearly differentiated into 5 dorsifixed which alternate with 5 long basifixed ones. In the other genera the carinal one tends to be intermediate in form.

Crotalaria species could also be recognised by their beaked and inflated legume. The following are examples of individual features of some Crotalaria species.

Botanical features of some Crotalaria species.

1. C. lachnosema, Stapt.

This species belongs to the section Chrysocalycinae, found generally in West Africa especially Nigeria, Liberia, Sierra Leone with isolated records in Angola and Zaire.

It is a bushy-woody herb or small shrub, with dense hair on all parts; about 0.7 - 2 (-3) m tall, tomentose with golden - greyish spreading hairs. Leaves all 3-foliolate or rarely the upper most is 1-foliolate, leaflets are oblong-narrowly oblong - elliptic in shape, rounded or truncate and apiculate, size 5-8cm (-10) x 1.6- 3.4 (-4) cm. Sericeous on both surfaces though more densely so on lower part. Short petiole, narrow stipules, 3-7mm long. Bracts attenuate - lanceolate, -6-12 (-18) mm long; bracteoles on the pedicel.

Calyx 1.4 -1.5 (-2) cm long, lobes are attenuate-triangular 2-3 times as long as the tube. Standard is subcircular, yellow sericeous outside, wings broadly oblong, a little shorter than the keel. Keel is rounded, shortly and bluntly

beaked, 2 - 2. 3cm (-2.6) long with dense indumentum on lateral surfaces. Legume is subsessile, cylindrical 2.5-3.5cm long with a dense furry tomentum, of slightly spreading hairs containing 26 - 30 seeds. Seeds are obliquely oblong - cordiform, 3 . 5 - 4mm long, Smooth to minutely granulate, colour ochre to shiny brown.

2. C. microcarpa, Benth.

This species belongs to section Geniculatae and is widespread over most of the Sudanian and Zambezian savanna regions of Africa, excluding the extreme west & south. Occurs in variety of habitats but generally in open places, on poor soils, in rocky and sandy sites. C.microcarpa is a much branched spreading annual or a short-lived perennial, 10-70cm tall, covered with long appressed or spreading hairs. Leaves have a short petiole, 3-foliolate. Leaflets are narrowly lanceolate-oblanceolate. Mostly 10-30 x 4–8 mm size. 1-2 mm long, stipules, setaceaous, 1-2mm long. Flowers few to numerous in heads or short dense, rarely lax racemes on a slender well developed peduncle, Bracts linear or linear - lanceolate, 1.5-3.5mm long, bracteoles inserted at base of calyx, setaceous, mostly 1 - 2mm long. Calyx 2.5 - 3.5 mm long, pilose; lobes triangular, may be as long as a tube. Standard elliptic - obovate, yellow, usually marked (or flushed), reddish; pubescent outside; wings may be as long as keel; keel angled in lower part, with a straight or slightly recurved narrow beak 4-5-7.5mm long. legume sessile, ellipsoid - oblong or conoid-oblong 4 - 7.5 x 2 - 4mm, pubescent, 8 - 12 seeded. Seeds oblique - cordiform, 1 - 1.2 mm long, smooth, brown.

3. C. macrocalyx, Benth.

This species belongs to section Chrysocalycinae, it is an annual or a short lived perennial, creeping, straggling or erect upto 30 - 90cm. Branches covered with long appressed hairs. Leaves shortly petiolate, all 3 - foliolate or in few cases upper are 1 - foliolate. Leaflets lanceolate or narrowly oblong - narrowly elliptic, mostly 3-8 x (0.5) 1-2cm, appressed, pilose beneath. Stipules linear to lanceolate, may be caudate, 3-6 (-8) mm long. Racemes short, dense subsessile, bracts linear - lanceolate caudate, 8 - 12m 10ng, very short pedicel. Bractioles basally fused to calyx tube, the free part directed forward, similar to the bracts. A deeply divided calyx that is as long as the keel, covered with long hairs, standard is subcircular or oblate, yellow, maturing orange, yellow, with several purplish lines shortly radiating from the throat. Wings as long as keel, keel is angled with a straight beek, slightly incurved at tip, 1 - 1.1 cm long, glabrous. Legume enveloped by the calyx, subsessile ellipsoid, 0.8 - 1.5 cm long with a short dense brown or purplish brown tomentum, 4 -6 (-8) seeded. Seeds broadly reniform to subcircular about 3mm across, rugulose, terra-cotta. C. macrocalyx is found in severel regions from Senegal - Chad and Cameroun.

4. C. senegalensis, Hutch.

It belongs to section Crotalaria, annual, erect and spreading, (0.1) 0.3 - 1m tall, branches appressed or subappressed pubescent. Leaves 3 - foliolate, leaflets mostly elliptic oblong varying from lanceolate or oblanceolate to elliptic 1.5-6.5 x

0.4-2.5cm appressed, pubescent beneath, petiole 1.5cm long. Stipules linear subulate, 1-3mm long. Racemes (5-) 12-45cm long, laxly (8-) 16-40 flowered, buds ascending; bracts linear - subulate to linear lanceolate 1-3.5mm long; bracteoles on calyx, 1-3mm long. Calyx 5-7 (-8)mm long, standard broadly elliptic to oblate, bright yellow, often finely lined and ageing reddish, pubescent outside. Wings oblong obovate, nearly as long as the keel, keel is angular. Legume is shortly stipitate, oblong ellipsoid, 10-17 x 5-7 (-8)mm, pubescent 6-16 seeds. Seeds are oblique - cordiform, 2.5-3mm long, smooth yellowish to brown sometimes mottled darker. Occurs from Senegal and Cape Verde to the Red Sea between 10 - 17°N - 22°N, and also found in Ethiopia, Tanzania, Zambia and Malawi. It is usually encountered in wooded, open grassland usually on sand and frequently in disturbed places.

5. C. arenaria, Benth.

It belongs to section Calycinae. A prostrate perennial herb, rarely shrub, 0.5m high. Stems, 30-50cm long, densely subappressed spreading, pubescent, with some longer more spreadly hairs. Leaves simple blade, ovate - elliptic, 0.7-2 x 0.5 - 1.1cm, rounded to the recurved tip, rounded to cordate at base, villous on both surfaces. Stipules absent. Racemes 5 - 12cm long, very laxly, (4-) 6 - 16 - flowered; bracts shortly stalked, bracteoles at base or below calyx. Calyx deeply divided into 2 -lips, 5.5 - 7mm long, villous, 10bes triangular-lanceolate, 3-4 x as long as tube, standard broadly elliptic - obovate, yellow, probably brownish lined. Wings nearly as long as keel, keel is subangular with a straight beak, 6mm long.

Legume subsessile, oblong - ellipsoid, 1 - 1.4 x 0.6 - 0.8cm, pubescent and with longer spreading hairs. (6-) 8-12 seeds. Seeds plump - oblique - cordi form, smooth, greyish green - brown, 2-5-3mm long. It is found in sandy places.

6. C. retusa, L.

An erect annual or a short - lived perennial, 0.5 1.5m tall. Well branched, stem is slightly ribbed, sub-densely covered with short hairs. Leaves simple; blade oblanceolate to oblong-obovate, 3.5-11 x 1.5-4cm, usually rounded or emarginate at the apex, rarely obtuse, finely appressed pubescent beneath; petiole ? 2 - 4mm long. Stipules 1 - 5mm long, linear or subulate. Many - flowered; bracts ? 2 - 6mm long, subulate - caudate, to lanceolate. Bracteoles on the pedicel ? 1 - 2mm long, calyx 1.1 - 1.4cm long, upper lobes broadly lanceolate - triangular, longer than the tube standard obovate - circular, pale yellow, often finely purplish veined inside and reddish purple diffused outside with darker veins. Usually with a few hairs along the midvein outside wings oblong-obovate, longer than the keel, bright yellow, keel rounded, with a rather short slightly incurved twisted beek, 1.3 - 1.5cm long. Legume shortly stipitate (3-) 4 - 5 x 1 - 1.8cm, glabrous, ? 12 - 20 seeds. Seeds oblique - cordiform with the narrow end, strongly incurved, ? 5 - 5cm long, finely papillose, yellowish or brown.

It is pantropical widely distributed as a fibre plant, readily naturalised. Most common around coasts of tropical Africa and the offshore islands, also in easten Africa. C. retusa has two varieties. Variety tunguensis seems to occur more in undisturbed places than retusa,this could also be easily recognised by its racemes

which are short and dense in flower, branches with spreading straight or slightly crisped hairs, calyx is subglabrous with hairs only at base of tube, whereas variety retusa has more pointed leaves, racemes long, flowers closely arranged only towards the top, branches with short appressed hairs, calyx thinly but uniformly puberulous.

7. C naragutensis, Hutch.

An erect, well branched, robust perennial herb, leaves 0.4 - 2- 5 cm tall. 3 - foliolate, leaflets are obovate-elliptic 3-7 (-10) x 1.5 - 4 (-6.5)cm, petioles ? as leaflets or shorter. Stipules are very small or none.

Racemes shortly pedunculate 13 - 4 5cm long with many flowers closely arranged. Calyx basically truncate and deflexed against pedicel, 5 - 7mm long, densely covered with short hairs, lobes are narrow. Standard are broadly elliptic or obovate, yellow lined with purple outside. Wings are shorter than the keel. Keel is rounded 9 - 12mm long, yellow, sometimes with a purplish brown, marked on either side or distally. Legume is shortly stipitate, subcylindrical, broadened upwards 1.8 - 2 . 5 x 0.6 - 0.7cm appressed, about 8 - 12 seeded. Seeds are oblique - cordiform, smooth ochre or pale brown C. naragutensis occurs from Mali to Ghana and also most abundantly in Nigeria. It occurs in open & wooded grasses on sandy soil or on rocky outcrops.

The features and characters of some Crotalaria species are shown in Figure 3. The places where these species could be found in Africa are shown in Figure 2 [Polhill, 1982].



[a] *Crotalaria macrocalyx*. 1, flowering branch, x ⅔; 2, flower, x 1½; 3, calyx, opened out, x 1½; 4 standard, x 1½; 5, wing, x 1½; 6, keel, x 1½; 7, pistil, x 2; 8, fruit, x 1; 9, legume, separated from calyx, x 1½; 10, seed, x 4½.



[b] *Crotalaria retusa* var. *tunguensis*. 1, habit, x ⅓; 2, flower, x 1; 3, calyx, opened out, x 1; 4, standard, x 1; 5, wing, x 1⅓; 6, keel, x 1½; 7, anthers, x 7½; 8, pistil, x 1½; 9, fruits, x ⅓; 10, seed, x 3

FIGURE 3: SOME NIGERIAN CROTALARIA SPECIES SHOWING THEIR VARIOUS PARTS AND FEATURES. (Adapted from Polhill, 1982)



[c] *Crotalaria ochroleuca*. 1, habit, x ½; 2, flower, x 1½; 3, standard, x 1½; 4, wing, x 1½; 5, keel, x 1½; 6, pistil, x 1½; 7, seed, x 5



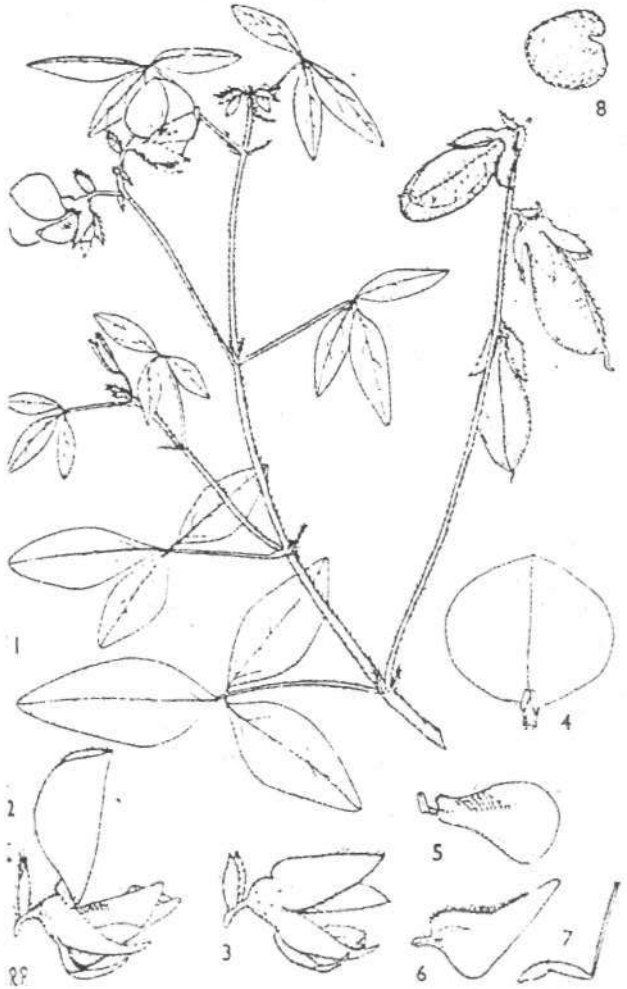
[d] *Crotalaria graminicola*. 1, habit, x ¼; 2, standard, x 3; 3, wing, x 3; 4, keel, x 3; 5, stamens, spread out, x 3; 6, calyx and pistil, x 3; 7, pistil, x 3; 8, tip of style, x 4½; 9, infructescence, x 1½; 10, legume, opened to show seeds, x 3; 11, seed, x 6

FIGURE 3 [CONTINUED]



Crotalaria glauca. A, habit, x ½; B, unopened flower, x 2½; C, standard, x 2; D, wing, x 2; E, keel, x 2; F, section of pistil, x 4; G, legume, x 1; H, seed, x 5

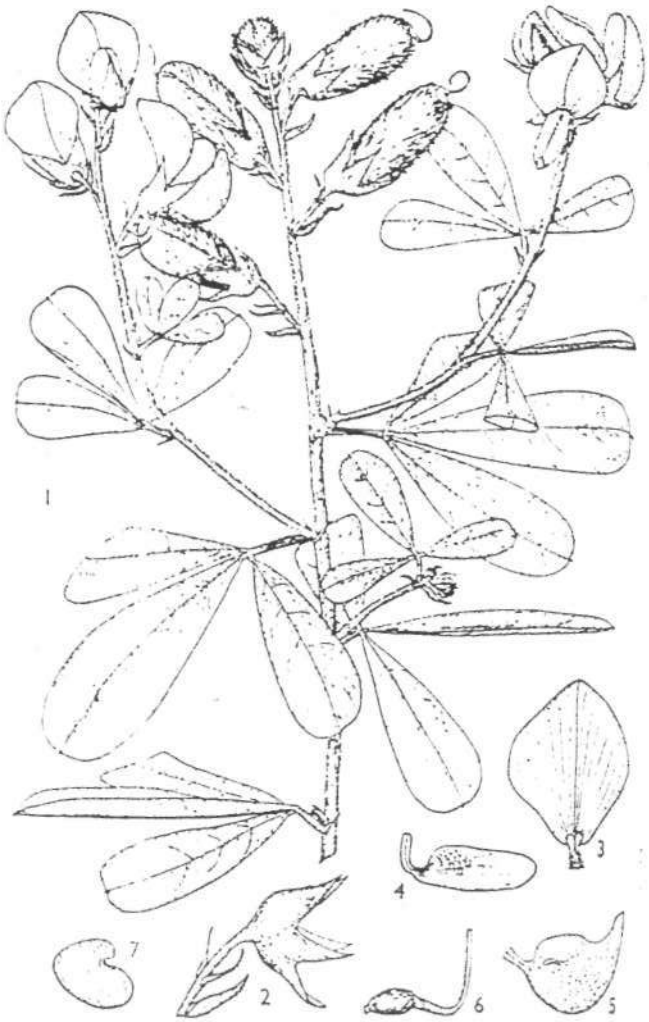
[e]



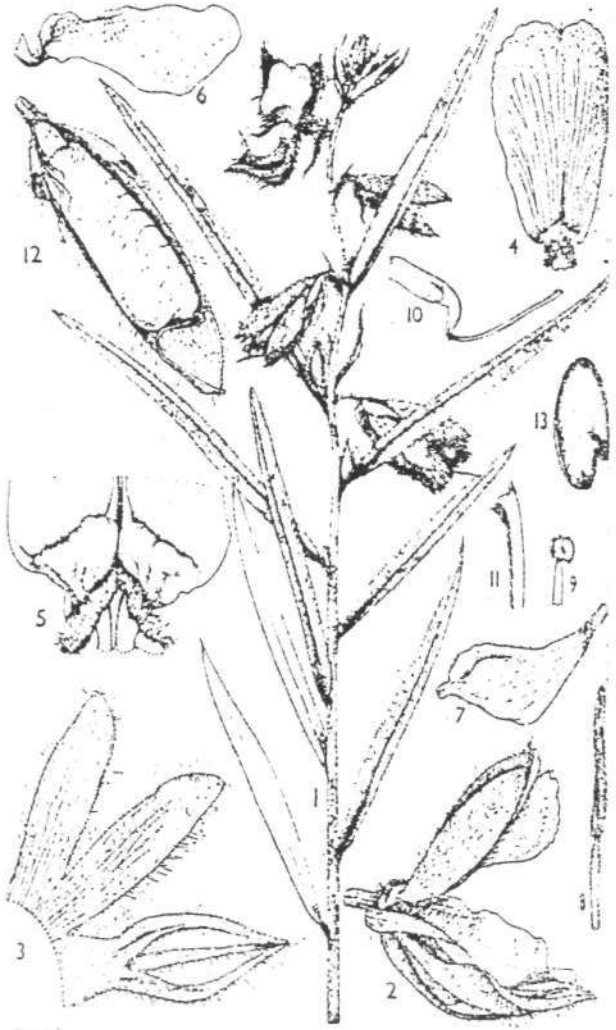
Crotalaria harkae subsp. *harkae*. 1, habit, x ½; 2, flower, x 1½; 3, calyx, x 1½; 4, standard, x 1½; 5, wing, x 1½; 6, keel, x 1½; 7, pistil, x 1½; 8, seed, x 2¼

[f]

FIGURE 3 [CONTINUED]



[g] *Crotalaria lachnosema*. 1, habit, x ½; 2, calyx, x 1; 3, standard, x 1; 4, wing, x 1; 5, keel, x 1; 6, pistil, x 1; 7, seed, x 2½.



[h] *Crotalaria calycina*. 1, habit, x ½; 2, flower, x 1¾; 3, calyx, opened out, x 1½; 4, standard, x 2; 5, detail of same, x 9; 6, wing, x 2¼; 7, keel, x 2; 8, 9, anthers, x 10; 10, pistil, x 1½; 11, tip of style, x 10; 12, fruit, with part of calyx cut away, x 1½; 13, seed, x 7

FIGURE 3 [CONTINUED]

1.2.3 Ecological, Geographical Distribution and Variations of Crotalaria Species.

The members of the genus Crotalaria have a wide climatic and soil tolerance,the genus displays a wide range of species abilities,suitable for different conditions and purposes such as vigorous early growth, good nodulation and reasonable pest resistance. Their main disadvantages are their potential toxicity to livestock, and liability to persist as weeds unless ploughed early. There are herbs and shrubs as well as annuals and perennials. Distribution pattern suggests geographical and ecological segregation. However, Boulter et al (1970) have established that size does not appear to be related in any obvious way to geographical or ecological factors.

1.2.4 Chromosomes and Breeding

Frahm-Leliveld in Boulter et al (1970) studied the karyo types of 57 mostly African species of Crotalaria. He noted a general feature and occurrence of 2n=16 except among members of section Chrysocalycinae and Incanae, where 2n=14 is usual, similarly both 2n=14 and also 16 was recorded for C.polysperma. There is no relationship between size of chromosome to geographical or ecological factors. Among African species C.massaiensis is the only reported tetraploid 4n=36 (2n = 18), though many American species are tetraploid. No work is reported on the breeding system of any African Crotalaria. However, a general pattern of variation suggests at least a modicum of outbreeding with a reasonable gene flow within populations.

There are also indications of self-compatibility (Morton 1972) which is higher in the tetraploids.

1.3 Importance of Pharmacognostic Research on Plants

The Plant Kingdom is made up of a large number of plants having different chemical compositions. However, not all plants are presently known to be medicinally useful. Even closely related plants, such as members of the same genus, may not all be recognised as official drugs. This, therefore, necessitates evaluation to determine correct identity, source, quality and purity of drug plants. Therefore pharmacognostic studies are very important for the identification of potential drugs among plants and correct identification of drug plants. The comparative pharmacognostic studies are based on the following characteristics of the drugs and determination of their various physico-chemical constants:

i) Gross morphology or macromorphological characters.

ii) Physical characteristics of the drug.

iii) Microscopical features.

iv) Chemical features.

v) Biological features.

The tests for determining the macromorphological characters use the organs of sense and they are generally called organoleptic tests.

The various features of a drug include its gross morphology, shape, size, texture, taste, colour and odour. By this, I mean organoleptic tests are more useful if crude drugs are in their whole form e.g. as fruits, leaves, etc. But once powdered then they loose some of their macro-features that are useful in evaluating them.

Similarly determination of various physical constants of a drug is important in evaluation of its identity and purity. These constants are important because, they are constant for a particular drug but vary from drug to drug. Therefore they serve as useful tools for identification of a drug in question. These parameters of natural drugs include extractive values, ash values and other physical constants.

Microscopical tests employ the microscope and its accessories in order to evaluate a drug. The microscope is indispensable in evaluating powdered drugs. Qualitative and quantitative microscopical tests are available. The former uses all qualitative characters such as presence of starch grains, calcium oxalate crystals, trichomes and their shapes and sizes. The latter uses some numerical microscopical constants such as stomatal number, stomatal index, vein islet number.

These parameters are constant for a particular drug but vary from drug to drug. Chemical tests are also very important in the identification of plant drugs. The chemical test is one of the most important in the proper identification of plants, because the plants are used as drugs because of their chemical constituents. Some of the qualitative chemical tests and quantitative chemical assays of the various chemical consituents of drugs are already available. For example, Vitali-Morin test for tropane alkaloids, Borntragers test for anthraquinone glycosides.

Biological tests are also used as a means of identifying plant drugs. It is used in determining the potency and extent of pharmacological and microbiological activity of the constituents of the drug. Many drugs such as antibiotics, toxins and vitamins are assayed and compared biologically.

In comparative pharmacognostic studies, plant drugs are compared on the basis of the above characteristics. It must be noted that for proper identification of a plant, using only one of these characteristics may not be sufficient to establish identity of a drug. Thus a combination of two to three different parameters may be necessary to establish the correct identity of a drug.

Among these five pharmacognostic features of plant identification, the chemical character is one of the most important, because the chemical nature of the constituents of plant drugs can be definately established by various chemical tests. The chemical parameter has thus received wide attention in comparative pharmacognostic studies. Many drug plants are now compared (comparative phytochemistry) and identified based on this character, i.e, their chemical constituents. This important aspect of comparative pharmacognostic studies is elaborated further below.

1.4 Comparative Phytochemistry and Pharmacognostic Evaluation.

Comparative phytochemistry simply means a method of comparison of plants based on the chemical nature of their constituents. It is the knowledge of the connection between the natural relationship of plant and their chemical composition. Comparative phytochemistry is a very important aspect of identification and classification of plant drugs.

The practice of comparative phytochemistry is very old. In the pre-Darwinian era, herbalists classified plants according to their virtues. McNair (1935) also applied comparative phytochemistry in his attempt to identify the angiosperms and to classify them. He used chemical characters such as alkaloids, glycosides, volatile oils as an aid to the taxonomy of this group of plants.

In the last thirty years, modern techniques of isolation, analysis and characterisation of substances have led to the chemical screening of many thousands of plant samples. This, coupled with renewed interest on the patterns of occurrence of compounds in plants, makes comparative phytochemistry a viable aid in plant identification and classification.

The plants produce a variety of chemical substances of different chemical nature. However, not all chemical compounds produced by plants are of equal interest. The secondary metabolites are the most important ones in comparative phytochemistry and pharmaceutical considerations. To the pharmacognosist, however, the active principles of plants deserve special attention, both because of their abundance in the plants and also due to their pharmacological or medicinal activity on the living system. In many cases,

the secondary metabolites are found to be the active principles. Other plant substances such as carbohydrates and fats are of dietetic importance, while gums. starch, calcium oxalate, lignin are also important in the identification of drugs and detection of adulterants in them (for quality and purity of drugs). (Trease and Evans 1992). Therefore plant drugs are often studied for their alkaloids, glycosides, volatile oils, etc., in comparative pharmacognostic studies.

However, chemical characters are only important in comparative pharmacognostic studies if they occur in significant quantities in the plant. Chemical constituents in trace amounts cannot certainly be taken as taxonomic or identification aids (Gibbs, 1963).

More recently comparative phytochemistry utilises even trace quantities of substance as an aid in taxonomy, especially in cases where the trace quantities make the difference between certain plants, (Bisby, 1973).

The quality of a drug is dependent on the quantity of its active principles. But even members of the same genus (i.e different species of same genus) have different quantities, spectrum or proportion of their constituents. This makes it necessary to evaluate and identify each species chemically. The use of various techniques of analysis is now employed in Natural Products research, namely chromatographic processes such as Gas-Liquid Chromatography (GLC), Gas Chromatography Mass Spectrometry (GCMS), High Performance Liquid Chromatography (HPLC), Paper Chromatography and Thin Layer Chromatography (TLC). Spectrometric analysis particularly Nuclear Magnetic Resonance Spectroscopy (NMR) is also extensively used in the analysis of compounds. X-ray crystallography has recently been accepted as the final solution to

problems of structural understanding of natural products (Chen, 1978). The most recent chromatographic technique, however, is the Liquid Chromatography Mass spectrometry (LCM) which was designed to overcome the limitations of GCMS and HPLC.

Comparative phytochemistry demonstrates the usefulness of the chemical character in the identification and classification of plants. It is also used in knowing the nature and relationship of different groups of plants. It has also served as an important tool to clarify some deadlocks in existing classifications of plants, For instance. Hegnauer (1955) made an extensive study of alkaloid distribution in the Solanaceae. He also recognised the potential significance of the chemical character of the group in their identification. Similarly, the comparative phytochemical study of the tropane alkaloids of the Solanaceae was done by Ghani (1976) to support the earlier classification of the family. Presently, comparative phytochemistry is extensively used in the identification and classification of plants. Two principal reasons contributed to this. The modern techniques of analysis which allow rapid and efficient screening of many plants, thereby making it possible to know the various constituents of plants. Secondly the chemical data have been found to be biologically important (Gibbs, 1963).

Members of the genus Crotalaria are quite numerous and owing to their potential toxicity and uses, it is necessary to properly distinguish each species as their level of toxicity would vary. The types and quantity of pyrrolizidine alkaloids which may be found in them would vary also with species and even geographical location. In fact, some species have been found to contain no pyrrolizidine alkaloids at all (e.g. members of family Orchidaceae), and as such are non-toxic. Pharmacognostic study would be a useful

tool in the proper identification of each species and its alkaloid contents. This would provide information on the Nigerian Crotalaria species for the purpose of comparison with same species grown elsewhere.

Pharmacognostic tests or analysis of drugs is very important in the search for new drugs, to cure existing ailments, or to provide alternatives to the present approach of treatment. The side effects of many drugs especially synthetic drugs are also an essential consideration in screening plants for their active constituents. Pharmacognostic evaluation provides the necessary information in considering plant species, phytochemical screening exposes the constituents of the plants, microscopical studies show the actual individual internal features of the plants for identification and quality control. Determination of physical constants is also principally for quality control. Pharmacognostic studies document important medicinal plants and provide important plant resources for the continuous medicinal needs of man. It also provides useful information in other sectors such as agriculture, medicine, chemical processes, economic botany and others.

1.5 Chemistry of Crotalaria Alkaloids

The most important chemical constituents of Crotalaria are the pyrrolizidine alkaloids (PAs). These and other pharmaceutically active principles suggest its many uses in folk medicine (Polhill, 1982). It is difficult to quote the exact number of pyrrolizidine alkaloids isolated to date from Crotalaria. More than fifty (50) have been isolated from some 45 species of Crotalaria (Mears and Mabry 1971, Kinghorn and Smolenski, 1981) and to date about 60 PAs have been detected. Pyrrolizidine alkaloids have infact been notable chemical markers for the genus Crotalaria among the genera of the Leguminosae as they have not been detected in other legumes apart from a few in Laburnum and Adenocarpus. In addition to the genus Crotalaria of the Leguminosae, families Boraginaceae (e.g Heliotropium, Trichodesma, Symphytum), Compositae (e.g Senecio, eupatorium) and Scrophulariaceae, (genus Castilleja) Ranunculaceae (Caltha), Apocynaceae (farnatha) all produce pyrrolizidine alkaloids. Table I shows the different families and genera that contain pyrrolizidine alkaloids [WHO, 1988].

There are variations as to the number and types of individual alkaloids found in parts of each Crotalaria species though it is unusual for a plant to contain a single PA. Large seeds in the section Grandiflorae and subsection Crotalaria have the highest recorded levels of accumulation of pyrrolizidine alkaloids and monocrotaline tend to be characteristic of that part of subsection which include the notably toxic species C. spectabilis and C.retusa.

TABLE 1: LIST OF GENERA CONTAINING TOXIC PYRROLIZIDINE ALKALOIDS (WITH NUMBER OF SPECIES INVESTIGATED). (W.H.O., 1988)

FAMILY	GENERA
1. Apocynaceae	Fernaldia (1), parsonsia (4)
2. Boraginaceae	Alkanna(1) Amsinckia(4), Anchusa(2), Asperugo(1), Borago(1), Caccinia(1), Cynoglossum(9), Echium(3), Hackelia(1), Heliotropium(25), Lappula(2), Lindelofia(7), Lithosperum(1), Macrotamia(1), Messerschmidtia(1), Myosotis (2), Paracaryum(1), Paracynoglossum(1), Rindera(5), Solennanthus(4), Symthytum(7), Tournefortia(2), Trachelanthus (2), Trachodesma(2), Ulugbekia (1).
3. Compositae	Adenostyles(3), Brachyglottis (1), Cacalia(4), Conoclinium (1), Crassocepharlum(1), Doronicum(2), Echinacea(2), Emilia (2), Erechites (1), Eupatorium (8), Farfugium (1), Gynura (2), Ligulavia (5), Petasites (4), Senecio (142), Syneilesis (1), Tussilgo (1).
4. Leguminosae	Crotalaria (60).
5. Ranunculaceae	Caltha (2).
6. Scrophulariaceae	Castilleja (1)

Pilbeam and Bell (1979) have studied the alkaloid levels for seeds of 85 African species and also surveyed 163 species for non-protein amino acids which when accumulated may have varied roles. They can serve as nitrogen reserves, alkaloid precursors or toxins. The distribution of amino acids shows a systematic pattern in the sections of the genus Crotalaria. Macrostachyae have a relatively low level of toxicity and amino acids accumulation whereas section Grandiflorae tends to have a high accumulation of both alkaloids and non protein amino acids. Broadly speaking there is a tendency for toxic amino acids to accumulate in seeds of groups with relatively unspecialized flowers. In general, Boulter et al (1970) shows that there are indications that proteins may provide distinctive systematic patterns which can be useful in the taxonomy of the genus. Table 2 shows the various individual pyrrolizidine alkaloids found in parts of Crotalaria species.

The pyrrolizidine alkaloids occur as free bases and as their N-oxides in the plant. Several basic alkaloids are present, which may or may not be closely related. These are often accompanied by N-oxides which may or may not be those of the alkaloids. Ratio of alkaloid bases to N - oxides may vary between different parts of the plant and with season e.g in C. retusa basic alkaloids accumulate in the seeds, while N-oxides accumulate in the green parts (Mattocks, 1971).

TABLE 2: PYRROLIZIDINE ALKALOIDS FOUND IN PARTS OF CROTALARIA SPECIES. (Compiled from various sources notably W.H.O. 1988)

Crotalaria Species	Constitutent Alkaloids	Plant Part
1. Crotalaria aegyptica, Benth.	Monocrotaline Crosemperine 7β-hydroxy-1-methylene-8β-pyrrolizidine	Ae Ae
2. C. agatiflora, Schweinf.	madurensine anacrotine 7-acetylmadurensine 6-acetylanacrotine 7-acetyl-cis-madurensine 6-acetyl-trans-anacrotine	Ae
3. C. anagyriodes, Humb. et al	1-methylenepyrrolizidine senecionine anacrotine	Sd Sd Sd
4. C. assamica, Benth.	Monocrotaline	Sd
5. C. axillarris, Ait.	Axillarine Axillaridine	Sd
6. C. barbata, R. Graham ex. Wight et Walk-Arn.	Crobarbatine	Sd
7. C. brevifolia	Integerrimine Usaramine	Sd Sd
8. C. burhia, Buch – Ham.	Crotalarine (croburhine) Monocrotaline	Ae
9. C. crassipes, Hook. (see C. novae-hollandiae, DC. Subsp. Novae-hollandiae)	Retusamine Monocrotaline Fulvine	Wh
10. C.crispata F. mueil. Ex Berth.	Monocrotaline crispatine	Wh

Table 2 Continued

11. C. dura, J.M. Wood et Evans.	dicrotaline	Wh
12. C. fulva, Roxb.	fulvine	Wh
13. C. globifera, E. Mey.	dicrotaline, globiferine, grantaline	Ae Ae
14. C. grahamiana, R. Wight et Walk-Arn.	monocrotaline grahamine monocrotalinine	Sd Sd Wh
15. C. grantiana, Harvey (C. virgulata)	grantianine grantaline l-hydroxymethyl-1β,2β-2ß-epoxypyrrolizidine	Sd Sd Sd
16. C. incana, L.	Integerrimine anacrotine usaramine	Sd Ae Sd
17. C. intermedia, Kotschy	Integerrimine Usaramine	Sd
18. C. juncea, L.	Senecionine Seneciphylline Riddelline Trichodesmine, Junceine	Sd Sd
19. C. laburnifolia, L.	Anacrotine (crotalaburnine) 1-methylenepyrrolizidine	Sd
20. C. laburnifolia, L. subsp. Eldomae.	madurensine anacrotine senkirkine hydroxysenkirkine crotatoline Crotaleschenine	Ae
21. C. leschenaultii	Monocrotaline Crispatine	Sd Sd
22. C. leiloba, Barth. (syn. C. ferruginea, Wall)	Monocrotaline	Ae
23. C. madurensis, R. Wight.	Madurensine	Sd

Table 2 Continued

	Crispatine Fulvine Cromadurine Isocromadurine	Sd Wh
24. C. mitchelli, Benth.	Monocrotaline Retusamine	Sd Sd
25. C. mucronata, Desv. (see C. pallida, Ait.)	Integerrimine Usaramine	Wh
26. C. mysorensis, Roth.	Monocrotaline	Sd
27. C. nana, Burm.	Crotananine Cronaburmine	Sd
28. C. novae-hollandia, DC. subsp. Lasiophylla (Benth) A. Lee....	Monocrotaline Retusamine	Sd Sd
29. C. novaehollandiae DC. subsp. Novae-hollandiae	Retusamine Usaramine Integerrimine	Sd Sd
30. C. pallida Ait. (syn. C. mucronata Desv., striata DC.)	Nilgirine Mucronatine	Wh Sd
31. C. paniculata Willd		
32. C. quinquefolia L	Crotastriatine Fulvine Monocrotaline	Sd Sd
33. C. recta Steud. ex A Rich.....	Monocrotaline Trichodesmine	Sd
34. C. retusa L.	Monocrotaline Retusine Retusamine Retronecine	Sd Sd Sd Sd, Ae

Table 2 Continued

35. C. rubiginosa Willd. (C. wightiana R. Graham ex R. Wight et Walk.-Arn.) ...	Junceine Trichodesmine	Sd
36. C. sagittalis L.	Monocrotaline	Sd
37. C. semiperflorens Vent.	Crosemperine	Sd
38. C. sericea Retz.	Monocrotaline Spectabiline	Sd
39. C. spartiodes DC.	Retrorsine	Ae
40. C. spectabilis Roth. (C. sericae Retz)	Monocrotaline Spectabiline Retusine	Sd Sd,wh Sd
41. C. stipularia Desv.	Monocrotaline	Sd
42. C. tetragona Roxb.	Integerrimine Trichodesmine	Sd
43. C. usaramoensis E. G. Baker.	Integerrimine Usaramine Senecionine Retrorsine	Sd Sd
44. C. verrucosa L.	1-methylenepyrrolizidine anacrotine	Sd Sd
45. C. walkeri Arnott.	crotaverrine acetylcrotaverrine	Sd
46. C. sp aff., Mitchelli.	retusamine	Wh
47. C. albida Heyne ex Roth. (syn. C. montana Roxb.)	croalbidine 1-methyloxymethl-1,2-dehydro- pyrrolizidine,	Ae Ae

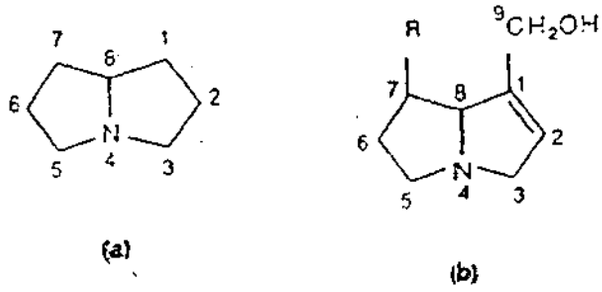
Table 2 Continued

48.	C. aridicola Domin.	7β-hydroxy-1-methoxy-methyl-1,2-dehydro-pyrrolizidine 7β-acetoxy-1-methoxymethyl-1,2-dehydropyrrolizidine isocromadurine, crispatine	Wh
49.	C. candicans W. and A.	crocandine, turneforcidine isocrocandine, cropodine	Sd
50.	C. damarensis Engl.	1-methylenepyrrolizidine	Wh,sd
51.	C. goreensis Guillem et Perrott.	7β-hydroxy-1-methylene-8β-pyrrolizidine, 7β-hydroxy-1-methylene-8α-pyrrolizidine,	Ae,sd
52.	C. grandistipulata Harms	1-methylenepyrrolizidine	Sd
53.	C. lachnophora A. Rich.	1-methylenepyrrolizidine	Sd
54.	C. maypurensis humb. et al.	7β-hydroxy-1-1-methylene-8β-pyrrolizidine 7β-hydroxy-1-methylene-8α-pyrrolizidine 1-methyoxymethyl-1,2-dehydro-8α-pyrrolizidine	Ae,sd
55.	C. medicaginea lamk.	7β-hydroxy-1-methoxymethyl-1,2-dehydro-8α-pyrrolizidine 1α-methoxymethyl-1β,2β-epoxy-8α-pyrrolizidine.	Wh,sd

Table 2 Continued

56.	C. natalitia Meissn.	1-methylenepyrrolizidine	sd
57.	C. podocarpa DC.	7β-hydroxy-1-methylene-8β-pyrrolizidine	sd
58.	C. rhodeside.	1-methylenepyrrolizidine	sd
59.	C. stolzii (Baf. F.) Milne Redh.	1-methylenepyrrolizidine	sd
60.	C. scassellatii chiov.	Axillarine Deoxyaxillarine	sd
61.	C. virgulata subsp. Grantiane.	Grantaline Grantianine	Wh
62.	C. zanzibarica Benth (C. usaramoensis).	Integerrimine Senecionine Usaramine	Wh
63.	C. cephalotes steud. Ex. A.Rich.	Retrorsine	Sd
64.	C. cunnighamii R.Br.	Monocrotaline	Sd
65.	C. nitens Kunth.	Monocrotaline	Sd
66.	C. paulina Schrank	Monocrotaline	Sd
67.	C. lunata Beddome expolhill.	Monocrotaline Trichodesmine	Sd
68.	C. lachnosema stapf.	Dicrotaline Acetyldicrotaline	L
69.	C. naragutensis Hutch	Nilgirine Integerrimine Usaramine acetyl integerrimine	Sd

KEY: **Ae -** ***aerial parts,*** **Sd -** ***seed*** **, Wh -** ***whole parts, L - leaf***



(A) BASIC STRUCTURE (B) 1-HYDROXYMETHYL -1:2-DEHYDROPYRROLIZIDINE

FIGURE 4: STRUCTURE OF PYRROLIZIDINE MOLECULE.

The alkaloid contents in most species vary from 0.1 g/kg dry weight to 40g/kg dry weight. A few others have higher amounts with a maximum of 180g/kg found in Senecio riddellis. Most hepatotoxic pyrollizidine alkaloids are esters of the molecule similar to those shown in Figure 4 (above) with the ring nucleus containing a double bond at the 1:2 position. However, a few are esters of the amino alcohol otonecine (e.g petasitenine). Hence the hepatotoxic pyrrolizidine alkaloids have 1,2-double bonds in the pyrrolizidine ring and branched chain acids esterifying a 9-hydroxyl and preferrably also the 7 -hydroxyl substituent. Similarly modified secondary pyrrolizidine alkaloids in which the central bond between the N and C8 atoms is broken are also hepatotoxic. Some Senecio species also contain non-basic derivatives that are 5-oxopyrroles. The toxicity of these has not been investigated though it is thought to be similar to that of the Senecio alkaloids. The unsaturated pyrrolizidine nucleus itself is not toxic but esters of branched-chain acids are. Some esters have an open molecular structure e.g heliotrine, whereas others are macrocyclic, e.g monocrotaline and retrorsine. The structures of some of these pyrrolizidine alkaloids are shown in Appendix I.

Toxic pyrrolic intermediates are formed by some alkaloids by the mixed - function oxidases of the hepatocytes of the liver. In this way the orally ingested N-oxides acquire their toxicity. The structure of the toxic pyrrolic intermediate is shown below. Both the substitution at the α-position of the acid and esterification of the C - 7 hydroxy group enhance the toxicity of the alkaloid (Robins, 1982).

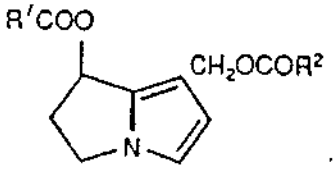


FIGURE 5: MOLECULAR STRUCTURE OF TOXIC PYRROLIC INTERMEDIATES.

Not much work is reported on the percentage of total alkaloids in the different parts of the Crotalaria species. However, in plants containing pyrrolizidine alkaloids it has been established that the alkaloid content in different parts of the plant (e.g roots, leaves, stalks, flowers, and buds) varies and is subject to fluctuations according to the climate, soil conditions (hence locality) and time of harvesting. (Danninger et al 1983, Hartmann and Zimmer, 1986). In related aspects it has been shown that there are several ways in which pyrrolizidine alkaloids might be altered during isolation procedures. Alkaloids may be changed by heat or by enzymic action when harvested plants are dried and stored. Bull et al (1968) reported the loss of 50 - 80% of alkaloids

during drying of some but not all of Crotalaria and Heliotropium species. On the other hand, Pendersen (1975) compared the alkaloid content of fresh leaves with that of dried leaves from several species of the Boraginaceae. Allowing for the loss in weight on drying, there were no changes in total alkaloid content, but changes were observed in the ratio of free bases to N-oxides, suggesting that enzymic oxidation or reduction of alkaloids could take place during the period. It has also been found among plants containing pyrrolizidine alkaloids, those with more N-oxides are less bitter to taste (Bull et al, 1968). Similarly pyrrolizidine alkaloids containing plants growing in warmer climates have been found to contain higher alkaloid levels than the same species grown in colder climates [WHO, 1988].

1.6 Pharmacological Properties and Uses of Crotalaria and its Alkaloids.

Crotalaria species have definite pharmacological actions due to their pyrrolizidine alkaloids content. These are encountered mainly through human and animal consumption of the plant.

1.6.1 **Pharmacological Action of Pyrrolizidine Alkaloids.**

Pyrrolizidine alkaloids (PAs) are noted mainly for the poisoning of livestock as a result of grazing on Pyrrolizidine Alkaloids - containing plants. The first noted cases

of Pyrrolizidine poisoning were described in Cattle in 1903 (Gilruth, 1903). Since then numerous reports on animals and man have been documented. In case of livestock poisoning, it is characterised by inflamation of horn-feeding membrane of the hooves causing deformity, lameness and occasional starvation (lamitic crotalism), these are especially attributed to species of subsection Incanae (e.g C. barkae, C. incana, C. polysperma). The more lethal form of crotalism in animals affects the nervous systems, lungs and liver. The principal action is the occlusion of smaller hepatic veins by subendothelial swelling. This is especially the case in species like C. retusa, C. spectabilis and C. berteroana (Watt & Breyer - Bradwijk, 1962, Verd-Court and Trump 1969).

In fact episodes of livestock poisoning and even large-scale outbreaks have been recorded from most parts of the world (Mohabbat, et al, 1976), in both temperate and cold climates (see Table3). In PAs poisoning, the liver is the principal target organ (Mattocks, 1971b, Schoentel and Magee, 1957, Clarke and Myra, 1985). Studies carried out on a wide variety of farm and laboratory animals have shown common features of toxicity with some species variations. In small laboratory animals an-almost lethal doses produce zonal haemorrhagic necrosis in the liver lobule, within 12-48hr of administration of PAs. In non-human primates (rats, chicken and swine), however, changes begin to occur after a short period of ingestion of the plant or alkaloid. This later becomes organised, in the subintima of the central or sublobular veins in the liver resulting in their occlusion. The reticulin framework in the central zone of the lobule collapses following necrosis leading to scarring. Repeated administration of suitable doses leads to chronic

liver lesion characterized by megalocytosis and increasing fibrosis, which may result in cirrhosis. Chronic liver disease including cirrhosis has been shown to develop in the rat following administration of a single dose of a PA. In a number of animal species the lungs develop vascular lesions characteristic of primary pulmonary hypertension with secondary hypertrophy of the right ventricle of the heart. in rats, appropriately low repeated doses of several alkaloids have been shown to induce tumours, mainly in the liver. In some studies, a single dose has been reported to be carcinogenic (W.H.O., 1988). The hazard to animals varies greatly with species and season. Animals are most at risk in the dry season when vigour is reduced and more palatable forage is scarce for a considerable period (especially for dry tropical African countries where Crotalaria species are quite abundant).

In man the ingestion of Pyrrolizidine alkaloids could be through any of the following:

1. Use of PAs containing plants as foods, beverages, or food related aspects.
2 Use of PAs containing plants as herbs or medicine, singly or mixed with other plants [as reported by Samuelson, et al, 1992; Nwude, 1986].
3. Contamination of Agricultural produce by PAs containing plants.
4. Contamination of other food products as a result of ingestion by the source animals, (i.e. eating animal products by man, e.g. milk; though this is quite insignificant and no case of any poisoning or outbreak is reported.)

Table 3 shows the various uses to which Crotalaria species are put in different countries.

It has been found that populations in some countries are already exposed to low levels of pyrrolizidine alkaloids in commonly used foodstuffs e.g comfrey (Symphytum officinale) (Culvenor et al 1980; Culvenor 1985) and honey in Australia (Culvenor et al 1981, Culvenor 1983, 1985). Human cases of acute disease following the brief ingestion of the alkaloids have been known to progress to cirrhosis (Stuart and Bras. 1951, Braginskii & Bobokhadzaev 1965, Stillman et al 1977, Tandon, B.N. et al 1977, Tandon H.D. et al 1977).

As shown above, the ingestion of PAs may be deliberate (as food or medicines) or as a result of contamination. In either case the initial disease may be cryptic (Braginskii & Bobokhadzaev, 1965) and may not be ascribed to herbal consumption and yet may later progress to cirrhosis (Huxtable, 1980). Veno-Occulsive Disease (VOD) was stated as the most common cause of cirrhosis in infants in Jamaica (Bras et al, 1961) and is also believed to be the most important etiological factor for adult cirrhosis, especially in developing countries (Gupta et al, 1963).

TABLE 3: USES OF CROTALARIA IN DIFFERENT PARTS OF THE WORLD AS HERBAL MEDICINE (M) OR FOOD (F), REPORTED CONTAMINATION (C) OR OUTBREAK (O) AND ALKALOIDS INVOLVED.

S/ No	CROTALARIA SPECIES	PRINCIPAL ALKALOIDS	REASON FOR INGESTION	COUNTRY	REFERENCES
1.	C. juncea	Trichodesmine and senecionine types	1. M 2. M & F haemoptysis	Ecuador India	Lyford, et al, (1976); Chopra (1933); Watt and Breyer-Bradwijk (1962)
2.	C. retusa	Monocrotaline	1. M 2. M & F haemoptysis, fever, colic. 3. M & F.	W. Indies Africa India	Bras et al (1954); A. Sofowora (1982); Watt and Breyer-Bradwijk (1962)
3.	C. fulva	Fulvine and monocrotaline	1. M 2. M	W.Indies Jamaica	Bras et al, 1954, (1957); Barnes et al 1964; Mc Lean (1970, 1974).
4.	C. brevidens		F	E. Africa	
5.	C. incana	Integerrimine	M	E. Africa	Schoentel & Coady (1968)
6.	C. laburnifolia	Anacrotine	1. M 2. F	Tanzania , Asia	Schoentel & Coady (1968);
7.	C. mucronata	Integerrimine	M	Tanzania	Coady, 1973.
8.	C. recta	Monocrotaline	M & F	Tanzania	Schoentel & Coady 1968;
9.	C. verrucosa		M	Sri Lanka	Arseculeratne, et al (1981)
10.	C. gorensis		M (sores)	Ghana	Irvine (1961)
11.	C. nana	Crotananine	C & O	India	Tandon, R.K. et al (1976); Krishnamachari et al (1977); Siddique et al (1978).
12.	Crotalaria Species		C & O	Afghani s-tan	Tandon, R.K. et al (1976); Krishnamachari et al (1977); Siddique et al (1978); Mohabbat et al (1976).

It is suggested that consumption of such herbs commonly used might contribute to the high incidence of chronic liver disease including primary liver cancer, in Asian and African countries, especially as they may act synergistically with aflatoxin and hepatitis B. virus. Risks are high in children (Schoentel 1959, Jago 1970), protein deficiency which exists in some countries, may potentiate the PAs action (Schoentel & Magee 1957). Even in countries with well developed health services and even among the higher economic and educated strata of society, the toxic incidence of PAs diseases are reported. Examples are reported in United Kingdom (McGee et al 1976, Ridker et al, 1985) and Hong Kong (Kumana et al 1985) and in U.S.A (Stillman et al 1977, Fox et al, 1978, Ridker et al 1985). In fact, Stillman et al 1977 in his reporting of cases in the U.S.A. called it the "iceberg disease" as there are likely more cases of the disease than is being noticed. He stated that PAs toxicosis might be more frequent than reported especially among people of Mexican - American origin where use of herbal remedies are common. In addition the use of herbal remedies is not elicited in clinical history and patients do not volunteer this information themselves.

In cases of contamination, these are more likely for food crops in parts of the world with arid climates, poor or uncertain rainfall and period of drought and poor irrigation facilities - all these promote the growth of PAs containing weeds as shown in the outbreaks in Afghanistan, India and the former USSR (Uzbekistan), the West Indies (W.H.O., 1988).

In cases of contamination of other foods through feeding by the source animals, Dickinson et al 1976, detected toxic metabolites being secreted in the milk of lactating

dairy cattle (who fed previously on PA containing plants). Suckling animals may appear to be in good health while their livers show toxic effects. Protein deficient and younger suckling animals are particularly vulnerable (Schoentel 1959).

Alkaloids have also been found in the honey secreted by bees feeding on the toxic plants (Deinzer et al 1977). The contamination through ingesting animals needs more data for proper understanding and strategy against PAs poisoning. In some experimental animals PAs are quickly metabolized and are almost completely excreted in 24h, so that no residual products are detectable in the biological fluids or body tissues after this period. Because of this, however, symptoms of PAs toxicity may take several days or months to appear. The effect is usually manifested as acute VOD characterised by a dull dragging ache in the right upper abdomen, rapidly filling ascites resulting in a marked distension of the abdomen and sometimes associated with oliguria and massive pleural effusion. It can also be a subacute disease with vague symptoms and persistent hepatomegaly. Children are particularly vulnerable. many cases progress to cirrhosis and in some instances a single episode of acute disease could lead to cirrhosis, inspite of removal of source of toxic exposure and even where symptomatic treatment, is given. Mortality can be high with death due to hepatic failure (in acute phase) or due to hematemesis resulting from ruptured oesophageal varices caused by cirrhosis. Less severely affected cases may show clinical or even an apparent complete recovery. No cases of primary pulmonary hypertension due to PAs toxicity have been reported even though this was found in test animals. Similarly in the report of outbreak of Trichodesma poisoning in former USSR, the symptoms were mainly neurological.

Other pharmacological effects of PAs are their antimitotic (anticancer) abilities and also their carcinogenic actions (Arnold, 1985), the conflict between these two properties of PAs is not resolved in the literature but both actions are reported for different PAs. The use of PAs containing plants in treatment of tumors has been reported since the fourth century A.D and also for cancer since the 10th century A.D. (Zalkow. L.H. et al 1979). Culvenor (1968) concluded that tumor-inhibitory activity was widely exhibited among PAs. Significant activity was also displayed by extracts of Heliotropium Indicum L (Boraginaceae) in several experimental tumor system and the active principle alkaloid was found to be indicine N-oxide. A recent report from China also confirmed the anti-tumor effect of monocrotaline and this alkaloid was reported to be more destructive to malignant cells than to normal cells. In fact, monocrotaline has been subjected to extensive screening for anti-tumor activity by the National Cancer Institute, Bethesda, Maryland NIH U.S.A. [Arnold, 1985]. It has indeed shown anti-tumor activity in several systems, where as crosemperine (also found in some Crotalaria species) was reported to have only minimal anti-tumor activity and is considerably more toxic than monocrotaline. The cytotoxic and antimitotic activity of many PAs has prompted investigations into their possible uses as anticancer agents. So far only one, indicine N-oxide, has reached clinical trial in cancer patients. It is however probable that other PAs or synthetic analogues will be tested for this purpose in the future.

Other actions of PAs include chromosomal aberrations, shown in experimental rats and humans with VOD (Martin et al 1972). Also in Soviet medicine the non-hepatotoxic PAs displacine and platyphylline have been used clinically for their

neuromuscular blocking and atropine-like activities. Recently also the action of series of semi-synthetic derivatives of PAs have been tested, they have been found to display a range of activity including hypotensive, local anaesthetic, ganglion blocking, neuromuscular blocking and antispasmodic properties. The different aspects of PAs activity shows that the pharmacological actions of the alkaloids vary with a number of factors. These include the species of animals (for example, guinea-pig was found to be resistant to PAs toxicity [Chesney and Allen, 1973]), its age and metabolic state, nature of the plant species and the amount (dose) consumed. In addition, the composition of alkaloids of each species of plant is different and the action of each plant species is a function of its individual alkaloids, hence species with similar alkaloids are expected to display similar pharmacological action under same circumstances. As for Crotalaria species, the bulk of the genus has not been investigated (see Appendix III). The research into the chemistry of each species is an important step in proper understanding of their various actions and possible exploitation as sources of drugs in the future, and this may also explain some of the actions, that Crotalaria displayed. Currently there is also a renewed interest of the anti-microbial/viral abilities of various pyrrolizidine alkaloids found among plant species.

1.6.2 **Other Uses of Crotalaria Species.**

Different Crotalaria species are being used in other non-medicinal aspects which include:

i} C. juncea (sunnhemp) - the bark of which provides high quality bast fibre for cordage and fine paper, about 130,000 metric tonne is produced annually mainly in the Indian subcontinent and Brazil (Vietmeyer, 1979). This plant has been introduced into Africa but is not yet exploited.

ii} C. retusa. C. pallida, C. brevidens, C. zanzibarica used as sources of fibre. The first two were also used for dyes.

iii} A few large-flowered shrubby species, notably C. agatiflora, C. capensis, C. laburnifolla and C. lunata have been grown for ornament in highlands & in South Africa.

iv} Among the shrubs C. micans (C. anagyroides can grow upto 5m in a year providing mulch, good shade or windbreaks and can be used to dry out wet soils in lowland tropical sites.

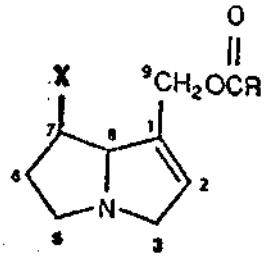
i) Agriculturally, Crotalaria species are used in soil conservation, erosion control, forestry and as organic manure.

1.7 Toxicity of Crotalaria Species and its Control

The hepatotoxic properties of PA_S have interested pathologists for a number of years. Studies have revealed that chemical (relating to individual alkaloids) and metabolic factors are all important in determining the toxicity of pyrrolizidine alkaloids. That is, the toxicity of an alkaloid depends on the extent to which it is converted into active metabolites and on the disposition and activity of these metabolites once formed.

(A) *Structural Features of a Toxic Alkaloid*

The essential structural features of a hepatotoxic pyrrolizidine alkaloid is shown in Figure 6.



where X = O, RCO, HO, or H

FIGURE 6: ESSENTIAL STRUCTURAL FEATURES OF A HEPATOTOXIC PYRROLIZIDINE ALKALOID.

These features are:

i) a - 1-hydroxymethylpyrrolizidine ring system unsaturated in the 1.2-position, preferably with a second hydroxyl group in the 7-position.

ii) esterification of at least one of the hydroxyls, though toxicity is greater when both hydroxyls are esterified.

iii) ester groups that are resistant to enzymic hydrolysis, which usually means that there is a high degree of chain branching in the acid moiety.

The most important feature is the right hand pyrroline ring, being the ring that is being metabolised to a pyrrole derivative. The structural requirements for N-oxides are the same as those for the hepatotoxic alkaloids. Though the pyrrolizidine alkaloid N-oxide is not hepatotoxic in itself, its toxicity depends on it being reduced to the corresponding basic alkaloid. This takes place mainly in the gut (Mattocks 1971, Mattocks and Bird, 1983) and other likely organs, such as the liver (Powis et al, 1979).

B} METABOLIC FACTORS DETERMINING TOXICITY.

The metabolic factors affecting the proportion of an ingested alkaloid that is converted into toxic metabolites in an animal are:

i) Lipid Solubility: highly water soluble alkaloids are easily excreted hence have low toxicity

ii) Susceptibility of Alkaloids to Hydrolysis: this is determined by the molecular structure of the alkaloid

iii) Susceptibility to N-oxidation: this depends on the relative amounts of an alkaloid

converted by hepatic microsomal enzymes to N-Oxides and to pyrrolic metabolites.

iv) Other features affect the toxicity of the active metabolites such as reactivity of the metabolite and the number of reactive groups it contains (Mattocks and Driver, 1983).

Generally the toxic effects are due to activation of the alkaloids in the liver. Metabolism of the alkaloids by mixed function oxidases leads to pyrrolic dehydro-alkaloids, which are reactive alkylating agents. Reactions of initial metabolites with constituents of the liver cell in which they are formed are probably the main cause of liver cell necrosis. Metabolites are released into circulation and they could pass beyond the liver to the lungs causing vascular lesions typical of primary pulmonary hypertension especially when alkaloids such as monocrotaline are involved, though relationship between rate of metabolism and expression of toxicity is uncertain.

The toxicity of Crotalaria species varies just as their alkaloid content. We shall not fully grasp or rather understand the toxicity variations of this genus unless all the species are chemically screened qualitatively and quantitatively and epidemiological studies carried out in individuals or populations consuming the species.

C) PREVENTION AND CONTROL OF CROTALARIA TOXICITY

The health menace of the toxicity of Crotalaria and other PAs species on livestock and humans is still universally insufficiently recognised (W.H.O., 1988) and this makes

it more difficult in the prevention and control of the disease. The only effective method of control of PAs poisoning is to prevent the intake of alkaloids, but once the plants are ingested, there is little that could be done presently. This is in fact a problem that can easily be fatal as such measures, preferably preventive are important for the control of the toxicity. The following are important measures in the control of pyrrolizidine alkaloids poisoning:

1. Public Health Campaign especially among Health care practitioners such as herbal medical practitioners and agricultural officers could be staged so that people can be educated to recognise poisonous plants. This has been effective in reducing VOD in Jamaica against Crotalaria fulva. Similarly health practitioners could be educated in order to recognise the symptoms of PAs poisoning, (especially in high risk areas), the sources of such poisoning could be identified, recognised and further cases prevented.

2. A set of agricultural legislative measures have been effective in preventing new outbreaks of poisoning due to Heliotropium and Trichodesma in the former USSR since 1947. Cereal crops should be assessed for possible contamination by weeds or PAs plants. Similarly commercially produced herbal teas, have to be assayed, screened and the plants properly identified before consumption.

3. Ethnobotanical and taxonomic investigations are needed universally so as to provide specific information on the use of plants containing PAs for medicinal and dietary needs. Their sale and prescription by herbalists and other health practitioners could be controlled.

4. In areas where poisoning is already documented, dairy products and honey should

be assayed for the level of PAs they contain.

In addition to the above, some few measures have been experimented on animals, and they have been found to have limited, though not so successful in protection against PAs poisoning. These measures include modified diets and the pretreatment with specific doses of certain chemicals. A protein rich diet and sucrose-only diet have been found to be protective against PAs toxicity. There are, however, many other considerations and further research is necessary before these protective measures could be fully utilised in the control of PAs poisoning.

1.8 Previous Research Methods and Scope on Crotalaria Species.

Literature review on Crotalaria species reveal that some work has been done on these plants, but only on a limited number of species. Many of the species are yet to be investigated. All most all Nigerian species, except a few, have not been studied. The NAPRALERT report, shown in Appendix III, indicates the scope of work done on Crotalaria species world-wide. The following are the key areas covered by previous workers on Crotalaria species.

1.8.1 Scope of Research on Crotalaria Species

The scope of research done on Crotalaria species could be grouped into macromorphological work, taxonomic studies, phytochemical investigations, toxicity studies and medicinal and pharmaceutical studies.

i) *Macromorphological work*

There is literature, though not quite abundant, which lists the various Crotalaria species and the macromorphological features of the parts of each species. To this Hutchinson & Dalziel (1958) in their flora of West Tropical Africa listed 51 species and their features. Polhill (1982), in describing Crotalaria species in Africa and Madagascar, noted the existence of about 600 species, 500 of which are in Africa. He reviewed the whole genus giving useful suggestions and addition of many species and the features/characters of each. Literature on Crotalaria species in Nigeria is very scanty indeed, only little work has been done on not more than two to three species (namely, C. lachnosema and C. naragutensis). Keay (1989) in his mention of Nigerian plants omitted these important species among the family Leguminosae.

ii) *Taxonomic studies*

Some work has been done on the taxonomy of Crotalaria species, notably by Polhill (1968, 1982) and Bisby (1973). Their work touched principally on the use of chemical and morphological characters in the classification of genus.

iii) Phytochemical Investigations

Owing to their toxicity, much of the research on Crotalaria species is centred on their chemical constituents. Even in this area much work was done only on detection, isolation and the chemical nature of individual alkaloids in the various parts of the different species. Less effort was seen on the percentage of total alkaloids in the parts, factors determining alkaloid accumulation or biosynthesis or the quantitative estimation of individual alkaloids found in the species. Many techniques have been developed on the extraction, separation and analysis of pyrrolizidine alkaloids. In this area the works of Mattocks singly and with others (Mattocks, 1961, 1967, 1968, 1971, 1986) and also Culvenor (1961, 1968, 1983, 1985) need to be mentioned.

iv) Toxicity studies

Various workers have made extensive studies on both farm-animals and experimental animals with regards to the nature of toxicity of Crotalaria species (and other plants containing PAs) and factors associated with it (Okopido and Ogunbiyi, 1976). Most of these were feeding experiments with the plants or individual alkaloids. The same study was not extended to man or rather long-term study of survivors of Crotalaria poisoning or related data is limited or in many cases missing. One of the basic problems with toxicity of PAs species such as Crotalaria is the general lack of awareness of the toxicity of the plants.

v) *Medicinal and Pharmaceutical studies*

Little work is documented on the various uses especially medicinal to which Crotalaria species are employed by the various communities/societies. Research into this aspect particularly the prescriptions by herbalists will give an insight into the likely occurrence of poisoning in a particular area, pattern of toxicity and possible future uses of the plants. Similarly pharmacognostic studies are unreported in Crotalaria literature. These studies are important because they will reveal other non-macromorphological features which could be useful in distinguishing these numerous species easily. The microscopy research for instance may provide characters to which Crotalaria species could be easily recognised both in whole form and in powdered form and identified using the simple microscope. So far in the pharmaceutical aspects only the pyrrolizidine alkaloid - indicine - N-Oxide have reached clinical trials in cancer patients. There is also a need for a study (especially where Crotalaria species are consumed) between relationship of liver and cancer diseases to Crotalaria consumption among populations. There are also reports of various activities of extracts of Crotalaria species, though most of these findings are preliminary, (Dhawan, et al, 1977).

vi) *Comparative Phytochemistry*

The comparative phytochemistry of the genus crotalaria is not extensive. This study intends to form the basis of comparative phytochemical studies of this genus.

1.8.2 Research Methods on Crotalaria Species

The following were found to be important considerations with respect to research methods on Crotalaria species as carried out by previous workers.

i) Preparation of Plants for Research

The species to be studied must be properly identified, especially because of the large number of species of the genus.. The plants should be prepared keeping in mind the type of investigation to be carried out, as for example, in phytochemical work, plants should normally be collected during their flowering/fruiting season, as the chemical constituents are normally highest in the plants during this period. The plants should also be properly prepared powdered and stored. Contamination and long periods of storage should be avoided.

ii) Extraction

Various workers have used different extraction methods. These included, among others, extractions from biological fluids and tissues by various chromagraphic techniques. Plant material could also be dried or crushed and extraction done with hot or cold alcohol. The alcohol is evaporated and the bases taken up in dilute acid, and fats extracted with ether. It is usual at this stage to reduce any N-Oxides to the corresponding basic alkaloids with Zinc, before making the solution alkaline and extracting the alkaloids with chloroform (Koekemoer & Warren 1951). Alternatively, the alcohol can be continuously circulated through the plant material and then cation exchange resin, and the alkaloid subsequently eluted from the resin (Mattocks, 1961, Deagen & Deinzer,

1977). PAs can also be extracted by soaking the plant material in dilute aqueous acid (Briggs et al 1965; craig et al, 1984).

iii} *Analysis of pyrrolizidine alkaloids*

The analysis of PAs has been carried out by various workers using thin layer chromatography (TLC) (Sharma et al, 1965, Chalmers et al, 1965, Mattocks 1967, and Wagner et al, 1981). Gas Chromatography (Weidenfeld et al, 1981, Culvenor et al, 1980 and Frahn et al, 1980), High - Performance Liquid Chromatography (HPLC) (Segall, 1979, Dimenna et al, 1980 and Mattocks, 1986) and Nuclear Magnetic Resonance (NMR) Spectrometry (Molyneux et al 1979, Pieters and Vlie tinck 1985, Mattocks 1986) have been employed in the determination of unsaturated PAs. The chromatographic techniques and NMR can both be utilised for qualitative and quantitative estimations of alkaloids. In addition to the above the Ehrlich reaction method devised by Mattocks (1967, 1971, 1968) have become most useful reaction tests for unsaturated pyrrolizidine alkaloids. It is also specific for PAs, because as little as 5μg of most PAs can be detected and measured by this method. Other methods of PAs analysis include the method of indicator dyes and direct weighing of the extracted alkaloids. Spectrophotometric analysis and estimations have also been documented (Mattocks, 1967).

1.9 Pharmaceutical and Other Possible Uses of Crotalaria Species in Nigeria

There is no doubt that Crotalaria species are used in Nigeria (Nwude and Ibrahim, 1980; Nwude, 1986) and world wide in traditional medical practice and as food. Their toxicity and effects in liver diseases to humans and livestock is also well documented elsewhere (Mattocks, 1985), though this has not been well recognised by the populace using the plants. There is need to examine the role and effects of these plants on humans and livestock. There may be other pharmaceutical potentials that the Crotalaria species could be exploited. Pyrrolizidine alkaloids accumulate in most Crotalaria species. This is important since the plants are consumed and traditionally utilised in medicine, for instance as anti-microbial agents. Traditional medicine in Africa serves more than 70% of the people (Moran, 1996).

The hazards to livestock is more evident since the Crotalaria species are very much abundant and varied, about 500-600 species in the genus in tropical Africa. They also grow easily under varying ecological conditions. They are utilised as food, traditional medicine, farming practices, for livestock and may also contaminate agricultural products. Crotalaria poisoning and even outbreaks have occurred in many countries. In view of the continuous indiscriminate use of Crotalaria and allied species, the World Health Organisation (1988) has called for a number of measures to be taken to combat arising health problems. These include ethnobotanical, taxonomic and epidemiological studies particularly in countries with high incidence of primary liver cancer in order to determine its association with pyrrolizidine alkaloids intake.

Other plants containing PAs are found largely in other geographical locations. Crotalaria species are largely African. Parameters for distinguishing the species, phytochemical screening and ethnomedical practices involving the use of the species and information on relationship of species, its chemical and toxicity pattern are important in understanding and preventing the hazards.

Crotalaria species are also potentially capable of being a source of future drugs. Their traditional uses in microbial infections might have prompted the search for other medicinal values of the species. Pyrrolizidine alkaloids have quite recently been reported to have both anti microbial and anti viral abilities [Mattocks, 1986]. Anti cancer properties of PAs are now under research in different parts of the world. So far only *Indicine-N-Oxide* has reached clinical trials in Russia (W.H.O., 1988). In agriculture and farming practices, and forestry, Crotalaria species are found to be good in soil conservation, control of erosion and as sources of organic matter, soil reclamation and increase fertility of soil. This is an important aspect especially here in Northern Nigeria where erosion and desertification is fast making fertile lands barren and valuable plant species largely undocumented are being lost. The ability of Crotalaria species to grow fast, they need little or no management requirement after planting and are less susceptible to animals. Their disadvantages include their toxicity to livestock. Evaluating the different species could be important in indicating which species could be most suitable for our specific needs. For instance species containing non toxic constituents could be most valuable in farming practices and agriculture.

The use of Crotalaria species should be highly considered especially with the

fertiliser crisis in Nigeria, the use of green manure could improve soil quality and regain worn out and toxic soils. The ability of some Crotalaria species to provide high quality fibre for especially production of paper is already being exploited in other parts of the world. The coloured flowers of Crotalaria species make them valuable ornamentals.

Literature survey has revealed that Crotalaria species are very promising plants and if properly exploited could be utilised for both pharmaceutical and agricultural sectors. Being naturally abundant in Africa, they could boost our economic potentials and reduce dependency in many sectors. There is also need to prevent contamination and poisoning outbreaks which have occurred in other parts of the World. Their likely role in primary liver cancer [WHO, 1988] adds another concern in the study and understanding of Crotalaria species.

1.10 RESEARCH OBJECTIVES AND SCOPE

Crotalaria species, though abundant and widespread in Nigeria have not been largely investigated with respect to their phytochemical constituents and potential toxicity. These species are extensively used in traditional medicine therapy and as fodder for livestock. There is no information yet available on the toxic and non-toxic species. In addition, there are no clear parameters using which the whole plants and powdered species could be properly recognised and identified among the numerous and widespread species of the genus. The information on their ethnomedical uses, illnesses for which they are utilised and possible side effects are scanty.

This study was therefore set out to investigate three Crotalaria species found commonly in Zaria, Nigeria, namely:

- C. microcarpa, Benth;
- C. naragutensis, Hutch and
- C. retusa, L.

This is a pioneering work on Crotalaria research in Nigeria especially with respect to the set out objectives listed below:

1.10.1 Research Objectives:

The objectives of this study are as follows:

1. To study the microscopical features and characters by which the three Crotalaria species and their powders could be identified. Both qualitative studies of their structural make up and quantitative estimation of the microscopical constants of the three species were to be carried out. The Crotalaria species are commonly utilised in traditional medicine as powders. There is yet no report of microscopical work done on any species of the genus.
2. To investigate the presence of some secondary metabolites in the three Crotalaria species, such as alkaloids, glycosides, flavonoids, anthraquinones and resins.
3. To determine the amounts of alkaloids in the three Crotalaria species and the presence of unsaturated or potentially toxic pyrrolizidine alkaloids.

4. To assess the toxicity of the Crotalaria alkaloids, especially the unsaturated pyrrolizidine alkaloid bases on living systems.

5. To investigate the ethnomedical parameters of the three species in terms of species choice, uses, occurrence and knowledge of toxicity among the populace and any toxic experience on man or animals.

6. To investigate the other features of the plants especially extractive values and macromorphological screening of all the plant parts. It is believed that these features along with microscopy would contribute in the preparation of pharmacopoeal standards for these important plants

1.10.2 Scope

The above objectives could be grouped into four major subheads, namely Identification, Phytochemical (alkaloid content), Toxicity and Ethnomedical aspects. The scope and limits for each of these are outlined below.

(a) Identification

All the three Crotalaria species were to be examined macroscopically in order to establish their common and individual features. The microscopic characters by which each Crotalaria species could easily be recognised and identified using the simple microscope were also to be investigated. The Crotalaria species are indeed quite numerous and abundant in tropical Africa, 600 species are known, 500 of them in Africa [Polhill 1982]. Crotalaria species are both medicinally and economically important.

Some of the species are also known to be toxic to humans, livestock and other animals. The toxicity of the species has been found to vary and some species are found to be non-toxic. In view of these, it is useful to provide a microscopic insight into each species, which will furnish more information and characters of the Crotalaria species. This would allow proper identification of each species among other plants and among the members of the genus Crotalaria. To this end, both analytical microscopical work involving determination of the size, shape and relative positions of the different cells and tissues, determination of the chemical nature of the cell walls and cell contents shall be carried out. Quantitative estimations of stomatal number, stomatal index, palisade ratio, vein islet and vein termination numbers of each species shall be carried out. There is no microscopical work reported in the literature on Crotalaria species inspite of the importance of the work. The World Health Organisation report on pyrrolizidine alkaloids [WHO, 1988] has stressed the health menance of the pyrrolizidine poisoning and called for more studies on the botanical, phytochemical and taxonomic aspects of plants containing pyrrolizidine alkaloids.

Literature review has revealed some features by which Crotalaria species could be identified in their whole form (that is, whole plant parts - either leaves, seeds or flowers). However, due to their toxicity and continuous prescription by herbalist worldwide who, in most cases, provide and keep these plants in powdered form, macroscopical features would not be enough for proper identification. It is important to add another parameter by which powdered Crotalaria could be recognised. It is in this respect that microscopical studies using the simple and easily available microscope

becomes important.

Qualitative microscopy, chemomicroscopy, quantitative microscopy, determination of the physical constants (moisture content, ash and extractive values) and the macroscopical features would all contribute towards identification and setting of pharmacopoeal standards of the Crotalaria species.

(b) Phytochemical Studies

The three Crotalaria species would be initially tested for the presence of alkaloids and other chemical contituents. The amounts of alkaloids in each species were to be determined. The qualitative assessment of the alkaloids would be carried out to determine possible presence of unsaturated groups/alkaloids - which are indicators of hepatotoxicity of the plant.

Literature reveals that most Crotalaria species contain the pyrrolizidine alkaloids(PAs), though some species have the PAs but are found to be non-toxic. Therefore, it is important to assess each species phytochemically, as there is a clear relationship between pyrrolizidine content and toxicity. This study will also provide data for the alkaloidal content of the Nigerian Crotalaria species compared to same species found elsewhere. The quantity of alkaloids especially could vary with the place it is grown.

(c) Toxicity Studies.

The phytochemical investigations would reveal which of the species may contain hepatotoxic pyrrolizidine alkaloid bases. These would then be tested on rats to determine

the actual degree of toxicity and the organs affected.

(d) Ethnomedical Studies

Presently the various uses to which Crotalaria species are put in Nigeria is not well documented and scanty in the literature. This research will document the various applications of the Crotalaria species in our traditional medical therapy and perhaps, other uses. This is important for further research into the plants. It could also be a useful tool or pointer into the relationship between consumption of Crotalaria in traditional medical practice and occurrence of some health related issues. Towards this end, oral interviews and written questionnaires would be administered to herbalists and traditional practitioners, farmers, cattle-rearers, veterinary and medical practitioners in and around Zaria.



CHAPTER TWO



CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Preparation of Plant Samples.

2.1.1 Identification

All the three Crotalaria species being investigated were identified and collected using descriptions given by both Polhill (1982), and Hutchinson and Dalziel (1958). Their identities were further confirmed by herbarium keepers using authentic samples in the Department of Biological Sciences, Ahmadu Bello University, Zaria.

2.1.2 Collection

Samples of C. retusa and C. naragutensis were obtained from the Botanical Garden of the Department of Biological Sciences of the Ahmadu Bello University, Zaria in early 1995. C. microcarpa was obtained at Jos - Zaria road. For the microscopical work, the samples were collected at different times from the above sources. For phytochemical work, samples were collected during their flowering season and no time lag was allowed between storage and investigation. This was done to minimise possible loss or change in the alkaloid and other chemical contents.

2.1.3 Preparation and Herbarium Specimens of Plants

A herbarium specimen of each of the species was prepared in duplicate using standard procedures. Each of the specimens was poisoned with merlury chloride for long term storage and the albums were kept for future reference.

The collected plant materials were separately processed as follows. The various parts were separated and air dried at room temperature until thoroughly dry. The dried parts were then powdered using mortar and pestle. The powders were sieved using sieve size No.40. Powders of leaves and powders of aerial parts (leaves, stems, fruits and seeds) were prepared separately. The powders were then packed in airtight containers, labeled and kept in a cool dry place until needed. During the microscopical work, samples of fresh whole plants were also collected whenever the need arose.

2.2 Equipment, solvents and reagents

The following equipment, consumable materials, solvents and reagents were used in the experiments. All solvents and reagents were of analytical grade (B.P). References to water mean distilled water.

- Compound microscope
- Camera lucida
- Oven
- Pestle and mortar
- Sieve, No.40.
- Slides and cover slips
- 250ml beakers

- 1 litre beakers
- Measuring cylinder (1 litre, 100 ml, 10 ml)
- Bunsen burner
- Micrometers
- Different sizes of conical flasks
- Nickel and porcelain crucibles
- Water bath
- Filter paper
- Ashless filter paper
- Burettes
- Funnels
- Separating funnels
- Weighing balance
- Razor blades
- Photographic camera
- Mechanical shaker
- Rotary evaporator
- Stand
- Silica gel plates (G, type 60)
- Questionnaires (designed to (a) Herbalist, (b) Herdsmen, (c) Medical Practitioners, (d) Veterinary Practitioners (see Appendix).
- Chloroform
- Methanol

- Ether
- Acetone
- 95% ethanol
- 90% ethanol
- N/50 iodine
- Chloral hydrate
- Hydrochloric acid
- Sulphuric acid
- Ferric chloride
- Ruthenium red
- Dilute glycerol
- Millon's reagent
- Chlor-zinc-iodine
- Picric acid
- Phloroglucinol
- Sudan IV
- Dimethyleneglycoldimethylether (Diglyme).
- Zinc dust
- Magnesium fillings
- Perchloric acid
- Acetic acid
- Boron triflouride
- Dimethylaminobenzaldehyde

- Ammonia
- Calcium hydroxide
- Acetic anhydride
- Hydrogen peroxide
- Alkaloidal reagents:

  Mayer's reagent (potassium mercuric iodine solution);

  Dragendorff's reagent (potassium bismuth iodine solution).
- Ehrlich reagents: (10mls of methanolic borontriflouride (14% BF_3) was diluted to 100mls with methanol and in it 4gms of dimethylamino-benzaldehyde was dissolved (Ehrlich reagent was prepared only on the day it was to be used).
- N/200 sulphuric acid (prepared by adding 54 mls of concentrated sulphuric acid to a litre of water)

2.3 Macromorphological Investigations.

An investigation using whole plants of each species was carried out and the general features and distinguishing characters were noted on all the parts of the plants, the appearance, seasonal occurrence, timing, size of leaves, flowers, stems, fruits and seeds. Each feature was noted and described with the appropriate botanical terms. The characteristic macro - features of the powders were also noted. The observations are reported in section 3.1.

2.4 Microscopical Study of the Leaves of the Crotalaria Species.

Qualitative and quantitative microscopy was carried out on the leaves of each of the three Crotalaria species using standard procedures (Evans, 1992).

2.4.1 Chemo-Microscopy of Crotalaria Leaves.

Microscopical analysis was conducted on the epidermal peel of each of the species. The epidermis was mounted in chloralhydrate. The epidermal characters were observed and drawn. Sizes of trichomes were measured using both stage and eyepiece micrometer. The various characters were noted and identified.

Powdered samples of each of the species were studied for sensory characters (odour, colour, taste and texture) as well as behaviour when shaken with water. Powdered samples were also mounted in different reagents, specifically, glycerol, N/50 iodine, ruthenium red, picric acid and chlor - zinc iodine solution. The various features were noted, identified and drawn.

Chemomicroscopical examinations were also carried out on the powders and the chemical natures of the powders were observed from colour and structural changes. The chemo-microscopical tests were done for the presence of lignin, cellulose, tannins, gums and mucillages, starch, calcium oxalate crystals, proteins, oils and fats. The details of these tests were as follows:

(i) Proteins

Powdered sample was treated with Millon's reagent. A red-violet colour was a positive indication of protein.

(ii) Cellulose

To a sample of the powder, chlor-zinc - iodine solution was added. Presence of cellulose was indicated by blue-violet; lignified showed yellow and starch grains - blue.

(iii) Lignin

Powder was stained with phyloroglucinol and concentrated HCl acid. All lignified walls stained pink-red.

(iv) Mucilage

To a sample of the powder Ruthenium red was added. Mucilaginous walls stained temporarily pink-red.

(v) $CaCO_3$

To a sample of the powder, dilute H_2SO_4 was added, effervescence with evolution of gas indicated positive.

(vi) Individual tests for Tannins

Powder was shaken with water, boiled gently (noted the odour), filtered and to the filtrate few drops of ferric chloride solution were added. Blue- black precipitate indicated

positive for hydrolysable tannis, brownish -green for condensed tannis, if both were present then blue was observed which would change to olive green as more ferric chloride was added.

(vii) Anthraquinones

Powder was shaken with water, boiled gently and filtered. The filtrate was shaken with ether, separated and added to ether solution NH_3 and shaken. Pink indicated positive.

(viii) Fats and Oils:

Powder + Sudan IV, followed by warming; red colour indicated positive.

The transverse sections of the leaves were also histologically prepared and assessed. The sections of the three species were photographed (see Plate 4).

2.4.2 Quantitative Microscopy of Leaves.

Five quantitative microscopical constants were determined for each of the three Crotalaria species. These were:

- stomatal number;
- stomatal index;
- palisade ratio;
- vein-islet number;
- vein termination number.

Details of the procedures for each of the above parameters are outlined below.

Stomatal Number

This gives the number of stomata per square millimetre of epidermis. Lower epidermal peel were obtained from each species. Using micrometre and camera lucida, area of field of view was calculated. The number of stomata in five fields of view were counted separately and the average calculated.

$$\text{Stomatal No.} == \frac{\text{Average Number of Stomata}}{\text{Area of Field of View in square millimeters}}$$

Stomatal Index

This was calculated for the lower epidermis. It gives the percentage proportion of the cells of the epidermis of a leaf, which has been converted into stomata. Procedure was as above but in each field of view both stomata and epidermal cells were counted and the index calculated as follows:

$$\text{Stomatal Index} == \frac{\text{Number of Stomata}}{\text{No . of epidermal cells + No. of stomata}} \times 100$$

Palisade Ratio:

The average number of palisade cells that occur beneath each upper epidermal cells is the palisade ratio. It was determined by counting the palisade cells beneath four contiguous epidermal cells.

Pieces of leaves were cleared by boiling in chloral hydrate, each piece was mounted and examined with a 4 mm objective, camera lucida was arranged so that epidermal cells and palisade cells were clearly visible. A group of four epidermal cells were traced clearly and the palisade cells below each. The average of the four was considered as the Palisade Ratio.

Vein Islet Number:

Vein - islet number denotes the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein-islets per square millimetres calculated from four contiguous square millimetres in the central part of lamina; midway between midrib and margin is the vein islet number.

A camera lucida was attached and by means of a stage micrometre the paper was divided into squares of a millimetre square. The stage micrometre was then replaced by the cleared preparation and the veins were traced in four contiguous squares.

The value obtained was then divided by four to give the vein islet number.

Vein Termination Number:

This is the number of veinlet terminations per square millimetre of leaf surface. A vein termination is the ultimate free termination of a vein islet or branch of vein. Procedure as above was followed but instead, vein termination in each square millimetre was counted. About five counts were taken and an average was the vein termination number.

2.5 Determination of Some Physical Constants of the Leaves of the Species.

The following constants were determined for each of the three Crotalaria species under study.

(i) Moisture content

(ii) Ash value

iii) Acid Insoluble Ash

(iv) Alcohol Soluble extractive value.

The procedure for each of these is detailed below:

2.5.1 Moisture Content:

The moisture content refers to the percentage quantity of water in a crude drug and it could be determined by various means. The moisture contents of the Crotalaria species were determined by the *loss on drying* method. 3.0g of leaf powder was accurately weighed into a tared evaporating dish. The dish was placed in an oven at 105 °C. After one hour the weight of drug and dish was measured and returned to oven. The weighing was repeated after every 30 minutes until the weight became constant. Then the weight of water lost during drying was calculated with reference to the original weight of the plant as follows:

$$\text{Moisture content (\%)} == \frac{\text{Weight of water lost}}{\text{Original weight of sample}} \times 100$$

2.5.2 Determination of Ash Value

The ash value is the percentage ash left by a plant after being completely burnt. 2.0g of accurately weighed samples of each species were heated in a tared crucible until charred. Heat was gradually increased until the drug became almost white (ash). The crucible was cooled and weighed. The ash was again heated and weighed. The heating and weighing was continued until weight became constant. The ash value was then calculated in the following way:

$$\text{Ash value (\%)} == \frac{\text{Weight of residual ash}}{\text{Original weight of sample}} \times 100$$

2.5.3 Determination of Acid Insoluble Ash Value

The acid insoluble ash value is the percentage of ash that remained undissolved after boiling the ash of the sample for 5 minutes in dilute hydrochloric acid. The ash obtained from 2.5.2 (i.e. Determination of ash value) was quantitatively transferred into a beaker containing 25ml of dilute hydrochloric acid. It was boiled for five minutes and filtered through an ashless filter paper. The beaker and crucible were rinsed with water. The water was passed through the same filter paper so that the ash was collected at the tip of the filter paper. The filter paper was dried in the oven at 105°C. The dried filter paper along with the residue was then transferred to a tared crucible and heated gently.

The heat was increased until the filter paper was completely burnt. Heating was continued for 5 minutes.

The crucible was cooled and weighed. The acid insoluble ash value calculated as follows:

$$\text{Acid insoluble ash value (\%)} = = \frac{\text{Weight of residual ash}}{\text{Original weight of sample}} \times 100$$

2.5.4 Alcohol Soluble Extractive Value

The alcohol soluble extractive value is the percentage of soluble materials obtained after dissolving the plant material in alcohol. This involved shaking, mixing and extraction of the plant material in 90% ethanol. 5.0g of each powdered leaf was accurately weighed into a stoppered flask, 100ml of 90% ethanol were added. The flask was shaken using a mechanical shaker for six hours and then left to stand for 18 hours. The solution was filtered. The filtrate was taken into a tared evaporating dish and evaporated just to dryness. The dish and residue were then placed in an oven to dry and then weighed. Weighing and heating was continuously taken at ten minutes interval until constant. The alcohol soluble extractive value was then calculated as follows:

$$\text{Alcohol soluble extractive value (\%)} = = \frac{\text{Weight of residual ash}}{\text{Original weight of sample used}} \times 100$$

2.6 Phytochemical Screening of the Crotalaria Species.

Phytochemical studies were carried out on the leaves and aerial parts of the three Crotalaria species. This involved initial tests for general chemical constituents, detection, extraction, quantitative estimations of the alkaloids content of each of the species and qualitative assessment of the alkaloids by TLC. The procedure for each is outlined below (Evans, 1992).

2.6.1 Phytochemical Screening

Tests were carried out for the presence of carbohydrates, flavonoids, tannins, cardiac glycosides, anthraquinone, resins and saponins on leaves of each species as follows:

2.6.1.1 Test for Carbohydrates:

5.0 gm of powders of Crotalaria species were accurately weighed and boiled in 50ml of distilled water on a hot plate for 3 minutes. The mixture was filtered while hot and the filtrate was cooled and used for these tests:

(i) *Molisch's General Test for Carbohydrate*

2.0 ml of extract were taken in a test tube and few drops of Molisch's reagent (α-naphthol) were added followed by a small quantity of concentrated sulphuric acid added

on the side of the test tube inclined at an angle 45° to form a lower layer. Purple to violet colour at interface indicates presence of carbohydrate. The mixture was then shaken gently and allowed to stand for two minutes and then diluted with 5ml water. A dull violet precipitate further indicates presence of carbohydrate.

(ii) *Barfoed's Test for Monosaccharides*

To 1.0 ml of filtrate was added 1.0 ml of Barfoed's reagent and the mixture was heated in a water bath for 4 minutes. A red precipitate indicates the presence of monosaccharides.

iii) Fehling's Test for Reducing Sugars:

2.0ml of water extract of each Crotalaria species were taken, 5.0 ml of Fehling solution A and B (1:1) were added and the mixture was boiled on a water bath for five minutes. A brick-red precipitate indicates the presence of free reducing sugars.

2.6.1.2 Shinoda Test for Flavonoids

1.0 g of powdered leaves was boiled in ethanol in a test-tube on a water bath for 5 minutes, filtered and cooled. Four pieces of magnesium fillings were added followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids.

2.6.1.3 Test for Tannins

2.0g of the powdered leaves of each Crotalaria species were boiled in 40 mls of water on a hot plate for 3 minutes. The mixture was filtered while hot. 1.0ml of the filtrate was diluted with 3.0mls of water. Few drops of Ferric chloride were added. A bluish black colour indicates the presence of hydrolysable tannins and greenish colour indicates condensed tannins (Trease and Evans, 1983). If both are present, initial colour would be blue which would change to olive green on addition of more Ferric chloride.

2.6.1.4 Borntrager's Test for Anthraquinones

1.0 g powdered leaves of each Crotalaria species was taken and 10.0ml of chloroform added. This was mixed thoroughly and heated for five minutes. The extract was filtered while hot and allowed to cool. Equal volume of 10% ammonia solution was added to the filtrate. The mixture was shaken. The presence of pink, red or violet colour in the ammonical layer indicated the presence of free anthraquinones.

2.6.1.5 Test for glycosides

(a) Keller-Killiani Test for deoxysugars in Cardiac Glycosides.

5.0g of each Crotalaria species powder were completely extracted with methylated spirit and then filtered. 2.0mls of the extract were mixed with 2ml of glacial acetic acid

containing one drop of Ferric chloride solution. 1.0ml concentrated sulphuric acid was added to the bottom by inclination of the test tube at an angle of 45°. A brown ring obtained at interface indicates the presence of a desoxy sugar characteristic of cardenolides. A violet ring may appear below the brown, also a greenish ring may form above the brown ring (Trease and Evans, 1992).

(b) **Salkowski test for steroidal ring.**

Ether extract of the leaves of crotalaria species was prepared and dried. 0.5 g of the extract of each species was dissolved in 2.0 ml chloroform. Sulphuric acid was carefully added to form lower layer. A reddish brown colour at the interface was noted as positive for steroid ring.

(c) **Guignard test for cyanogenetic glycosides**

2.0 of each powder of aerial part of the crotalaria species was used to test for cyanophoric glycosides. The powder was placed in a teat-tube and moistened with water. Sodium picrate paper was suspended above the drug. This was allowed to stand for an hour. Brick-red colour was taken as positive for cyonogenetic glycosides.

2.6.1.6 Test for resins

0.5g of powdered leaves of each Crotalaria species was weighed and mixed with 10mls of acetic anhydride. 1.0ml of concentrated sulphuric acid was added. A purple to violet colour indicates the presence of resins.

2.6.1.7 Test for Saponins

Presence of saponins was tested in the leaves of the Crotalaria species. 10 g of the powder of leaves of each species was mixed with 20.0 ml of water. This was mixed thoroughly. Frothing indicates presence of saponins.

2.6.2 General Test for Presence of Alkaloids

The presence of alkaloids in leaves and aerial parts was tested. 2.0g of each Crotalaria powder were placed in 30ml of methanol and shaken for 30 minutes. The mixture was filtered and the filtrate was evaporated to dryness on a water bath. 5ml of dilute hydrochloric acid were added to the dry residue portions. 1.0ml of this mixture was tested respectively with Mayer's reagent, Dragendoff's reagent, Wagner's reagent and saturated solution of picric acid for the presence of alkaloids.

2.6.3 Extraction of Alkaloids of the Crotalaria Species.

Normal extraction procedures for pyrrolizidine alkaloids were used for the extraction of the alkaloids of the leaves and aerial parts of the Crotalaria species being investigated (Mattocks and Nwude, 1988). The extractions were done for the purposes of quantitative estimations, qualitative assessments and toxicity studies. A general procedure used for the extraction is presented in scheme 1. Some slight variations were introduced as discussed in the relevant sections; for example, in carrying out the extraction for the quantitative estimation of total alkaloids, N-oxides were reduced with Zinc dust.

50g Powdered Crotalaria

↓

Extracted with 1.5 litre methanol

↓

Solvent was evaporated

↓

Residue mixed with N/200 H_2SO_4
And the mixture extracted with sufficient ether.

-------- → ether layer

↓

Aqueous solution
Zn dust added to reduce any N-Oxides
[If only basic alkaloids are needed]

↓

Basified with strong Ammonia solution and the alkaloids extracted with excess CH_2Cl_2

-------- → aqueous layer

↓

CH_2Cl_2 layer

↓

The alkaloid fraction
was concentrated to obtain the alkaloid.

SCHEME 1: EXTRACTION OF ALKALOIDS OF THE CROTALARIA SPECIES.

2.6.4 Quantitative Estimations of the Alkaloids of the Crotalaria Species.

Titrimetric methods were used for the quantitative estimation of the alkaloids in the leaves and aerial parts of the Crotalaria species. 50g of powder were accurately weighed and the alkaloids in the powder were exhaustively extracted with 1.5 litre solvents in the same manner as shown in Scheme 1. Total alkaloids were determined by titration as explained below.

The extracted alkaloids were dissolved in 2.0ml of acetone and 10.0ml of ether. The mixture was quantitatively transferred to a flask and 5ml of bromocresol green indicator solution were added. This was then titrated with N/200 sulphuric acid. The titration was carefully carried out until a light greenish colour persisted after shaking (end-point). At this point the titration was stopped. Volume of acid used was noted. The percentage of alkaloids was then calculated as follows:

Each ml of N/200 sulphuric acid is equivalent to 0.00165g of alkaloid. The amount of alkaloid was calculated for each sample. The percentage of total alkaloids was finally calculated with reference to the original weight of the sample as follows for each species.

$$\text{Total alkaloids (\%)} == \frac{\text{Amount of alkaloid in Sample}}{\text{Weight of plant sample used}} \times 100$$

The total alkaloid content of the leaves and aerial parts of each Crotalaria species were thus determined.

2.6.5 Qualitative Assessment of the Alkaloids

Specific tests for Tropane, purine and pyrrollizidine alkaloids was carried out. The alkaloids of the Crotalaria species were extracted as shown in Scheme 1. However, the oxidation of the N-oxides was omitted for the sake of qualitative analysis. This assessment was aimed at identification of the types of alkaloids and their number as found in the leaves and aerial parts of the plants. It was also intended to determine the presence of unsaturated pyrrolizidine alkaloids in the species, which is an indication of the toxicity of the species. The procedure used for each of the above is described below.

2.6.5.1 Specific tests for tropane, purine and pyrrolizidine group

(a) Detection of Unsaturated Pyrrolizidine Alkaloids and N-oxides of the Crotalaria Species.

The Ehrlich reaction method of Mattocks [1971] is the most useful colorimetric method for potentially hepatotoxic pyrrolizidine compounds. It is specific for pyrrolizidine alkaloids and is not useful for any other alkaloids. This qualitative test was used for detection of unsaturated pyrrolizidine alkaloids in the leaves and aerial parts of the three Crotalaria species as shown in Scheme 2.

(b) Test for presence of Tropane Alkaloids

The powder of the aerial part of each Crotalaria species was tested . Alkaloid extract was obtained as shown in scheme 1. Extract was dissolved in two drops of

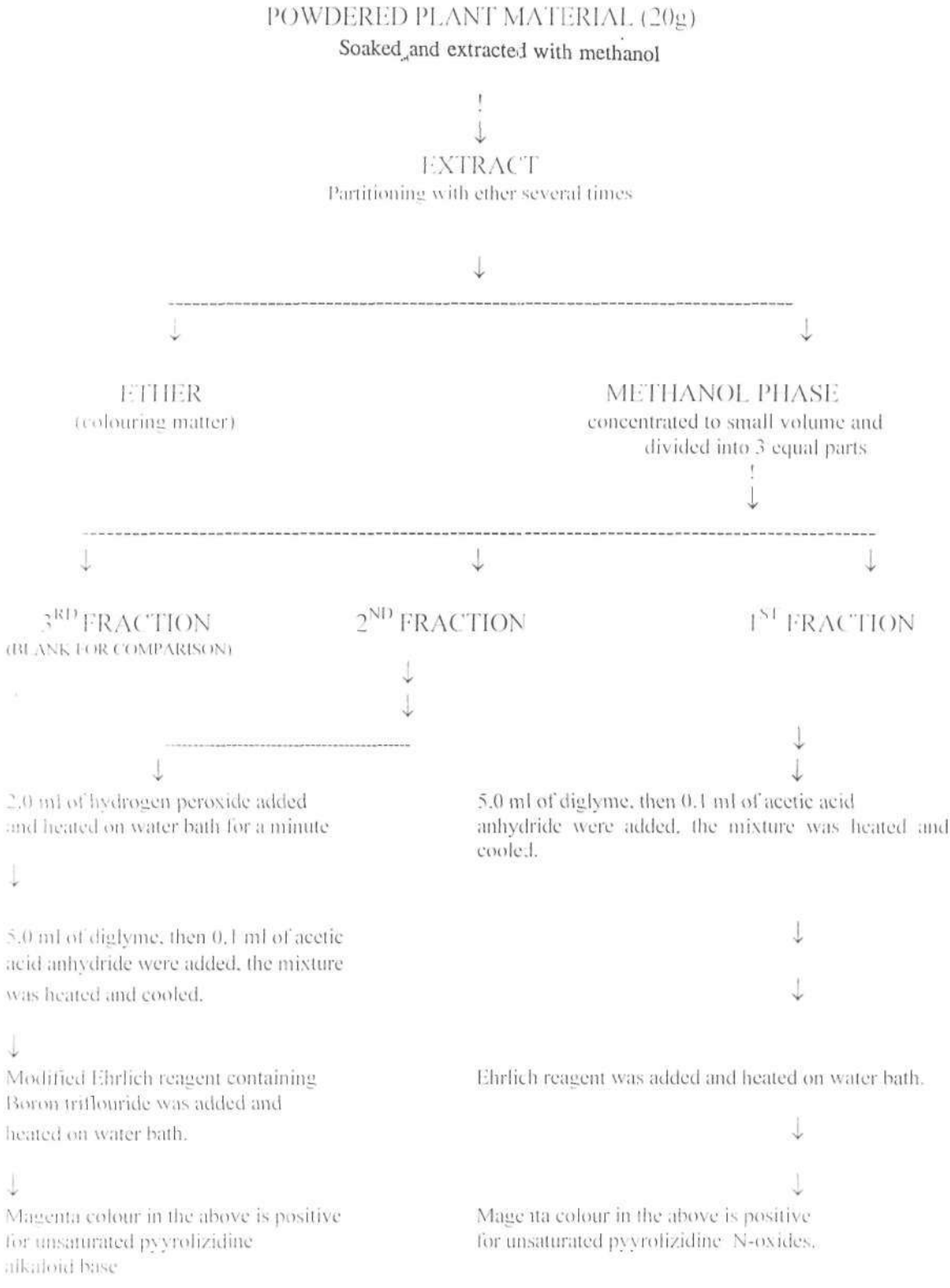
fuming nitric acid. This was evaporated to dryness. The residue was dissolved in 3% methanolic potassium hydroxide. Development of a characteristic diminishing purple colour was taken as positive for presence of tropane alkaloids.

(c) Test for presence of purine alkaloids

Alkaloid extracts of aerial parts of each crotalaria species were tested as follows. On a watch-glass the extract was moistened with three drops of hydrochloric acid. A crystal of potassium chlorate was added. This was evaporated to dryness on water bath. After cooling, two drops of dilute ammonia solution was added. Purple colour indicates purine alkaloids.

2.6.5.2 Identification of the Number and Types of Alkaloids

The number and identity of alkaloids in the aerial parts of the three Crotalaria species were investigated by Thin Layer Chromatography (TLC). However, the problem faced was the lack of pure reference alkaloids. This problem has been recognised by other workers [Bull, et al, 1968; WHO, 1988]. Therefore, R_f values of the pure pyrrolizidine alkaloids noted by other workers under the same parameters were used to identify the alkaloid monocrotaline.



SCHEME 2: EXTRACTION AND TESTS FOR PYRROLIZIDINE ALKALOID BASES AND N-OXIDES.

(i) TLC of the Alkaloids of the Leaves

Test Samples:

A Alkaloids of C. microcarpa leaves

B Alkaloids of C. retusa leaves

C Methanol extract of C. naragutensis leaves (this species does not contain alkaloids according to previous tests on the leaves).

Adsorbent: Silica gel G (Type 60)

Technique: Ascending

Solvent System: Chloroform : methanol : conc ammonia (85:14:1)

Visualisation: Dragendorff's reagent (spray reagent)

(ii) TLC of the Alkaloids of the Aerial Parts

Test Samples:

D Alkaloids of C. microcarpa aerial parts

E Alkaloids of C. naragutensis aerial parts

F Alkaloids of C. retusa aerial parts

Adsorbent: Silica gel G (Type 60)

Technique: Ascending

Solvent System: Chloroform : methanol : conc ammonia (85:14:1)

Visualisation: Dragendorff's reagent (spray reagent)

2.7 TOXICITY ASSESSMENT OF THE EXTRACTED CROTALARIA ALKALOIDS.

Toxicity tests on the Crotalaria alkaloids was carried out using the extracted alkaloids of C. retusa L., as this was found to be the species with significant quantity of alkaloids among the other species. It was also the species that was positive to Mattocks test for unsaturated pyrrolizidine alkaloids. The alkaloids were extracted as indicated in Scheme 1, omitting the reduction of the N-oxides. The toxicity tests were carried out in order to determine if there was any toxic effect of the Pyrrolizidine alkaloids of the Crotalaria species as reported in literature and the organs affected. Therefore parameters for acute toxicity were selected, namely by mode of administration and dosage.

Six healthy albino rats, Rattus species, three males and three females, were obtained from the animal house, Pharmacology Department of the Ahmadu Bello University, Zaria. The male rats weighing 148g, 146g and 141g were designated as U, V and W respectively, while the female rats weighing 139g, 136g, 137g were designated X, Y, and Z. Samples were reconstituted in 50%. These rats were dosed with 40mg/kg body weight of the alkaloids of C. retusa for interperitoneal administration. Behaviour of the animals and general body conditions were noted after administration. Early necropsy examination was conducted after subsequent death of the animals. Gross features of affected organs were noted and photographed. Histopathological examination of the affected organs was also carried out and the observations were recorded and photographed.

2.8 Study and Documentation of Ethnomedicinal Uses of Crotalaria Species, Toxicity Awareness Among Medical and Veterinary Practitioners, Herbalists and Herdsmen in Zaria.

This study was carried out using two approaches:

(1) Questionnaires in English distributed among medical and veterinary practitioners and in Hausa among herbalists and herdsmen in Zaria.

(2) Oral interviews of herbalists and herdsmen.

This study was conducted in view of the world-wide lack of knowledge of the toxicity of the Crotalaria species. The study was aimed at determining any toxic experience among the identified groups and the ethnomedical uses of Crotalaria species among the population. These are important information for future exploitation of the plants and study of toxicity occurrence in Nigeria. Traditional ethnomedical information offers a short cut to the discovery of new medically or industrially useful compounds [Moran, 1996].

The questionnaires were therefore designed and administered to local herbalists, herdsmen, medical doctors and veterinary doctors in Zaria and environs. Samples of the questionnaire are shown in Appendix II.



CHAPTER THREE



CHAPTER THREE

RESULTS AND DISCUSSIONS

3.1 MACROMORPHOLOGICAL FEATURES OF THE VARIOUS PARTS OF CROTALARIA SPECIES

3.1.1 General Features of the Crotalaria Species.

(1) Crotalaria microcarpa

General appearance and habit:

Found in and around Zaria; grows in both sandy and loamy soils; short plant, a herb about 10-50 cm tall; morphological parts are small, densely branched, stems are slender with short hairs and reddish appearance. Crotalaria microcarpa is shown in Plate 1.

Leaf:

Colour: lightly green; type: compound trifoliate; outline: narrowly lanceolate; margin: entire, apex: slightly pointed, acute; base: unequal; size: 1-2 cm long, 0.4-0.9 cm broad; venation: unclear, tiny; stipule present, 1-2 mm long petiole: short, 0.5-0.8 cm long, thin; texture: smooth with short, tiny hairs; midrib: not prominent, clear at lower surface; duration: deciduous; appearance after drying: golden brown.

Flowers:

Occurrence: few, dull appearance, racemes, sessile; timing: November - December; colour: dark-yellow orange; calyx: 5; corolla: hairs present; androecium: 10 stamens; gynaecium: 1 carpel, superior; ovules: 2, ovuled.

Fruits:

Appearance: tiny legume; occurrence: few to many; size: 0.8-1.2 cm long; shape: oblong-ellipsoid; dehiscence: dry indehiscent; opening: regular; seeds No: 2; seed colour: brown; seed shape: oblique-cordiform; seed size: about 1 mm long.

(2) Crotalaria naragutensis

General appearance and habit

Found amply in Zaria; generally found on loamy soil; a shrub of about 70 cm height; many flowers; perennial; a much branched shrub covered with tiny soft hairs. C. naragutensis is shown in Plate 2.

Leaf:

Colour: green, under surface paler; type: compound - trifoliate; outline: narrowly obviate-elliptic; margin: smooth; apex: rounded, to tiny emerginate in some; size: 5-5.5 cm long and 1.5 - 1.7 cm broad; venation: alternate; stipule: none, absent; petiole: long; texture: no hairs visible but upper softer; midrib: clear; base: unequal; duration: deciduous; appearance after drying: pale green to brown.

Flowers:

Occurrence: not many, spread out; timing: July to October; colour: bright yellow; calyx: small 5-7 mm long with oppressed hairs; corolla: 4.

Fruits:

Appearance: inflated, green, lightly brown when dry with many seeds; occurrence: numerous; size: 1.3 cm long 0.5 - 1 cm broad; shape: sub cylindrical; dehiscence: dry indehiscent; opening: regular; seeds No: 18-20 in mature fruits; seed colour: pale brown; seed shape: oblique-cordiform; seed size: 2-3 cm long.

(3) Crotalaria retusa .

General appearance and habit:

Found abundantly in Zaria along roadsides, on abandoned lands and in farms; evergreen with dense yellowish orange conspicuous flowers; found in both sandy and loamy soils; a shrub of about 1 meter height; perennial; well branched, thick ribbed stems; covered with very tiny hairs; has a taproot system with hairs present. Both varieties of retusa and tunguensis occur abundantly. However, this study was carried out on variety retusa. Crotalaria retusa is shown in Plate 3.

Leaf:

Colour: dark green, under surface paler; type: simple - 1 - foliolate; outline: oblanceolate to retuse; margin: entire, smooth; apex: rounded, to slightly emerginate; size: 5-8 cm long, 1.5-4.5 cm broad; venation: alternate; stipule: stipulate, minute, (1-3 cm long); petiole: present thick about 2-4 mm long; texture: even, upper softer than

lower; midrib: prominent; base: equal; duration: deciduous; appearance after drying: greyish.

Flowers:

Occurrence: numerous (1-12) arranged in vertical racemes; timing: July through to October; colour: yellow; calyx: 5 large with dark markings 1-1.5 cm long puberulous; corolla: 3, yellow.

Fruits:

Appearance: inflated, lightly green; clear black when dry with numerous seeds. Occurrence: numerous fruits arranged along a thick stems; size: 4-5 cm long 1.2 broad; shape: cylindrical; dehiscence: dry indehiscent; opening: regular; seeds No: 15-23 for mature fruits; seed colour: yellowish brown; seed shape: oblique-cordiform, indented, one edged longer; seed size: 5-6 cm long.

Features of Dry Powdered Leaves of the Three Species:

C. microcarpa:

Appearance:	Hairy with cotton like appearance.
Colour:	Dull green to greyish on aging.
Taste:	Plain taste, slightly slimy.
Odour:	Slight, not distinct
Powder in Water:	Mucilaginous swelling, absorbs water.

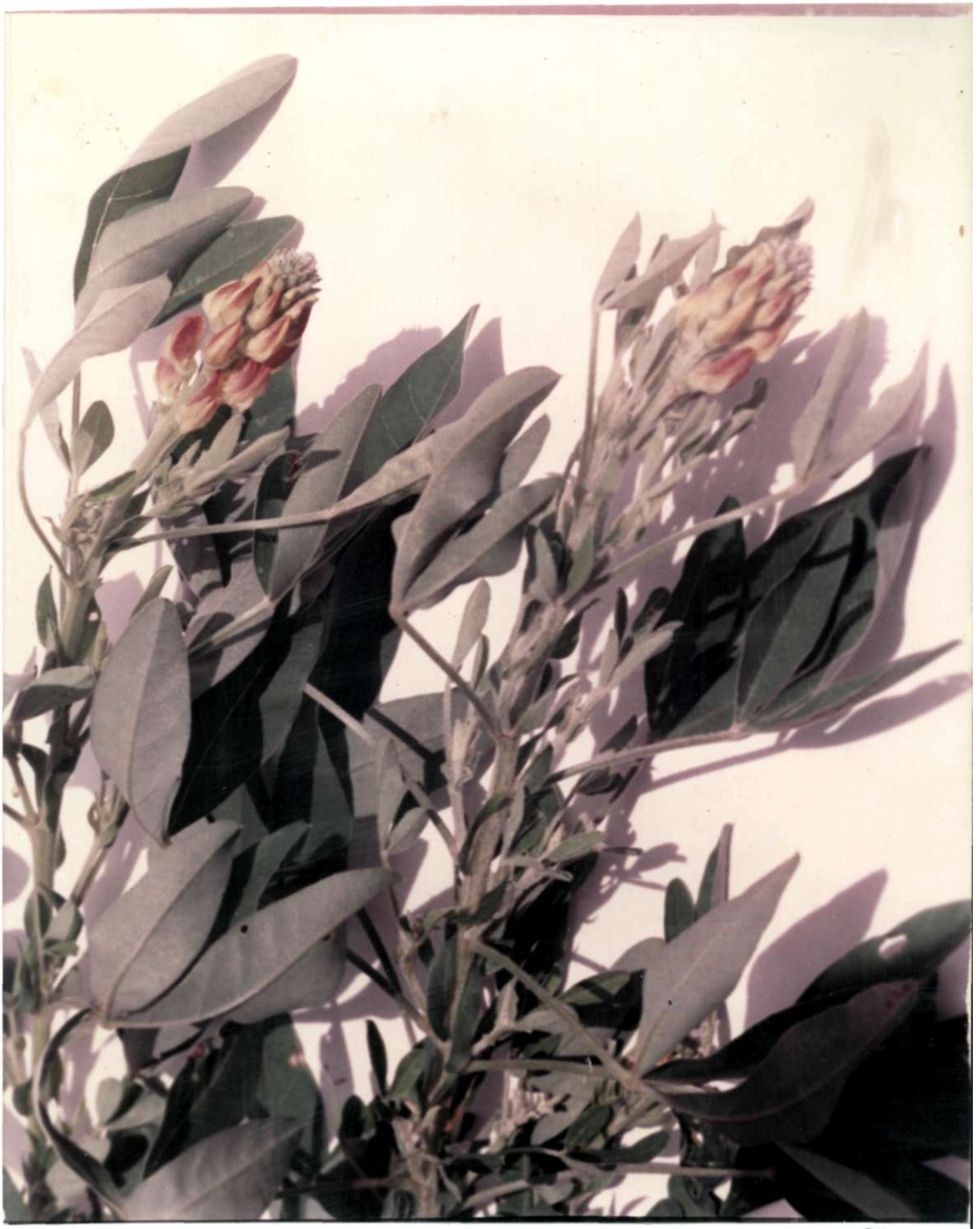
C. naragutensis:

Appearance:	Coarse.
Colour:	Green to brownish tint on aging.
Taste:	Plain taste, slightly slimy.
Odour:	Slight, not distinct
Powder in Water:	Clear swelling, absorbs water.

C. retusa:

Appearance:	Coarse in appearance.
Colour:	Dark green, which gets darker on aging.
Taste:	Slightly bitter.
Odour:	Clear characteristic odour.
Powder in Water:	Slight swelling, tends to displace water down.







3.1.2 Distinguishing Macro Features of the Crotalaria Species.

There are macromorphological features by which the three Crotalaria species could be distinguished. These features are presented in Table 4.

TABLE 4: DISTINGUISHING MACROMORPHOLOGICAL FEATURES OF THE CROTALARIA SPECIES.

PARA METER	C. microcarpa	C. naragutensis	C. retusa
Appearance	Small herb with relatively smaller morphological parts.	Shrub	Shrub, with dense colourful flowers
Leaves	Trifoliate	Leaves have no stipules at base, trifoliate.	Simple, single leaves, greyish when dry.
Flowers	Flowers are dull with the presence of hairs.	Flowers terminally in clusters	Numerous, colourful and vertically arranged
Fruits	Smallest fruit with least number of seeds in mature fruit, usually 2.		Many fruits with numerous seeds, distinctly black when dry.
Stem	Slender with reddish appearance	Brownish	Thick and green

DISCUSSIONS

A study of the macro features of the three Crotalaria species has been carried out. These features have been noted, measured and documented for each species. A comparative survey of distinguishing characters has also been done as shown in Table 4. The macro features of the Crotalaria species investigated have displayed identical features with the same species mentioned by *Polhill (1982) and Hutchinson and Dalziel*

(1958).

These features are important in assessment and identification of whole plants or plant parts. This study has shown that it is possible to distinguish Crotalaria species macroscopically using a combination of two to three macroscopic features. Sizes, colour markings and the presence of hairs on morphological parts were found to be useful criteria in the assessment of whole plants of the Crotalaria species for toxicological and epidemiological purposes. The macro features and distinguishing features are also important in setting macroscopical standards for pharmacopeial documentation of Crotalaria species.

3.2 Microscopical Studies of the Crotalaria Species.

The microscopical studies carried out on the Crotalaria species were both qualitative and quantitative microscopy on the leaves. The results are presented below.

3.2.1 Qualitative Microscopical Features of the Crotalaria Species.

Qualitative microscopical analysis was carried out on the leaves of each species and consisted of the following:

(i) Epidermal characters, general structural features of the powders of the three Crotalaria species.

(ii) Histological sections of the leaves of the plants (transverse section).

(iii) Chemomicroscopy on the powder of each species.

The observations made on each of the above are presented below.

(i) **Structural Features of the Leaves of Crotalaria Species.**

Analytical microscopy shows that the three Crotalaria species possess some common features and specific differences between them. In the lower and upper epidermis of all the three species, covering trichomes were observed. The number of subsidiary cells in the stomata of the species was, however, different. Three subsidiary cells were observed in stomata of C. microcarpa, C. naragutensis and C.retusa while four were also observed in C. retusa. There was abundance of intercellular spaces between cells and abundant fibres of varying types and sizes. The fibres observed were found to be different and this could serve as a distinguishing feature of the species. Group of distinct fibres, centrally joined together with a lumen are a distinct characteristic feature of C. naragutensis . All the species possess simple fibres which are grouped only in C. naragutensis. Simple fibres of C. retusa were observed to be septate. Vessels were also found to be of three types in C. naragutensis (reticulate, spiral and annular), two types in C. microcarpa (annular and spiral) and only annular vessels were observed in C. retusa. The vessels were usually lignified and occur in groups. Vessels in C. naragutensis were much elongated and lignified compared to the other two species. The structural features of the three Crotalaria species are shown in Table 5 and Figures7, 8 and 9.

TABLE 5: MICROSCOPICAL OBSERVATIONS ON STRUCTURAL FEATURES OF THE LEAVES POWDER.

PARAMETER	SPECIES		
	C. microcarpa	C.naragutensis	C. retusa
A. EPIDERMAL CHARACTERS:			
(a) Trichomes: Presence	+	+	+
(a) Occurrence	Both surfaces, usually at stomatal openings.	Both Surfaces	Both surfaces
Type	Covering, bent at end	Covering	Covering
Size (length)	230-500μm	215-350 μm	80-160 μm
Inclusions (cystolith)	+	+	+
Lignification(wall)	+	+	+
(a) Stomata: Upper/Lower surface	More on lower.	More on lower.	More on lower.
No. of subsidiary cells	3	3	3 and 4 on lower usually.
(a) FIBRES:			
2. Types	Simple & in groups	Simple are rare; in groups, distinct	Simple, septate
2. Abundance	++	++++	++
Sizes	320-480 μm	263-430 μm	124-173 μm
(a) VESSELS (Xylem)			
Types	Annular & spiral.	Reticulate, spiral, annular.	Annular.
Abundance	++	+++	+
Lignification	Significant	Heavy	Slight
(b) CALCIUM OXALATE CRYSTALS:			
Types	3, tetragonal & prism.	Single, prism type.	3, tetragonal & prism.
Abundance	Abundant	Few/rare	Abundant
5. STARCH GRAINS: Presence	Absent	Absent	Present
(a) ALEURONE GRAINS:			
6. Presence	+	+	+
Occurrence	Numerous in mass	Few aggregates	Numerous in mass
7. SCLEREIDS	Few	Abundant	Abundant

KEY: + : Present

- : Absent

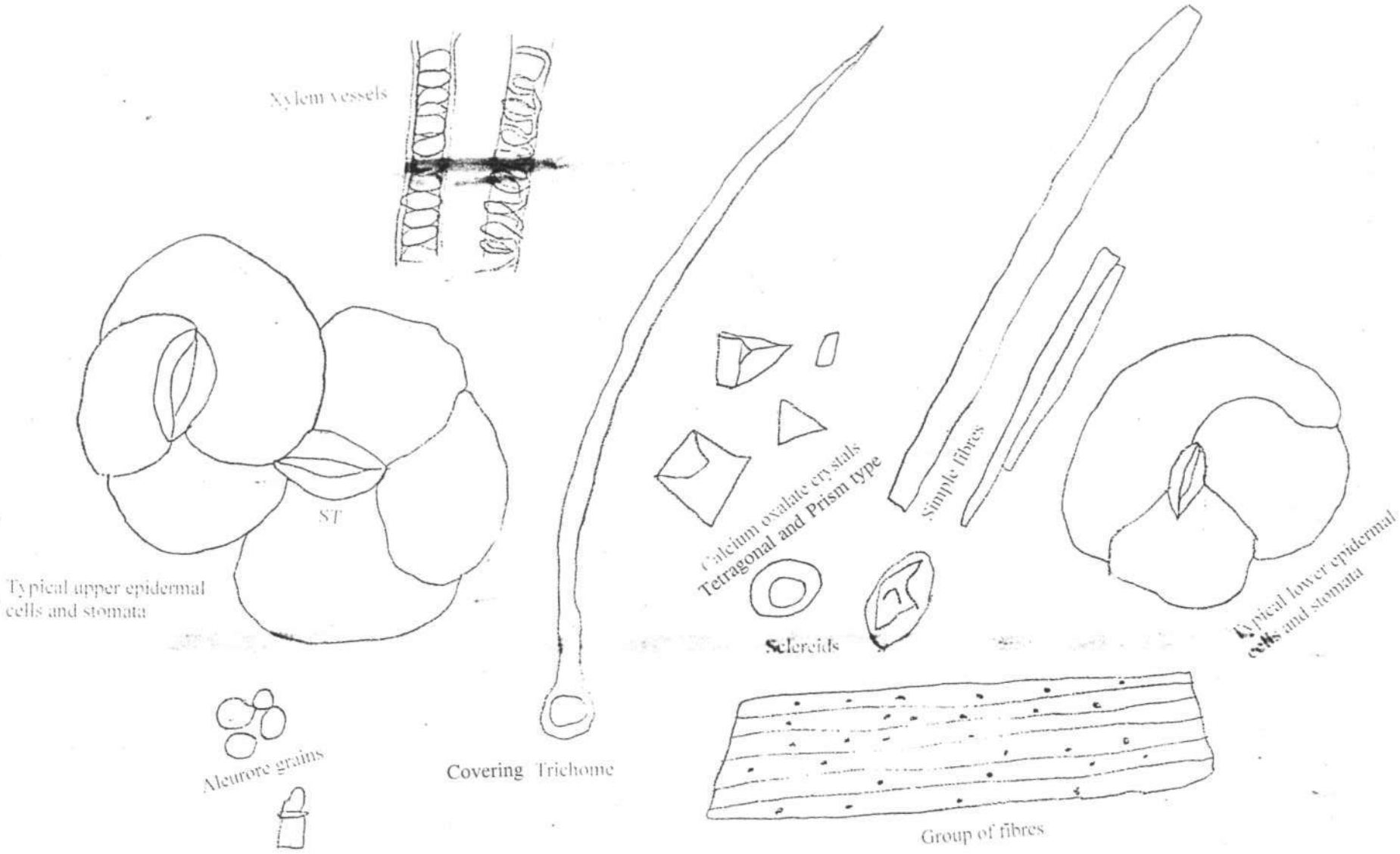


FIGURE 7: EPIDERMAL CHARACTERS AND GENERAL STRUCTURAL FEATURES OF C. microcarpa LEAVES X 400.

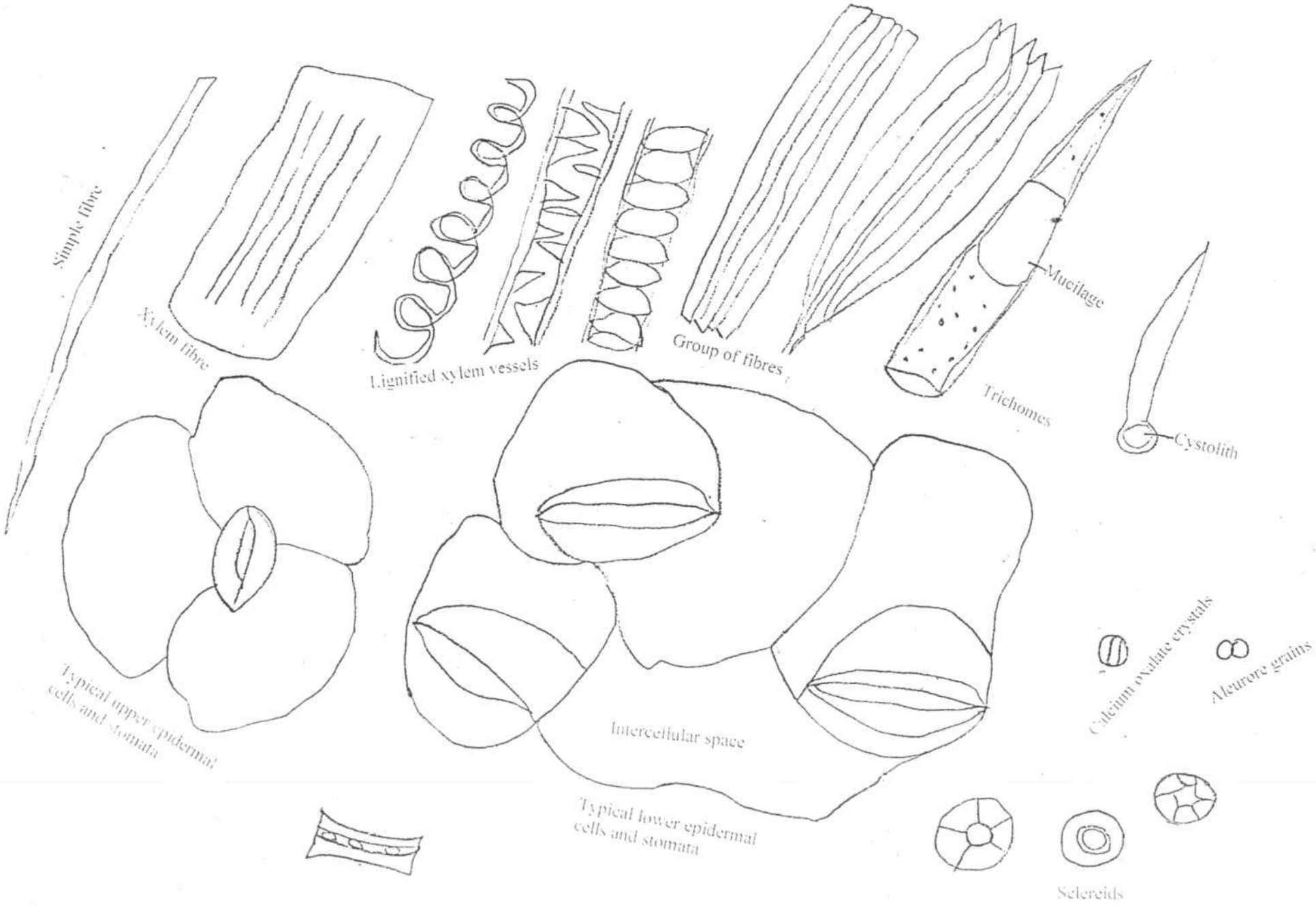


FIGURE 8: EPIDERMAL CHARACTERS AND GENERAL STRUCTURAL FEATURES OF C. naragutensis LEAVES X 400.

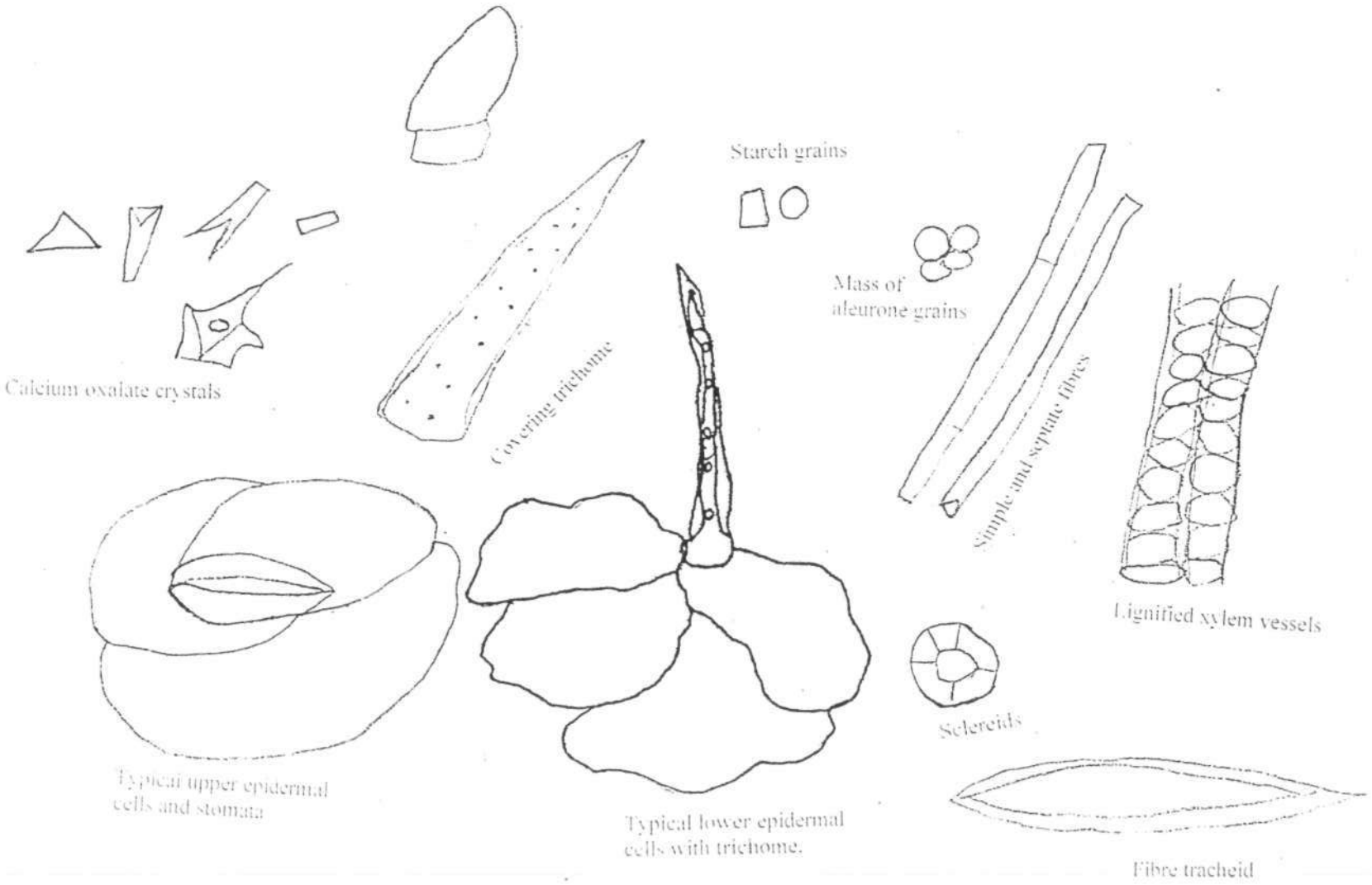


FIGURE 9: EPIDERMAL CHARACTERS AND GENERAL STRUCTURAL FEATURES OF C. retusa LEAVES X 400.

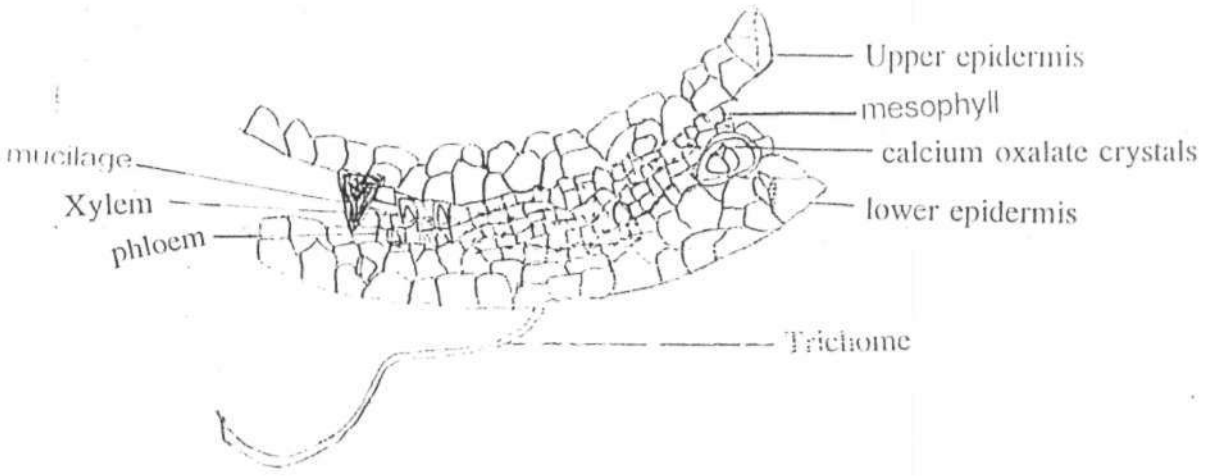
ii) Histological Sections of the Crotalaria Species.

Transverse sections of the leaves of the three Crotalaria species were prepared, magnified and drawn. These are shown in Figure 10. The leaves sections reveal important structural and chemical features of the Crotalaria species. A single epidermal layer was seen in C. naragutensis and C. microcarpa. Cells of the epidermal layers of C. retusa are much smaller than in the other two. The mesophyll displayed significant specialisation. A single layer of palisade tissue is present. A well developed internal spaces could be seen within the mesophyll of notably C. retusa and C. microcarpa. Calcium oxalate crystals are present in all the plants along with abundant tannins and mucilage containing cells, which are more in C. microcarpa. Vascular tissues especially Xylem elements are clearly abundant. Vascular bundles are accompanied by sclerenchyma. Schizogenous spaces and canals were noted in C. retusa and C. naragutensis. Veins in C. retusa are accompanied by well developed phloem fibres.

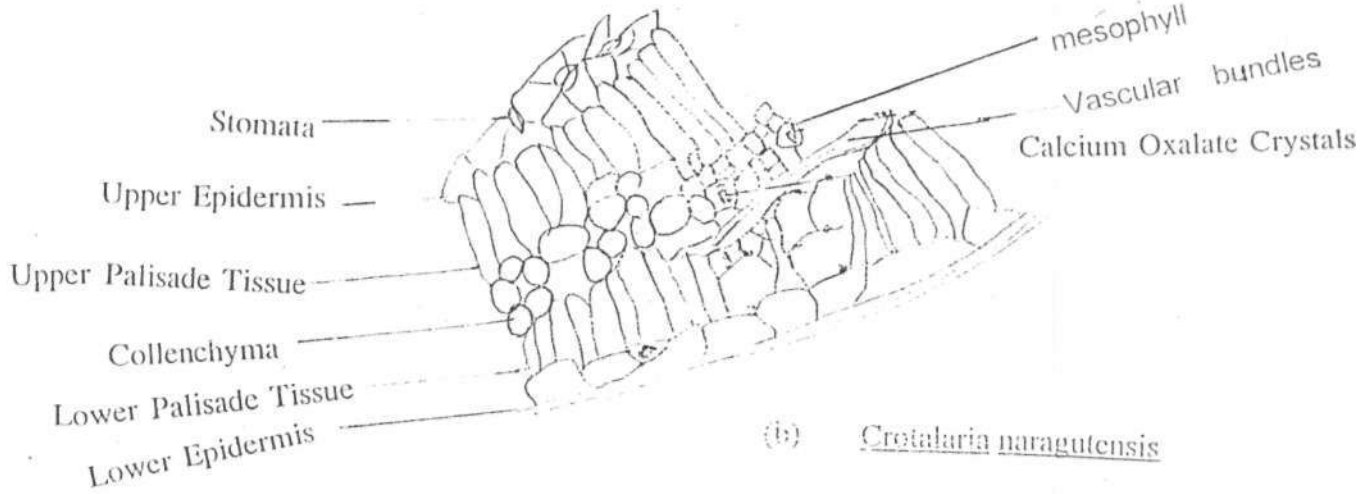
iii) Chemomicroscopy of the Powdered Leaves

Chemomicroscopical examination of the powdered leaves of the three Crotalaria species detected the presence of proteins, lignin, cellulose, fats and oils, mucilage, tannins and calcium oxalate crystals in all the species. Variations were, however, noted in the amounts and type of these substances. The results are shown in Table 6.

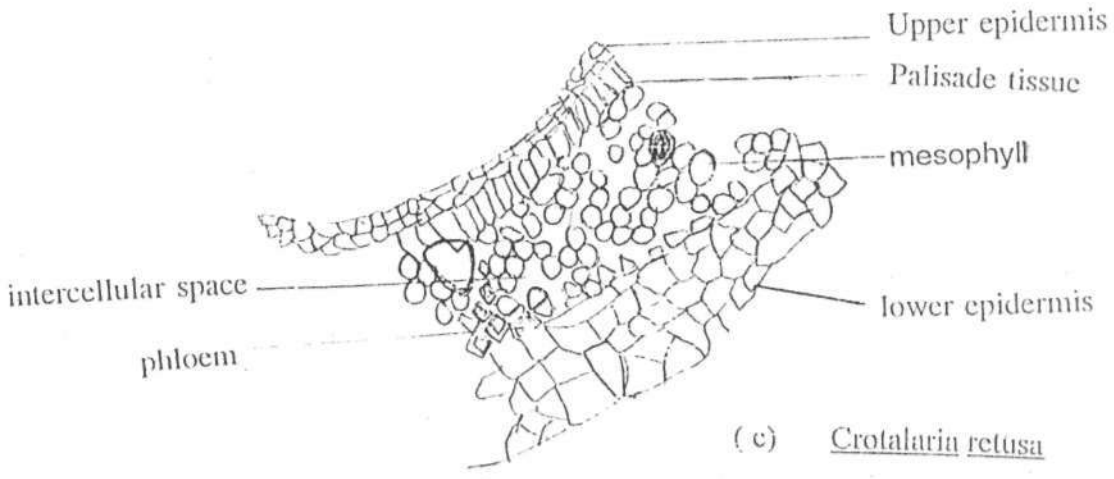
C. microcarpa and C. retusa appear to contain condensed tannins. Starch was also observed in C. retusa only and abundance of diosmin deposits was noted in C. microcarpa only.



(a) Crotalaria microcarpa



(b) Crotalaria naragutensis



(c) Crotalaria retusa

FIGURE 10: TRANSVERSE SECTIONS OF THE LEAVES OF CROTALARIA SPECIES. X 100.

TABLE 6: SUMMARY OF THE RESULTS OF THE CHEMOMICROSCOPICAL EXAMINATION ON THE LEAVES OF THE THREE CROTALARIA SPECIES.

CONSTITUENTS	C. microcarpa	C. naragutensis	C. retusa
LIGNIN	+	+	+
CELLULOSE	+	+	+
TANNINS	+ (X)	+ (Y)	+ (X)
GUMS & MUCILAGES	+ + +	+ +	+
STARCH	-	-	+
CALCIUM OXALATE CRYSTALS	+ +	+	+ +
FATS & OILS	+	+	+
PROTEINS	+	+	+
DEPOSITS OF DIOSMIN	+	-	-

KEY:

+ : Present
- : Absent
(X): Indicates Condensed tannins
(Y): Condensed and hydrolysable tannins.

3.2.2 Quantitative Microscopy.

Quantitative microscopical determinations of stomatal number, stomatal index, palisade ratio, vein islet and vein termination numbers were carried out on the Crotalaria species. The average results are presented in Table 7. The diagrams from which these results were deducted are shown in Appendix IV.

TABLE 7: QUANTITATIVE MICROSCOPICAL CONSTANTS OF THE CROTALARIA SPECIES (LOWER EPIDERMIS).

CONSTANT	C. microcarpa	C. naragutensis	C. retusa
6.STOMATAL NUMBER	83	37	66
STOMATAL INDEX	25.2	15	23
PALISADE RATIO	3.8	3.6	5.1
VEIN ISLET NUMBER	13	7	6
VEIN TERMINATION NUMBER	7	10	9

.Discussions:

Microscopical examinations of Crotalaria species has, for the first time, been undertaken and the various microscopical features and constituents of the species have been determined. The Microscopical examinations revealed the presence of anisocytic stomata in all the species. Number of stomata is more numerous on lower surface as is normally the case in most plants. Epidermal features indicate distinct presence of covering trichomes with a characteristic deposit of cystolith at the swollen base. A lignified reticulate xylem vessel is present in all the species. Numerous fibres of varying length were also observed in the specie. Chemomicroscopical examinations revealed the presence of proteins, lignin, mucilages, tannins and cellulose in all the species while differences were observed in the presence of starch, tannins and calcium oxalate crystals. Abundance of fibres was also noted.

Quantitative microscopy indicated that stomatal number could be a good criteria for the identification of the species, especially C. naragutensis. Other distinguishing microscopical parameters found were the sizes of the trichomes and types and sizes of

the fibres found in the plants (see Table 5 for details). Starch grains were noted only in Crotalaria retusa. Transverse sections of the plants confirmed some of the above observations.

The microscopical features of the Crotalaria species are typical of the Leguminosae with slight species variations. These have been reported by some workers who investigated other plants of this family. Metcalfe and Chalk [1950] explained that vascular bundles accompanied by sclerenchyma (observed in sections of the plants), is common in all tribes of Leguminosae. Esau [1971] also noted that Tanninferous idioblasts occurs in Leguminosea and gum ducts. Similarly, Fahn [1982] noted idioblasts with crystal in other Leguminosae and also the presence of osteosclereids in their leaves. The numerous air spaces within the mesophyll facilitate rapid gas exchange for photosynthesis. The abundant air spaces noted in C. retusa and C. microcarpa could be an important criteria in the assessment of the species, as this feature has been utilised in other plants [Turrel, 1944].

3.3 Determination of Physical Constants of the Crotalaria Species.

Four physical constants were determined for each Crotalaria species, namely :

- Moisture content
- Ash value
- Acid insoluble ash
- Alcohol soluble extractive value

The average values obtained for each species are given below in Table 8. The details of the calculations of the physical constants are shown in Appendix IV.

TABLE 8: PHYSICAL CONSTANTS OF THE THREE CROTALARIA SPECIES (% W/W).

Physical Constant	C. microcarpa	C. naragutensis	C. retusa
1. Moisture content	5.6	17.5	14
2. Ash value	20	7.5	12.5
3. Acid insoluble ash	15	2.5	2
4. Alcohol soluble extractive	15	3	2.2

Discussion:

The physical constants of the Crotalaria species show significant variation. The only reference to similar works could only be traced to Bartha (1970) who found the ash values of some other Crotalaria species namely, C. macrocalyx and C. arenaria to be 6.2% and 12.1% respectively. His results indicate the wide range of variation of the ash values of different Crotalaria species as noted here. Among the three species being investigated, C. microcarpa has distinct physical values that could be used in its assessment.

3.4 Phytochemical Study of Crotalaria Species.

The phytochemical investigations carried out on the three Crotalaria species covered the following areas:

(i) Initial phytochemical screening for presence of carbohydrates, flavonoids, resins, anthraquinones, tannins and cardiac glycosides.

(ii) Detection of alkaloids and specific tests for some alkaloids.

(iii) Extraction and quantitative estimation of the alkaloids.

(iv) Thin Layer Chromatography (TLC) of the alkaloids.

The results are presented below in tables 9-11.

3.4.1 Phytochemical Screening of the Crotalaria Species.

The Crotalaria Species studied were found to contain varying amounts of chemical constituents. These are shown in Table 9.

TABLE 9: PRELIMINARY PHYTOCHEMICAL CONSTITUENTS OF THE CROTALARIA SPECIES.

CONSTITUENT	C. microcarpa	C. naragutensis	C. retusa
1. Carbohydrates	+	+	+
2. Reducing sugar	+	+	+
3. Monosaccharides	-	-	+
4. Flavonoids	+	+++	-
5. Resins	-	-	-
6. Anthraquinones	+	-	-
7. C-glycosides	+	-	-
8. Tannins	+	+	+
9. Saponins	-	-	-
10. Alkaloids	+	+	+
11. Steroidalring	+	+	-
12. Cyanogenetic glycosides	-	+	-

KEY: + : Present - : Absent

3.4.2 Extraction and Quantitative Estimations of Total Alkaloids.

Powdered leaves (50g) were exhaustively extracted, and the alkaloids dried. The extracted alkaloids were dissolved in acetone and ether and titrated with N/200 sulphuric. Bromocresol green solution was used as an indicator. The volume of acid used was noted and equivalent amount of alkaloid was calculated.

The volume of acid used for each method is presented here, and the calculated percentages of the alkaloids. Details about the calculations are given in Appendix IV.

TABLE 10: THE QUANTITATIVE PERCENTAGES OF TOTAL ALKALOIDS IN THE LEAVES OF THE CROTALARIA SPECIES.

Crotalaria Species	Volume of Acid Used (Average), ml	Amount of Alkaloid	
		Gramme	Percentage (%)
C. microcarpa	18	0.030	0.06
C. retusa	52	0.086	0.172

NOTE: C. naragutensis contains no alkaloids in the leaves

TABLE 11: THE PERCENTAGES OF TOTAL ALKALOIDS IN THE AERIEL PARTS OF THE CROTALARIA SPECIES.

Crotalaria Species	Volume of Acid Used (Average), ml	Amount of Alkaloid	
		Gramme	Percentage (%)
C. microcarpa	23	0.038	0.08
C. naragutensis	16.5	0.027	0.06
C. retusa	93	0.154	0.31

3.4.3 Qualitative Assessment of the Alkaloids.

(i) Specific tests indicated that purine alkaloids are absent, tropane were detected in C. microcarpa while pyrrolizidine alkaloids was detected in all the three species.

(ii) **Tests for the presence of unsaturated pyrrolizidine alkaloids and N-oxides.**

Mattock Tests for unsaturated pyrrolizidine alkaloids in the leaves and aerial parts of the plants revealed that only C. retusa contains both unsaturated pyrrolizidine alkaloids and N-oxides. Since only unsaturated pyrrolizidines are known to be toxic, C. retusa

was, therefore, assumed to be a potentially toxic species and its toxic effects were accordingly studied on rats (section 3.5). Table 12 gives the results of the Mattocks Test for the three Crotalaria species. The method is specific for pyrrolizidine alkaloids.

TABLE 12: DETECTION OF UNSATURATED PYRROLIZIDINE ALKALOIDS AND N-OXIDES.

CONSTITUENT	C. Microcarpa	C. naragutensis	C. retusa
Unsat P.A. base in leaves	-	-	+
Unsat P.A. N-oxides in leaves	-	-	+
Unsat P.A. base in aeriel parts	-	-	+
Unsat P.A. N-oxides in aeriel parts	+	+	+

KEY: + : present; - : absent

(iii) TLC Separation of the Alkaloids

Thin Layer Chromatography (TLC) of the species revealed the presence of alkaloids in the leaves and aeriel parts of the plants, except the leaves of C. naragutensis. A total of three alkaloids were detected in C. retusa , four in C. microcarpa while two were detected in C. naragutensis. The R_f values of these alkaloids which showed Dragendorff's positive spots are presented in Table 14.

TABLE 13: TLC SEPARATION OF ALKALOIDS OF CROTALARIA SPECIES.

SAMPLES	PARTS AND R_f VALUES		NUMBER OF ALKALOIDS DETECTED
	Aeriel parts	Leaves only	
Alkaloids of C. microcarpa	0.36 0.39 0.45 0.51	0.36 0.39 0.43 -	4
Alkaloids of C. naragutensis	0.36 0.43	No alkaloids	2
Alkaloids of C. retusa	0.46 (monocrotalaine) 0.58 0.68	0.45 0.58 -	3

DISCUSSIONS:

Phytochemical study of Crotalaria species gave valuable information on the plants. Initial screening showed that the plants contain carbohydrates, flavonoids and tannins. Resins and saponins were absent. However, anthraquinones and cardiac glycosides were detected in C. microcarpa only. Alkaloids were detected in the leaves and aerial parts of the three species except the leaves of C. naragutensis. Tropane alkaloids was also for the first time detected in C.microcarpa.The parcentage of alkaloids was found to be highest in the aerial parts of C. retusa 0.31% and 0.17 % in the leaves. Amount of alkaloids in the others is minimal with the least in the aerial parts of C. naragutensis (0.06%) and C. microcarpa leaves, while the leaves and aerial parts of C.

microcarpa contained 0.08% of total alkaloids. Specific Mattocks test for toxicity indicated that only C. retusa contains unsaturated and potentially toxic pyrrolizidine alkaloid bases and N-oxides. C. naragutensis and C. microcarpa do not contain unsaturated pyrrolizidine alkaloid bases but only N-oxides in their aerial parts.

Further separation of these alkaloids by TLC revealed the presence of a total of three alkaloids in C. retusa, four in C. microcarpa and only two in C. naragutensis. An attempt has been made to identify these alkaloids using R_f values of previous workers obtained under similar conditions. As explained earlier, reference samples of pure pyrrolizidine alkaloids could not be obtained but R_f values of pure monocrotaline under this set up was known from other workers as 0.46 [Pilbeam, et al, 1983]. From this, the presence of monocrotaline in C. retusa has been confirmed. Some of the alkaloids of C. naragutensis appear to be similar to those of C. microcarpa, while those of C. retusa are different with higher R_f values. The alkaloids detected may be bases or N-oxides.

The above results agree with the work of many others with little variation especially in the case of C. retusa and C. naragutensis. There is no reported work on the phytochemical features of C. microcarpa Initial phytochemical screening of the Crotalaria species shows the typical features of many leguminous plants, namely presence of tannins and mucillages. Flavonoids have earlier been detected by Ogbeide and Parvez [1992] in the shoots of C. retusa , while saponins were not detected in the shoots of the plant by Sinha and Dogra [1985]. C. naragutensis leaves show abundance of flavonoids which is reported in this study for the first time. Cardiac glycosides and anthraquinones were also detected in C. microcarpa only. Yadava and Thakur [1995] detected a

cardenolide in the seeds of C. retusa .

Alkaloid percentage in the plants varied with many factors. It was found to be highest in the aerial parts of C. retusa. These species have been reported to normally contain high content of alkaloids up to 1% by Bull et al [1968] who, however, noted that low amounts of alkaloids could be as a result of decomposition of alkaloids during drying of plant material. Losses of 50-80 % of alkaloids were observed in many species of Crotalaria by Culvenor and Smith [1967]. Sinha and Dogara [1985] reported no alkaloids in the shoots of C.retusa grown in Bhagalpur, India. Kumari et al [1966] reported a high monocrotaline strain of C. retusa, containing about 9% of alkaloids in the seeds. Normally, alkaloid percentage is highest in seeds than aerial parts of Crotalaria species. Similarly, N-oxides were found to accumulate more in the leaves while basic alkaloids are more in the seeds[WHO, 1988]. In the case of C. naragutensis, Mattocks and Nwude [1988] did not detect any alkaloids in the leaves of this plant, but detected four in the seeds, namely nilgirine, usaramine, acetyl integerrimine and integerrimine. In C. retusa, the presence of monocrotaline, retusamine, retusine and retronecine have been confirmed by various workers and the toxicity of this plant has been attributed to its monocrotaline content. The relationship of structure to toxicity has been explained in Chapter 1. The percentage of alkaloids in a plant is not critical to its toxicity, but what determines the toxicity is the nature of the individual alkaloids present. Therefore, the toxicity study of the alkaloids of C. retusa on rats was undertaken as presented in section 3.5.

3.5 TOXIC FEATURES OF C. retusa ALKALOIDS.

The alkaloids of Crotalaria retusa proved to be toxic on all the Albino rats used in the experiment. The toxic effects observed could be categorised into three:

- Behaviourial and body symptoms displayed after I.P. injection of the alkaloids.
- Gross morphology of body organs after autopsy examination.
- Histopathological studies/examination of body organs.

Though male and female rats were used for this study, both displayed identical symptoms in all the results of each of the above categories. The details of each is presented below.

3.5.1 Behavioural and Body Symptoms Displayed After I. P. Injection of the Alkaloids:

The gross toxic effects observed could be summarised in Table 14.

TABLE 14: SIGNS DISPLAYED BY HEALTHY ALBINO RATS AFTER I.P. INJECTION OF C. RETUSA ALKALOIDS.

S/NO.	SPECIES	SEX & WEIGHT (g)	BEHAVIOUR	ONSET OF SYMPTOMS (min)	SURVIVAL TIME (hr:min)	TOTAL DOSE mg/kg
1.	Albino rat U	Male, 148	Restlessness, inactive, loss of vigour, sleep, quiet death and apparent loss of weight.	15	7:00	40
2.	Albino rat V	Male, 146	--do--	20	9:00	40
3.	Albino rat W	Male, 141	--do--	15	8:00	40
4.	Albino rat X	Female, 139	In addition to above, wrinkling and loss of hair	29	25:00	80
5.	Albino rat Y	Female, 136	--do--	19	9:00	40
6.	Albino rat Z	Female, 137	--do--	21	9:20	40

3.5.2 GROSS MORPHOLOGY OF BODY ORGANS AFTER NECROPSY EXAMINATION.

Gross necropsy findings indicated that the symptoms displayed by the rats were quite similar. It is therefore appropriate to take any single one to illustrate the toxic effects of Crotalaria alkaloids. The gross pictures are shown in Plate 4 and details are in the Pathologist's report. Gross appearance indicated that the liver, lungs and intestine were the affected organs. Necrotic foci were observed in the liver, with the liver being firm and mottled dark. Pulmonary congestion was observed in the lungs while the intestine was clearly congested. No growth lesion was observed in the spleen, stomach,

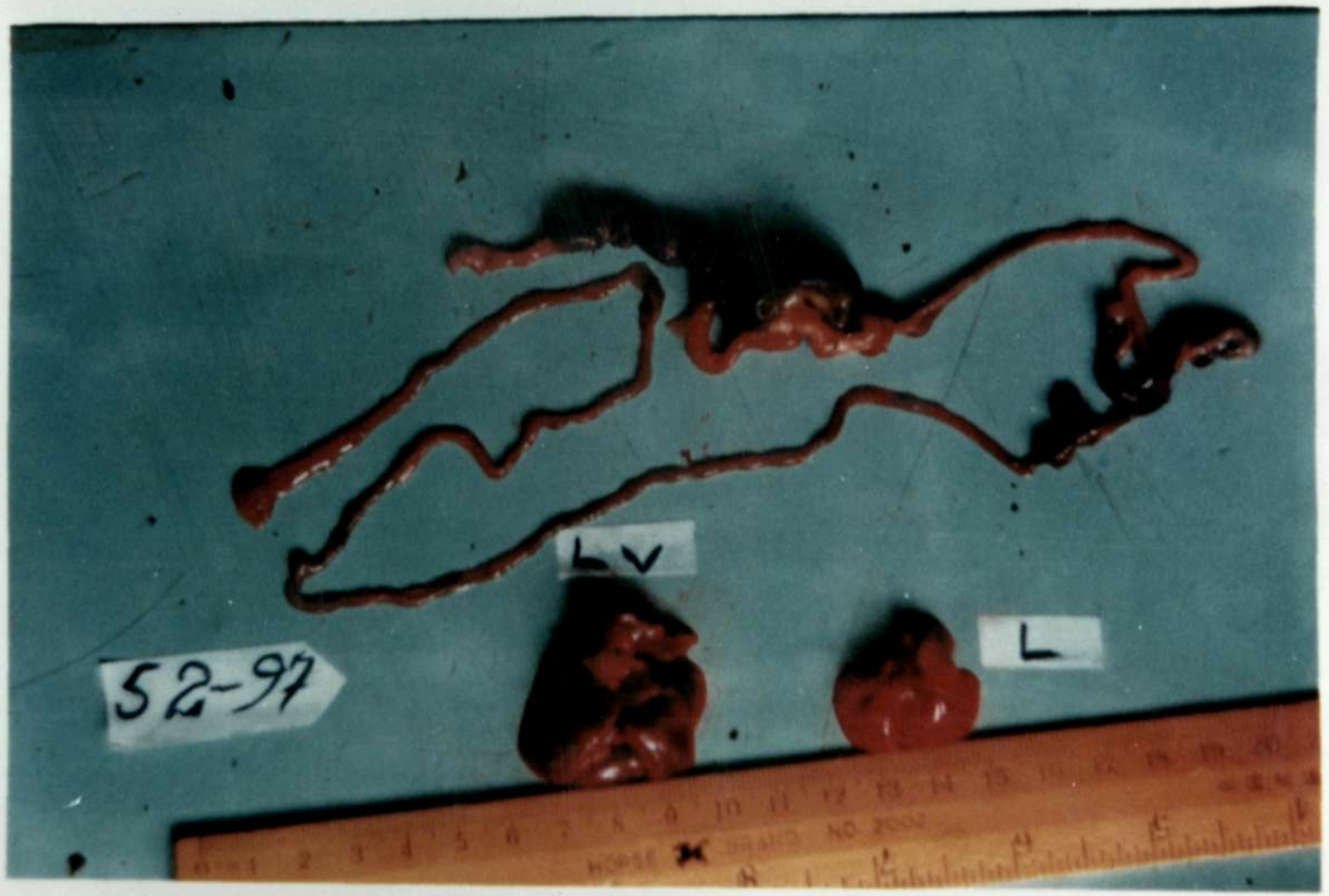
uterus and testis. Specimens of these major organs were histopathologically examined in plate 5.

3.5.3 HISTOPATHOLOGICAL ASSESSMENTS OF BODY ORGANS

A detailed histopathological analysis of the various organs of the rats dosed with the alkaloids of C. retusa reveals interesting results. These results are better illustrated separately for each organ.

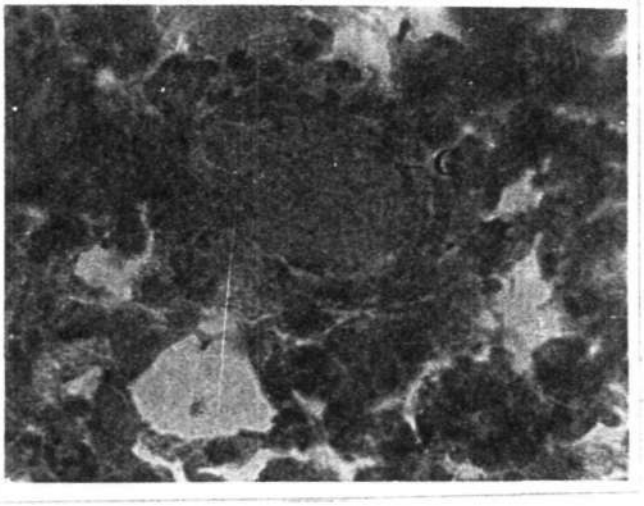
LIVER:

The liver proved to be the principal target of attack by the alkaloids. It was found to be the site of extensive changes. Necrosis, haemorrhage and hyperaemia were noted in the liver. Necrosis of hepatocytes was at portal areas (PA) as well as non-suppurative centrilobular necrosis (CN) were observed, as shown in Plate 6. The necrosis of the liver was clearly significant as shown in Plate 5(a).

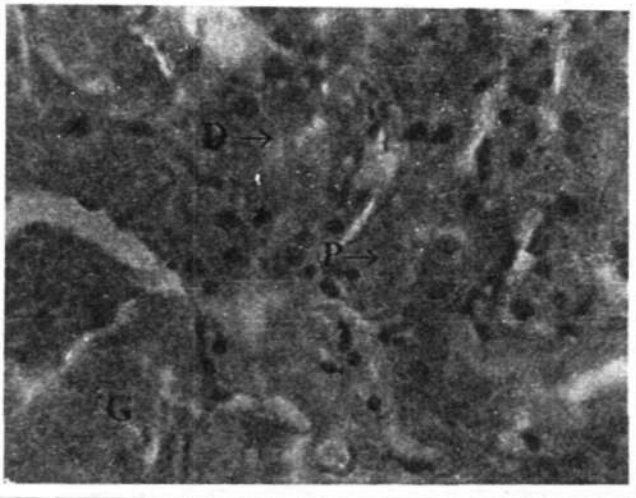

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(a) **THE LIVER**: Note the non-suppurative centrilobular necrosis (CN) and focal areas of necrosis of the hepatocytes at the portal areas in the liver. H & E. X 400.



(b) **THE LUNGS:** Note the congested blood vessels (C) and haemorrhage in the lung parenchyma. H & E. X 400



(c) **THE KIDNEY**: Note the non-suppurative necrosis of the glomeruli (G), proximal (P) and distal convulated tubules (D) of the kidney. H & E. X 400.

PLATE 5: HISTOPATHOLOGICAL FEATURES OF TOXICITY OF C. retusa ALKALOIDS IN RATS.

AHMADU BELLO UNIVERSITY
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY

Necropsy No. 52-97

Date : 22/10/97 Clinic No. GPI

Animal _____ Species Rattus Age _____ Sex M & F Breed Albinus

Owner Mrs Hadiza Nuhu Clinician Dr. Tahir

History : Experimentally injected intraperitoneally with 40mg/kg body of crude alkaloid on mixture of C. retusa on 20th October, 1997.

Gross Necropsy Findings :

Liver :- Necrotic foci in the liver.
Lung :- Pulmonary congestion.
Intestine :- Slightly congested intestine.
NAD :- Kidney, spleen, stomach, uterus and testis.

Tentative Diagnosis Experimental toxicity studies with C. retusa.

Comments

1. Specimens for histopathology :- liver, lung, intestine, heart, spleen, brain.
2. Gross photograph.

Pathologist Dr. N. D. G. IBRAHIM

Name of Owner: Mrs. Idris Bugaji

Address: Faculty of Pharmaceutical Sciences

Animal specie: Albino rate
(Rattus sp)

History: Experimentally dosed with 40mg/kg body weight of Pyrrolizidine alkaloids from Crotalaria retussa for toxicity studies.

RESULT
HISTOPATHOLOGY REPORT

Non-suppurative centrilobular necrosis and focal areas of necrosis of the hepatocytes at the portal areas (Fig A) and haemorrhage in the liver. Non-suppurative necrosis of the glomeruli, proximal, distal and collecting tubules of the kidney (Fig B). Congested blood vessels and haemorrhage in the lung parenchyma (Fig C). Non-suppurative necrosis of the intestinal villi. No significant microscopic lesions in the brain and spleen.

DR. N.D.G. IBRAHIM
HISTOPATHOLOGIST

LUNGS:

The blood vessels of the lungs were congested. Haemorrhage was evident in the lung parenchyma as shown in Plate 5(b).

INTESTINE:

The intestine displayed significant hyperaemia (congestion) and non-suppurative necrosis of the intestinal villi. This is shown in Plate 4.

KIDNEY:

Non-suppurative necrosis was also noted in the kidney at the glomeruli (G), proximal (P) and distal convoluted tubules (D) as shown in Plate 5(c).

BRAIN AND SPLEEN:

There was no significant microscopic lesions detected in the brain and spleen.

DISCUSSIONS:

Toxicity evaluation of Crotalaria species (or any pyrrolizidine alkaloid plants) is essential for the following reasons:

1) Direct testing of plants or their extracts allow observation of not only their hepatotoxicity but other pathological effects which it might have. It was by

pharmacological testing of the extracts of Crotalaria incana and Crotalaria mucronata that the neuro-lathyrogen α-amino-β-oxalyaminopropionic acid was detected [Bell,1968].

2) To assess the toxicity of the species singly, especially for plants or families known to produce potentially toxic compounds.
3) Pure alkaloids may not be available for analysis such as chromatographic comparisons and there are limited number of pyrrolizidine alkaloids isolated to date. Testing allows the detection and analysis of pyrrolizidine alkaloids or their derivatives.

The toxicity of Crotalaria alkaloids was therefore evaluated using the alkaloids of Crotalaria retusa L. as it was the species which not only has an appreciable yield of alkaloids among the three species under this investigation, but tests for unsaturated hepatotoxic alkaloids showed that this species contains toxic basic alkaloids. The method used for toxicity assessment was discussed in section 2.7 and results presented in the above. These results clearly indicate the cytotoxicity of the alkaloids in all the rats used in this investigation.

The effect of alkaloid mixture of C. retusa on white rats was assessed in this work for the first time. Alkaloids of C. retusa L. proved toxic to rats at the experimental dose. These findings agreed with the work done on other related species by others (Mattocks, Culvenor, Schoentel) with slight variations. This confirms that C. retusa contains toxic pyrrolizidine alkaloids. All researchers indicated that the liver was the principal target organ of pyrrolizidine alkaloids toxicosis. However, differences arise as to the toxicity in other body organs such as lungs and kidney. Sub-acute and chronic liver changes were

also observed in rats after a single dose of various pyrrolizidine alkaloids of Senecio (Schoentel and Magee, 1959). Toxicity pattern in these organs is dependent on the nature of the individual pyrrolizidine alkaloids which is difficult to confirm by toxicity tests alone.

An exposure to C. retusa in a piggery was observed to cause poisoning in Australia. Animals consume the plant at the rate of about 0.1% weight for three weeks. Symptoms displayed by the animals were mainly reduced weight, inapetite while pathological features were megalocytosis of liver and kidney, nephrosis, interstitial pneumonia accompanied by microscopic diseases (Hooper and Scanla, 1977). Schoentel (1968) and many others confirmed that some of the pyrrolizidine alkaloids could induce chronic pathological lesions in organs other than the liver. In experimental rats, especially in white rats lesions could be induced after only one to a few doses. Similarly, changes in the structural and functional integrity of endothelium have been recognised as early features of progressive pulmunary vascular injury caused by the pyrrolizidine alkaloid monocrotaline; Hoorn and Roth (1993) concluded that high concentration of monocrotaline pyrrole decreases endothelial activity.

Monocrotaline is an important pyrrolizidine alkaloid found mainly in section Calycinae and Crotalaria among the sections of the genus Crotalaria (Pilbeam et al, 1983). In a related work, Culvenor et al (1976) screened 62 pyrrolizidine alkaloids for hepatotoxicity and pneumotoxicity. He noted that lung lesions are produced by most pyrrolizidine alkaloids that induce liver lesions, especially at higher doses. He attributed toxicity to the macrocyclic retnonecine diesters (such as crispatine, senecionine, seneciphylline and usaramine), crotonecine diesters (such as anacrotine, madurensine)

and heliotridine esters (such as heliosupine, lasiocarpine, rinderine). There is no doubt that pyrrolizidine alkaloid toxicosis is related to the structure of the individual alkaloids. In other words, individual pyrrolizidine alkaloids produce characteristic toxic patterns and the pattern is found to be structure dependent.

Mattocks (1973) has demonstrated that acute liver necrosis of pyrrolizidine alkaloids is caused by primary pyrrolic esters (or dehydroalkaloids). He argued that the liver is exposed to them in high concentration as there is strong correlation between amount of pyrroles bound to liver tissue and acute hepatotoxicity. Further more, the pyrrolic alcohols are not acutely hepatotoxic even at large doses. Schoentel (1968) also indicated that hepatotoxic pyrrolizidine alkaloids are esters of 1,2,dehydro-1-hydromethyl pyrrolizidine, its 7-hydroxy derivatives (necin) and their N-oxides. The esterifying acid known as necic acids contain various branched chains which are often unsaturated and hydroxylated. Examples of these basic moeities are heliotridine, retronecine, supinidine, and crotanecine. The latter contains an additional hydroxyl on the 6-Carbon found in cyclic diester alkaloids of Crotalaria species (Atal, et al, 1966).

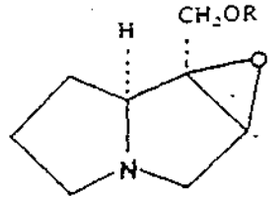
Mattocks (1968) also found anacrotine from C. incana to be hepatotoxic. Diesters of retronecine with derivatives of glutaric acid have been detected mainly among the Crotalaria species and they often induce lung and vascular lesions (in addition to liver lesions), but rarely liver tumors. Relation between structure to toxicity is also evident in the LD_{50} of the alkaloids. The LD_{50} of cyclic diester alkaloids of retrorsine which are most toxic have LD_{50} below 100mg/kg body weight and the LD_{50} differ among individual alkaloids of this group by a factor of no more than 2 to 3 (Elilly, et al, 1949).

Pyrrolizidine N - oxides have been observed to be less toxic. They could

however be changed to toxic components if they are converted to their corresponding bases. Therefore, route of administration is important since reduction of N-oxides to free bases takes place in the gastro-intestinal tract after oral administration. This also suggests that parental administration may be less toxic though to a lesser extent, reduction of N-oxides also occur in the liver (Mattocks, 1971).

Non-ester pyrrolizidine alkaloids isolated may not be hepatotoxic though some may cause pathological effects (other than in the liver). This is examplified by epoxy alkaloids isolated from C. trifoliastrum Wild and C. aridicola Domin which were found to cause esophageal lesions in some regions in Australia (Culvenor, Donovan and Smith, 1967).

Carcinogenicity of various pyrrolizidine alkaloids have also been reported [Schoentel, 1968; Schoentel and Magee, 1957]. In this investigation, signs of tumor were not detected in any of the experimental animals used. Perhalps early death is a major reason why tumor and carcinogenicity was not encountered in acute toxicity though it has been reported in chronic toxicity.



R = H : 1β - 2β -epoxy-1α - hydroxymethyl pyrrolizidine
R = CH_3 : 1β - 2β -epoxy-1α - methoxy- methyl pyrrolizidine

FIGURE 11: CHEMICAL STRUCTURE OF EPOXY ALKALOIDS FROM SOME CROTALARIA SPECIES.

Various factors also influence toxicity of pyrrolizidine alkaloids. Different species display variable susceptibility and toxicity symptoms. The rats and mouse are susceptible, farm animals differ widely, goats are fairly resistant, cattle and horses less resistant, poultry are sensitive while sheep show delayed toxicity. Symptoms of toxicity in horses is largely neurological, non-specific gastric and oesophageal lesions may be present [Kalkus et al, 1925; Gibbons, et al, 1950]. Man is also susceptable. The young are more at risk and low protein diet increases vulnerability. Liver necrosis produced by pyrrolizidine alkaloids in rats could be ameliorated by addition of cystein before or after exposure to the alkaloid (Hayashi and Lalich, 1968).

Response to pyrrolizidine alkaloids depend on balance between rate of formation of relevant metabolites, rate of degradation, excretion of the metabolites and the survival or fate of the cells that interacted with it. While C. retusa from the above experiment has demonstrated that it is a potentially toxic plant, with toxicity pattern typical of monocrotaline poisoning, many species of the genus have not been assessed in this manner. The recognition of the hepatotoxicity and possible carcinogenicity of pyrrolizidine alkaloids, their etiology in primary liver cancer in man has added new interest to the study of other members of the genus Crotalaria.

In conclusion, extracts of Crotalaria retusa alkaloids have demonstrated toxicity in experimental rats typical of pyrrolizidine alkaloid. This result agrees with the work of many others. The possible chemistry of the alkaloids has also been discussed in the above. It has been observed that I.P. administration of the alkaloids presents similar toxicity symptoms to oral administration or poisoning by the plant as reported in the literature. This may suggest that:

1) That the plant C. retusa contains unsaturated pyrrolizidine alkaloids.
2) That the presence of monocrotaline identified by TLC is confirmed by these toxicity tests.
3) Severe necrosis and lesions displayed by pathological studies in liver, kidney, lungs and intestine suggests that C. retusa found in Nigeria is equally or more toxic than the same species grown elsewhere. The loss of hair by female rats after alkaloid adminstration is reported here for the first time among the effects of pyrrolizidine alkaloids.
4) Some toxic C. retusa alkaloids are fairly stable, and do not decompose in isolation procedures.
5) Species variation in pyrrolizidine alkaloid toxicity suggests that animals should be grouped for specific study types. The rat, which displays close species toxicity pattern to man, could be the most suitable for toxicity assessment.
6) Though the percentage of total alkaloids may be small, plant could be highly toxic because of the types of individual alkaloids present.

3.6 Ethnomedical Studies Relating to Crotalaria Species

An oral and questionnaire exercise was undertaken on Crotalaria species among local herbalists and herdsmen, Veterinary Practitioners of the Veterinary Teaching Hospital and Medical Doctors of the Ahmadu Bello University Teaching Hospital, all in Zaria on information relating to Crotalaria species. At the same time an

investigation into the different Crotalaria species found in Zaria and environs was attempted. This was necessary since the species likely to be used by the herbalists and herdsmen are the ones that are available around them. Sample copies of the questionnaires used are shown in Appendix II). The results of this investigation are presented below.

3.6.1 Occurrence of Crotalaria Species in Zaria

About 25 (twenty five) Crotalaria species were identified to grow in Zaria. The abundance of the species and spread vary. However, a random survey was done for the purpose of this studies and the species found with their relative abundance are shown in Table 15.

3.6.2 Awareness, Uses and Practices Relating to Crotalaria Species.

The oral and questionnaire exercise revealed some results on the awareness, uses and practices relating to Crotalaria species. The following are the number of respondents to this information exercise among the various groups of the participants:

(i) Herbalists and Herdsmen, Oral interview: 10

(i) Herbalists, Written Questionnaire: 8

(ii) Herdsmen, Written Questionnaire: 9

(iii) Veterinary Medical practitioners: 10

(iv) Medical Doctors dealing with liver diseases: 10

TABLE 15: CROTALARIA SPECIES FOUND IN ZARIA AND RELATIVE ABUNDANCE

Species	Relative abundance
1. C. anthyllopsis , Welw.	Available, not abundant
2. C. arenaria Benth	Many, well distributed
3. C. barkae Schwenf	Available, not abundant
4. C. bongensis Bak	Available, not abundant
5. C. calycina Schrank	Available, not abundant
6. C. comosa Bak	Available, not abundant
7, C. cylindrocarpa DC.	Available, not abundant
8. C. doniana Bak	Available, not abundant
9. C. falcata Vahl	Abundant, well distributed
10. C. galuca Willd	Rarely available
11. C. glaucoides Bak	Available, not abundant
12. C. goreensis Guill Perr	Available, not abundant
13. C. graminicola Taub	Available, not abundant
14. C. hyssopifolio Klotzsch.	Available, not abundant
15. C. intermedia Kotschy	Numerous, limited distribution
16. C. lachnosema Stapf	Abundant,distributed
17. C. macrocalyx Benth.	Abundant well distributed
18. C. microcarpa Hochst	Not many, limited distribution
19. C. mucronata Desv.	Available, not abundant
20. C. naragutensis Hutch	Numerous, well distributed
21. C. ochroleuca G.	Available, not abundant
22. C. retusa L.	Most abundant, well distributed
23. C. senegalensis Hutch	Not many, limited distribution
24. C spectabilis Roth	Available, not abundant
25. C. vogelii, Benth	Available, not abundant.

The oral interview among herbalists and herdsmen was necessary because of the general dislike of paper work and inability of some of them to read or write. The overall results obtained could be categorised as follows:

(a) Local names of species.

(b) Traditional uses of Crotalaria species.

(c) Crotalaria species in Veterinary practices in Zaria.

(d) Crotalaria toxicity awareness among Medical Practitioners.

(a) Local and Vernacular Names of Crotalaria Species:

Hausa: General name: *Bi-rana* or *Biya-rana*.

Other specific names are: *Bakar biya rana* (C. retusa), *farar bi-rana* (C. lachnosema), *yauki* (C. microcarpa), *furan saura*, *biranar awaki, gyadar yara* (C. retusa), *bi-ranar zomo*.

Yoruba: *Korupo*

Igbo: *Akedimwo*

Fulani: *Biriji-bei*

From the oral and questionnaire exercise, it was confirmed that the populace are well aware of differences in Crotalaria species. Two important features are utilised to distinguish them, and these are (1) colour and size of leaves; (2) presence of hairs. Three Crotalaria species were also found to be very well familiar to the populace. These

are:

(1) C. retusa.

(2) C. lachnosema,

(3) C. naragutensis.

(b) Traditional uses

From the results of this exercise, it is clear that C. retusa is a well known species and is utilised in various practices in Zaria. The Crotalaria species were being utilised in traditional medicine but not as food by humans. However, sheep and cattle are being fed Crotalaria species notably C. retusa,. C. lachnosema and C. naragutensis by herdsmen. This is especially during the dry season when palatable forage is scarce. The various uses to which Crotalaria species are put into are indicated in Table 16.

(c) Crotalaria Species in Veterinary Practices:

Crotalaria species are encountered in veterinary practices in two ways:

(i) Eaten by sheep and cattle voluntarily (there is species choice here);

(ii) Given to many domestic animals for medical reasons (see Table16).

This study has uncovered that there were incidences of toxicity occurring in the utilising of the Crotalaria species as outlined above. These incidences were noticed by Veterinary Doctors of the Ahmadu Bello University Veterinary Teaching Hospital who go on field trips to treat animals among the local populace. The usual symptoms recorded

were loss of appetite, diarrhoea, gastro-intestinal problems, foamy discharges from mouth, congested mucous and even sudden death. Local remedies for Crotalaria poisoning in animals include certain herbs (not identified) while veterinary teams usually administer groundnut or palm oil, atropine sulphate or other anti-diarrhoeal agents. The veterinary team also acknowledged the fact that Crotalaria poisoning is much under reported.

TABLE 16: THE TRADITIONAL USES AND RECIPES OF CROTALARIA SPECIES IN ZARIA.

USES	RECIPES	REMARKS
1. Scabies	Whole plant, decoction (bathing)	C. retusa C. lachnosema
2. Venereal disease*	Whole plants plus potash, boiled – drinking	Any single species
3. Loss of menses	Flower squashed and potash added, cooked in soup (drinking)	C. retusa
4. Stomach colic and flatulence*	Roast black caraway, mixed with powdered plant, drink small quantities.	C. retusa
5. Cardiac cases*	Decoction of whole plants, small bits in soup or tea.	Any species except C. retusa
6. Highly sought in love matters, acceptance, etc.*	Written and washed Qur'anic verses are combined with the powder of any species of Crotalaria.	C. retusa, C. lachnosema or C. naragutensis
7. Driving away snakes	Seeds are sprinkled in flames to produce smoke.	C. retusa
8. Liver diseases in animals	Whole plant grounded in water and fed to animals	C. lachnosema or C. microcarpa
9. De-worming of animals	Not explicit	--
10. Heart diseases	Decoction (drinking)	--
11. Difficult rashes*	Decoction (bathing)	--

Those with asterisks (*) indicate the uses that are very popular.

(d) Crotalaria Toxicity Awareness in Medical Practice.

The toxicity of Crotalaria species appears to be known among Veterinary Practitioners only. Among the Medical Practitioners, usually information on consumption of herbs by patients is not much taken into account. Where recognised, patients are only advised to stop taking the herbs. On the other hand, though Crotalaria species are not eaten as food, their toxic potentials are not known to the populace and different species are continuously being utilised in different ways.

DISCUSSIONS:

Ethnomedical study on Crotalaria species has been carried out and the Crotalaria species found in Zaria have been identified. Three species are most common. These species could be recognised by the people based on their morphological features. The species were found to be used in traditional medical practices and veterinary practices but not as food. Three species, C. retusa, C. lachnosema and C. naragutensis were found to be the most widely utilised. Toxicity of Crotalaria species though highly documented in other parts of the world is not recognised in Nigeria. There is also a clear reported correlation between Crotalaria consumption and liver diseases [WHO, 1988]. There is, therefore, need to make further research on consumption of herbs containing Crotalaria species and/or other plants containing pyrrolizidine alkaloids and its relationship with liver disease.

In animals it has now been identified by this study that Crotalaria poisoning does occur. Nwude and Ibrahim (1980) had reported that Crotalaria species are used in veterinary practices. It is, therefore, necessary that appropriate measures be taken to educate the populace, especially herdsmen on this. The methodology of this ethnomedical study (i.e. interviewing herbalist, herdsmen, etc) has been recognised by Sofowora (1993) as an important method of obtaining information on local medicinal plants in Nigeria.

This study has also shown that consumption of herbs by patients has not been largely recognised by the medical circle in Nigeria especially its relationship with liver diseases. This factor needs to be recognised especially when over 70% of Nigerians utilise traditional herbal remedies, many times taken together with the modern medical remedies. The reported cases of hepatotoxic effects of Crotalaria species and ability to induce Veno-Occulusive Disease (VOD) in other countries of the world are well documented [WHO, 1985] and Nigerian medical doctors need to take note.

3.7 CONCLUSIONS AND RECOMMENDATIONS

Detailed pharmacognostic evaluation of the three Crotalaria species has been undertaken. This study is a pioneering work on the pharmacognostic evaluation of Crotalaria species and has revealed very useful information and results. At the end of the study, the following important conclusions and recommendations are made:

3.7.1 CONCLUSIONS

This study has conclusively demonstrated that the three species, C. microcarpa, C. naragutensis and C. retusa, contain varying proportions of pyrrolizidine alkaloids. This confirms the taxonomical relationship of the species. The alkaloids were present as bases and as N – oxides . The highest accumulation was in the aerial parts of C. retusa. The leaves of C. naragutensis contain no detectable alkaloids. Other phytochemical constituents were flavonoids, tannins, carbohydrates, proteins, gums and mucillages. The nature and amounts of these constituents also differ with species.

The features of the plants were examined and clear macro- and microscopical differences were established. These differences could be useful in recognition, identification and pharmacopoeal standards of these important and widespread species.

The pyrrolizidine alkaloids of C. retusa species were found to be toxic with marked microscopical lesions principally in the liver, lungs, kidney and intestine. Their toxicity is typical of monocrotaline toxicity. TLC shows the presence of monocrotaline in the leaves and aerial parts of C. retusa only, among the three species. Other alkaloids were also detected in the other two species.

The consumption of these plants by livestock and in human population was also confirmed. Cases of poisoning also do occur in livestock, though not well reported. The general population in Zaria [and by extension Nigeria], seem to be unaware of the potential toxicity of these plants. The prevalent use of herbs in our traditional medicine is also not much taken into account by the Medical circle. This is important especially since studies have shown very small doses could produce toxic effects sometimes long

after consumption.

The extensive literature search on these plants revealed the recent renewed interest on the pharmaceutical potentials of these plants, their etiological role in V.O.D, carcinogenicity, primary liver cancer, anti-carcinogenicity, etc. The number of the species of the genus that have been studied are minimal (about 95 out of 600). Even for those species reportedly screened, the depth of work done is in many cases limited. Research on C. microcarpa reported here is undertaken for the first time.

This study has presented important microscopical, phytochemical and macroscopical data useful in the identification and pharmacopoeial standards of the three species. It has also provided valuable toxicological, veterino-medical and other information on the Crotalaria species in general.

3.7.2 RECOMMENDATIONS

The genus Crotalaria holds a lot of potentials. There is, however, the need for further research on the different species in order to generate more information and improve our understanding of this important genus for their optimum economic exploitation. The following recommendations with regards to further work on Crotalaria species are therefore made:

(1) General screening of all species of Crotalaria for pyrrolizidine alkaloid content is recommended. The amount of alkaloids in all species, percentage alkaloid base

and N-oxides in the morphological parts and factors affecting amount of alkaloids needs to be evaluated.

(2) The qualitative testing of the alkaloids of all the species individually, so that the spectrum of alkaloids of each plant could be known and studied. While pyrrolizidine alkaloids are widely recognised in the Crotalaria species, there is need to study possible presence of other groups of alkaloids.

(3) There is also need to carry out toxicity tests on the extracted alkaloids of each species.

(4) Carcinogenicity of some pyrrolizidine alkaloids and anti-carcinogenicity as reported in the literature needs to be further investigated and resolved.

(5) Possible use of the non-toxic species of Crotalaria as organic manure should be investigated.

(6) There is need to evaluate some of the Crotalaria species as sources of gums and mucilages.

(7) The presence of C-glycosides in some species indicates the need to assess all the species for this compound and possible utilisation.

(8) The effect of consumption of pyrrolizidine alkaloids by the human population in Nigeria needs further study. There is also need to investigate epidemiological information on survivors of pyrrolizidine poisoning. Antidote for Crotalaria poisoning needs to be developed.

(9) Mass campaign on the potential toxicity of these plants and government regulations are needed to be put in place to educate the population, especially herbalists and herdsmen. This will go along away in preventing poisoning in livestock and humans as well as contamination of food and agricultural products. These measures have been found to be effective in some countries.

(10) The various reported activities of some species of Crotalaria (e.g. their fungicidal, molluscidal and spermatocidal abilities) need to be further investigated. This is important in our search for the development of new drugs and exploitation of bio-resources for the future.



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APPENDICES



APPENDIX I

CHEMICAL DATA FOR SOME PYRROLIZIDINE ALKALOIDS.

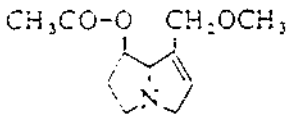
The following represent some important chemical data related to the pyrrolizidine alkaloids as adapted from Bull, et al [1968]. The listing has been done alphabetically and consists of the following:

(A) Molecular structure of the pyrrolizidine alkaloids (Table 17).

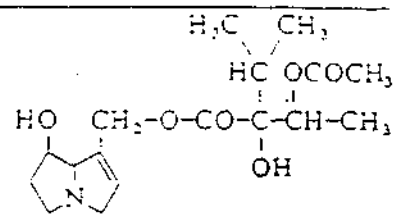
(B) Physical properties of the pyrrolizidine alkaloids (Table 18).

(C) Median lethal dose of pyrrolizidine alkaloids for rats (Table 19).

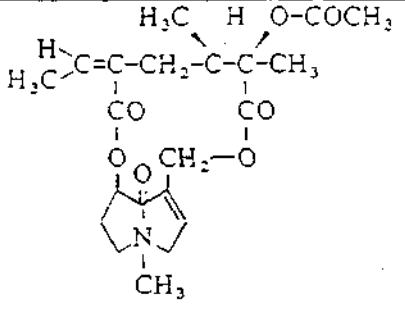
TABLE 17 : MOLECULAR STRUCTURE OF PYRROLIZIDINE ALKALOIDS.



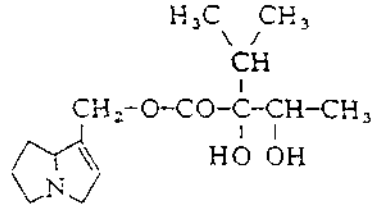
1. 7*B*-Acetoxy-1-methoxymethyl-1,2-dehydropyrrolizidine



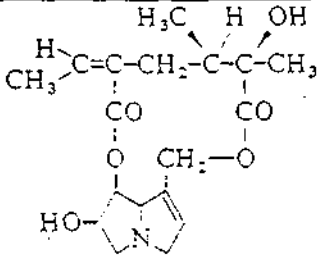
2. Acetylindicine
(retronecine, β-acetyl-(−)-trachelanthic acid)



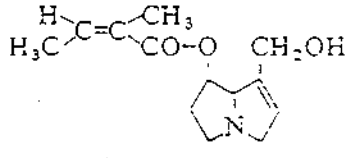
3. O-Acetylsenkirkine
(otonecine, acetylsenecic acid)



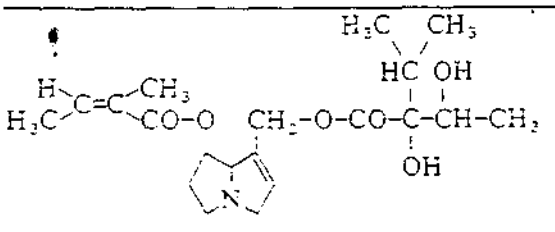
4. Amabiline
(supinidine, viridiflonic acid)



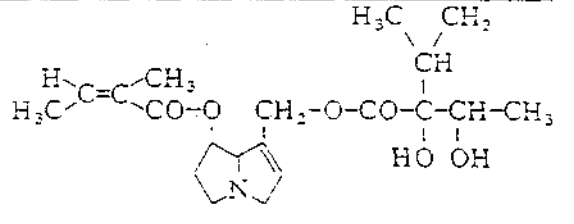
5. Anacrotine (crotanecine, senecic acid)



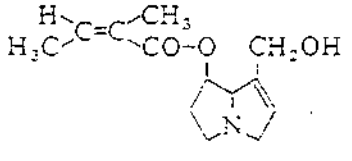
6. 7-Angelylheliotridine (rivularine)



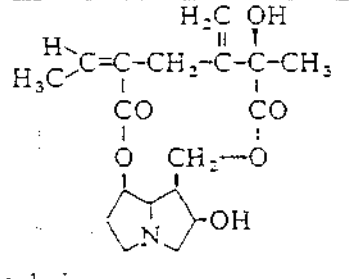
7. Angelylheliotridine trachelanthate



8. 7-Angelylheliotridine viridiflorate



9. 7-Angelyiretronecine

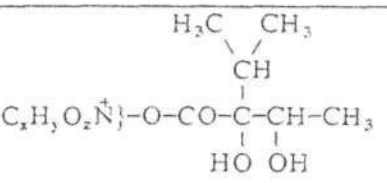


10. Angularine
(rosmarinecine, seneciphyllic acid)

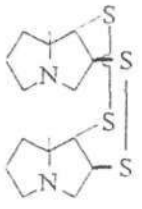
$C_{13}H_{19}O_3N$

Structure unknown

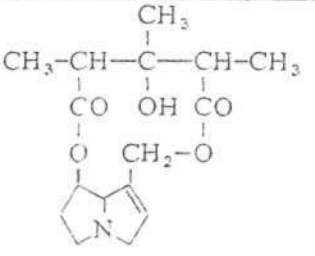
11. Campestrine



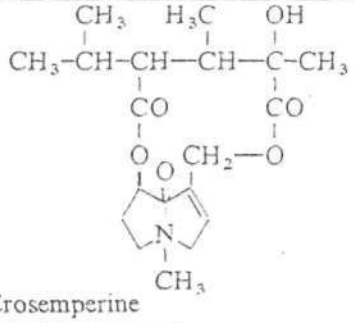
12. Caratégine
(unknown aminoalcohol, viridiflorique acid)



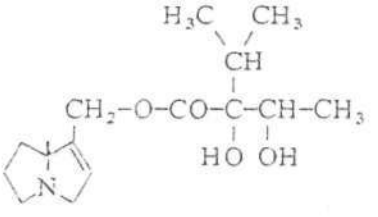
13. Cassipourine



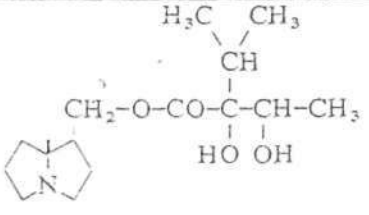
14. Crispatine
(tretronecine, crispatic acid)



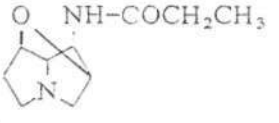
15. Crosemperine
(otonecine, incanic acid)



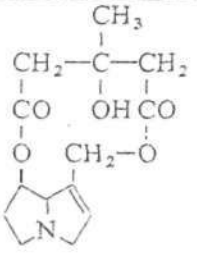
16. Cynaustine
((−)-supinidine, viridiflorique acid)



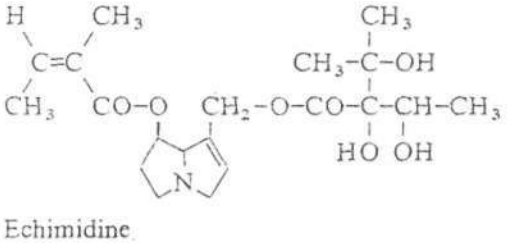
17. Cynaustraline
((+)-isoretronecanol, viridiflorique acid)



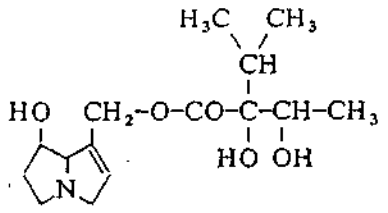
18. Decorticasine
(norioline, propionic acid)



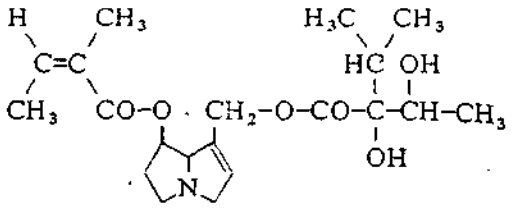
19. Dicrotaline
(retronecine, dicrotalic acid)



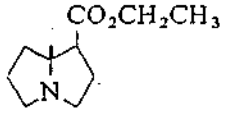
20. Echimidine
(tretronecine, angelic and echimidinic acids)



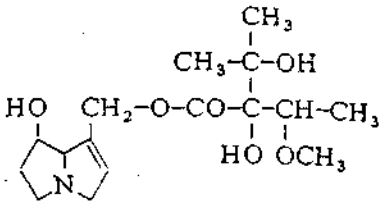
21. Echinatine
(heliotridine, viridifloric acid)



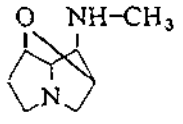
22. Echiumine
(retronecine, angelic and trachelanthic acids)



23. 1α-Ethoxycarbonyl-8β-pyrrolizidine



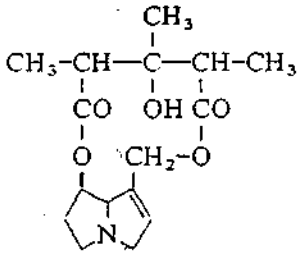
24. Europine
(heliotridine, lasiocarpic acid)



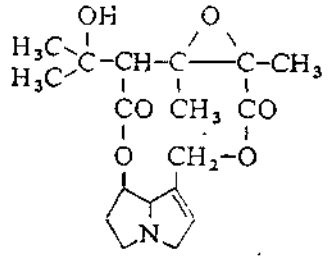
25. Festucine

$C_{12}H_{21}O_3N$
Structure unknown

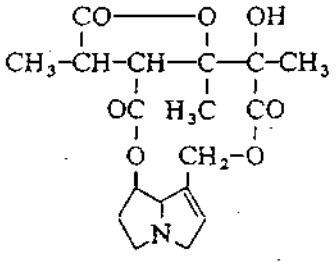
26. Fuchsisenecionine



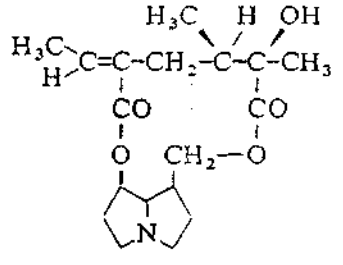
27. Fulvine
(retronecine, fulvinic acid)



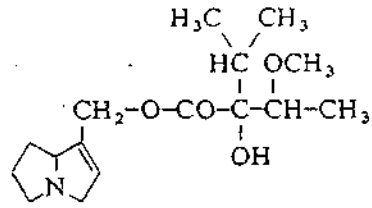
28. Grantaline (?)
(retronecine, grantalic acid)



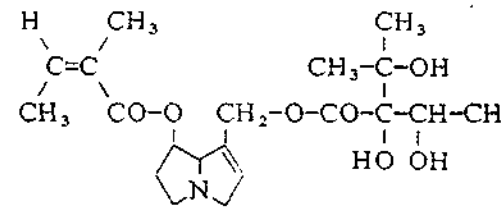
29. Grantianine (?)
(retronecine, grantianic acid)



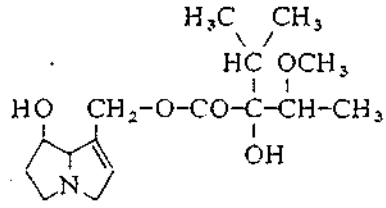
30. Hastacine
(hastanecine, integerrinecic acid)



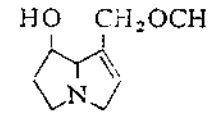
31. Heleurine
(supinidine, heliotric acid)



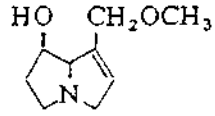
32. Heliosupine
(heliotridine, angelic and echimidinic acids)



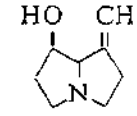
33. Heliotrine
(heliotridine, heliotric acid)



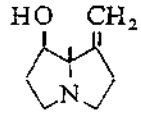
34. 7α-Hydroxy-1-methoxymethyl-1,2-dehydropyrrolizidine



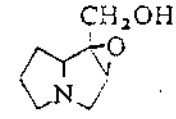
35. 7β-Hydroxy-1-methoxymethyl-1,2-dehydropyrrolizidine



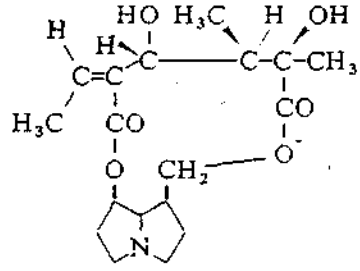
36. 7β-Hydroxy-1-methylene-pyrrolizidine



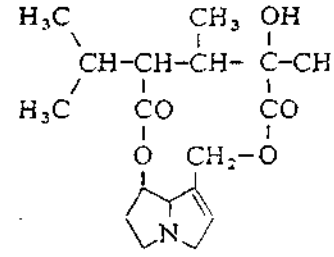
37. 7β-Hydroxy-1-methylene 8β-pyrrolizidine



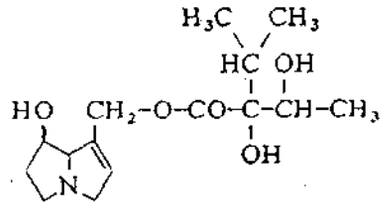
38. 1-Hydroxymethyl-1β,2β-epoxypyrrolizidine



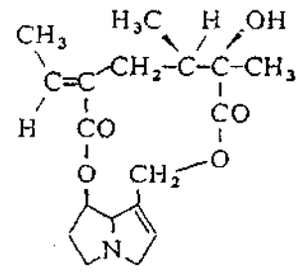
39. Hygrophylline
(platyphylline, hygrophyllinecic acid)



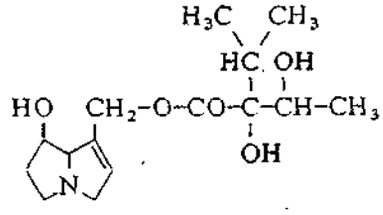
40. Incanine
(retronecine, incanic acid)



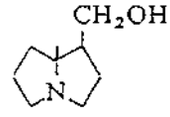
41. Indicine
(retronecine,
(−)-trachelanthic acid)



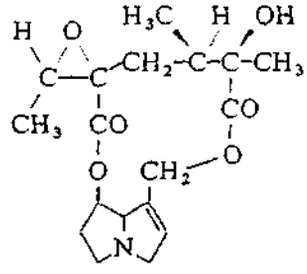
42. Integerrimine
(retronecine, integerrinecic acid)



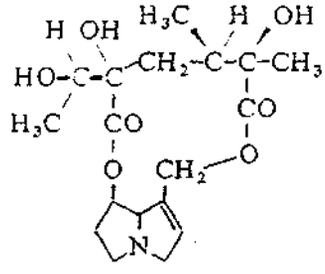
43. Intermedine
(retronecine, trachelanthic acid)



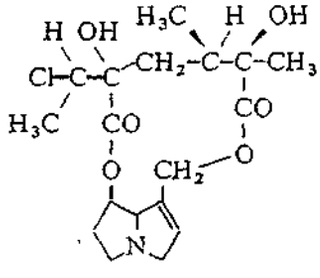
44. (+)-Isoretronecanol



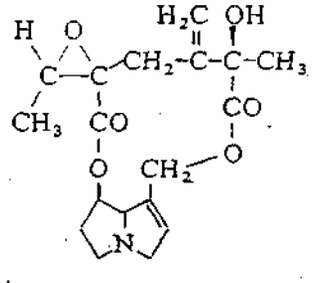
45. Jacobine
(retronecine, 'jacobinecic' acid)



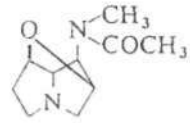
46. Jacoline
(retronecine, 'jacolic' acid)



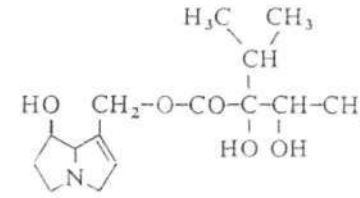
47. Jaconine
(retronecine, 'jaconinecic' acid)



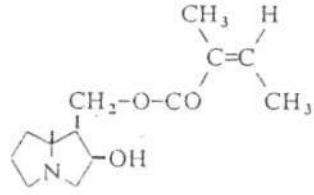
48. Jacozine
(retronecine, 'jacozic' acid)



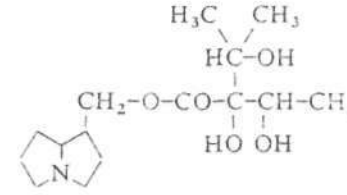
59. Lolinine



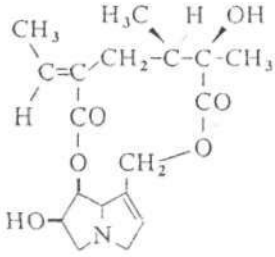
60. Lycopsamine
(retronecine, viridifloric acid)



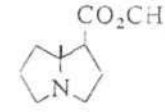
61. Macrophylline
(macronecine, angelic acid)



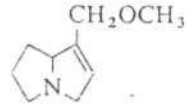
62. Macrotomine
(trachelanthamidine, macrotomic acid)



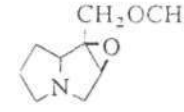
63. Madurensine
(crotanecine, integerrinecic acid)



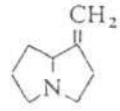
64. 1α-Methoxycarbonyl-8β-pyrrolizidine



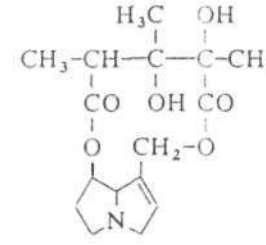
65. 1-Methoxymethyl-1,2-dehydropyrrolizidine



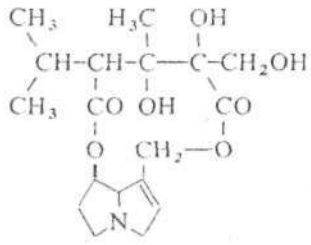
66. 1-Methoxymethyl-1β,2β-epoxypyrrolizidine



67. 1-Methylenepyrrolizidine



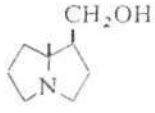
68. Monocrotaline
(retronecine, monocrotalic acid)



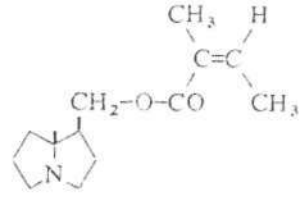
49. Junceine
(retronecine, junceic acid)



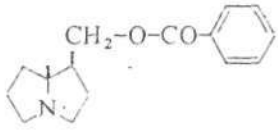
50. Laburnamine



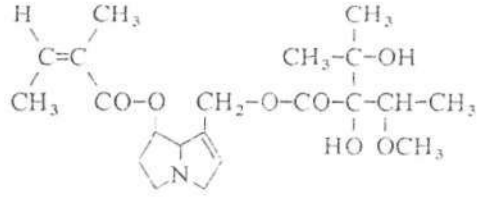
51. Laburnine



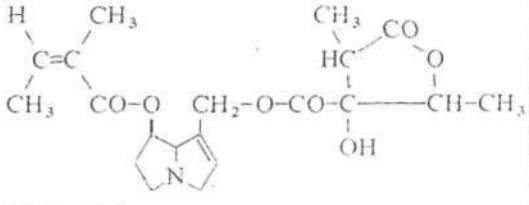
52. Laburnine angelate



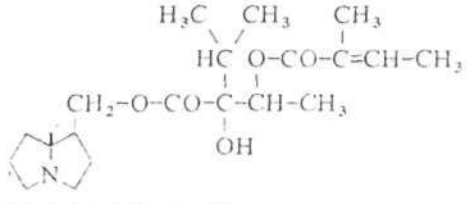
53. Laburnine benzoate



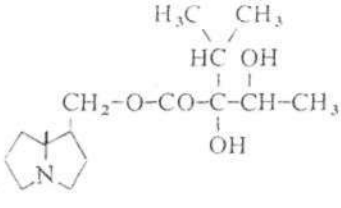
54. Lasiocarpine
(heliotridine, angelic and lasiocarpic acids)



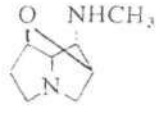
55. Latifoline
(retronecine, angelic and latifolic acids)



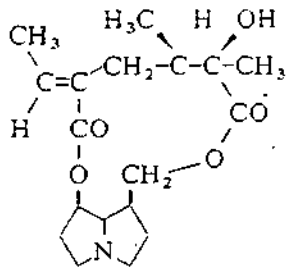
56. Lindelofamine (?)
((+)-isoretronecanol, trachelanthic and angelic or tiglic acids)



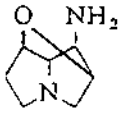
57. Lindelofine
((+)-isoretronecanol, trachelanthic acid)



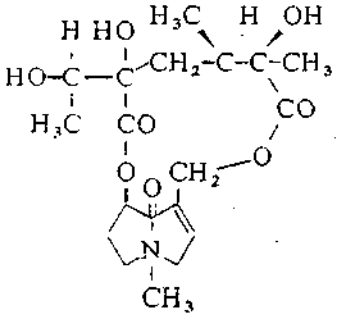
58. Loline



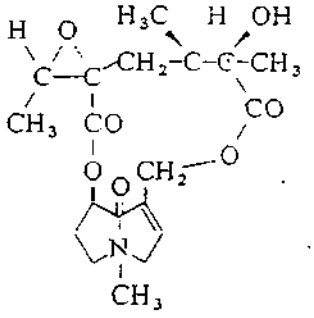
69. Neoplatyphylline
(platynecine, integerrinecic acid)



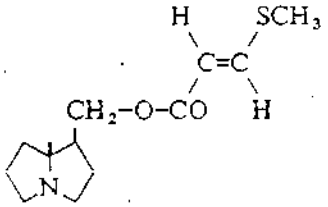
70. Norloline



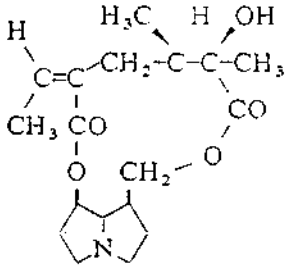
71. Onetine
(otonecine, 'jacoline' acid)



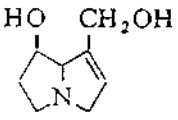
72. Otosenine
(otonecine, 'jacobinecic' acid)



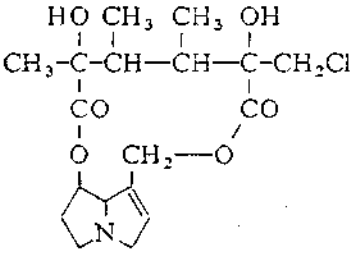
73. Planchonelline
(laburnine, methylthioacrylic acid)



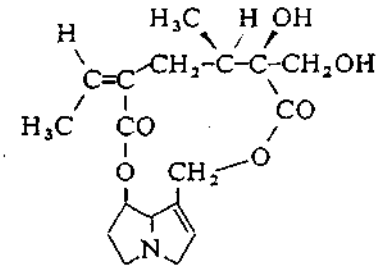
74. Platyphylline
(platynecine, senecic acid)



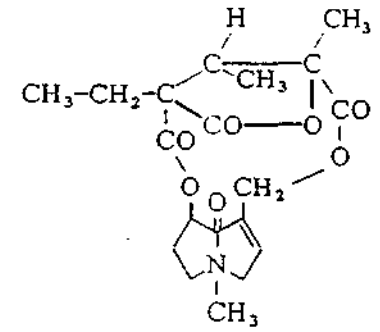
75. Retronecine



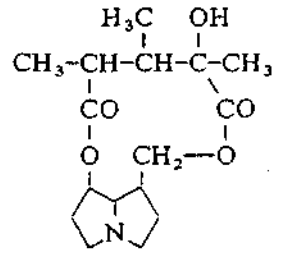
76. Retronecine-sceleratinic ester



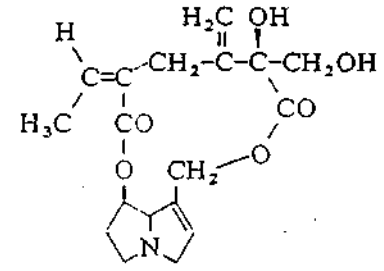
77. Retrorsine
(retronecine, isatinecic acid)



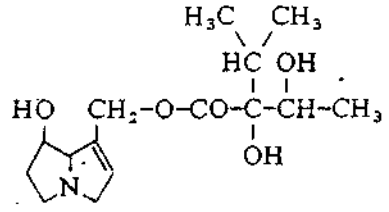
78. Retusamine
(otonecine, retusaminic acid)



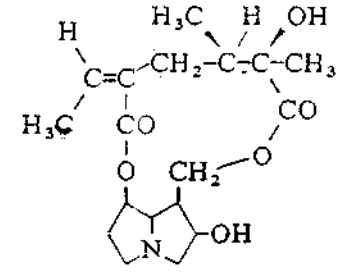
79. Retusine
(turneforcidine, α-dihydro-anhydromonocrotalic acid)



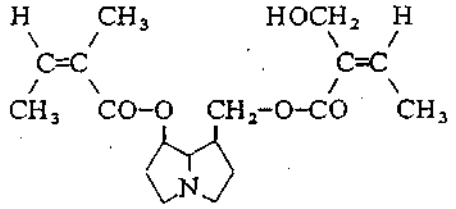
80. Riddelliine
(retronecine, riddelliic acid)



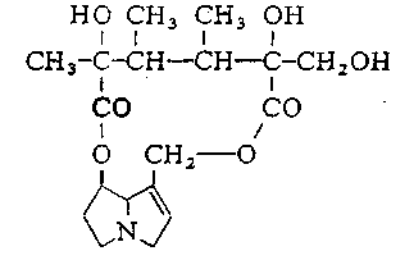
81. Rinderine
(heliotridine, trachelanthic acid)



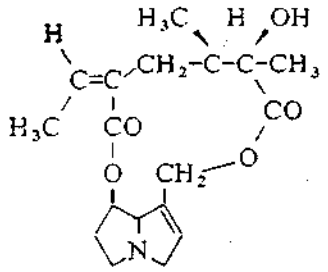
82. Rosmarinine
(rosmarinecine, senecic acid)



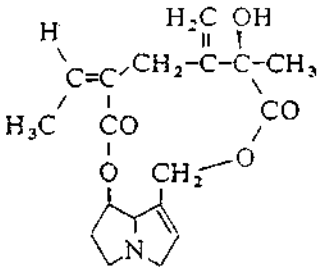
83. Sarracine
(platynecine, angelic and sarracinic acids)



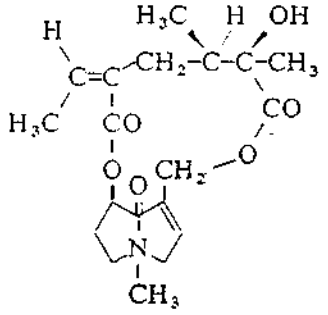
84. Sceleratine
(retronecine, sceleranecic acid)



85. Senecionine
(retronecine, senecic acid)



86. Seneciphylline
(retronecine, seneciphyllic acid)

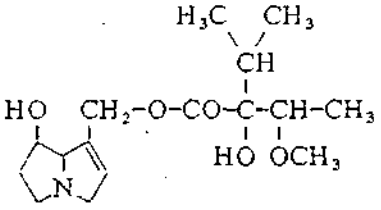


87. Sénkirkine
(otonecine, senecic acid)

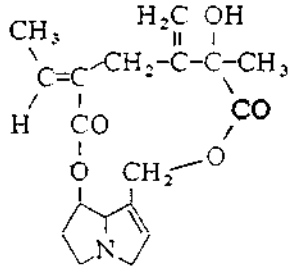
$C_{12}H_{21}O_4N$

Structure unknown

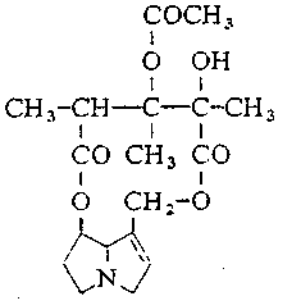
88. Silvasenecine



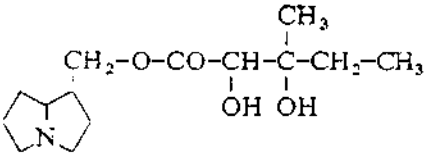
89. Sincamidine
(retronecine, isomer of heliotric acid)



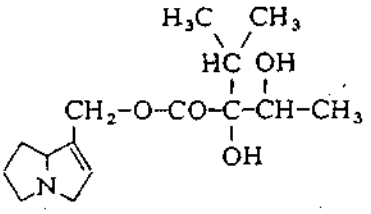
90. Spartioidine (?)
(retronecine, isomer of seneciphyllic acid)



91. Spectabiline
(retronecine, β-acetyl-monocrotalic acid)



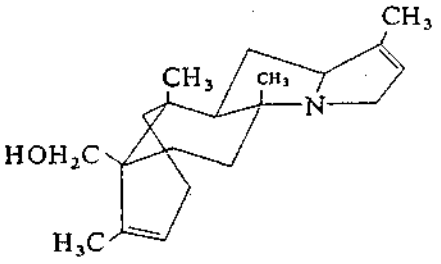
92. Strigosine
(trachelanthamidine, αβ-dihydroxy-β-methylvaleric acid)



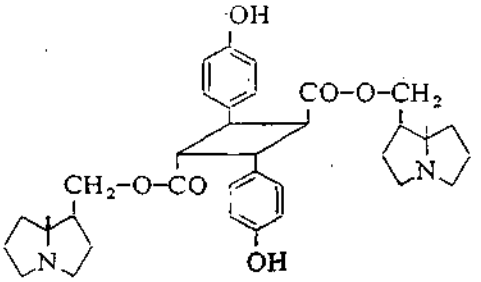
93. Supinine
(supinidine, trachelanthic acid)

Structure unknown

94. Thelepogidine



95. Thelepogine

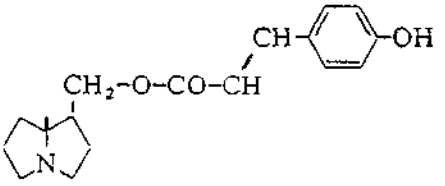


96. Thesine
((+)-isoretronecanol, thesinic acid)

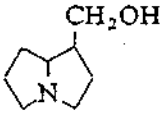
$C_{10}H_{11}O_2N$

Structure unknown

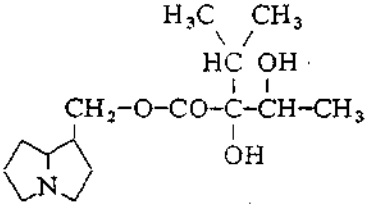
97. Thesinicine



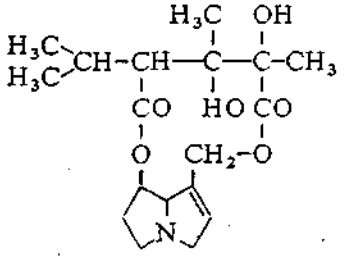
98. Thesinine
((+)-isoretronecanol, p-hydroxycinnamic acid)



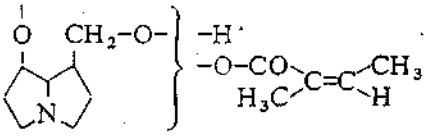
99. Trachelanthamidine



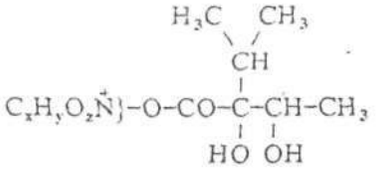
100. Trachelanthamine
(trachelanthamidine, trachelanthic acid)



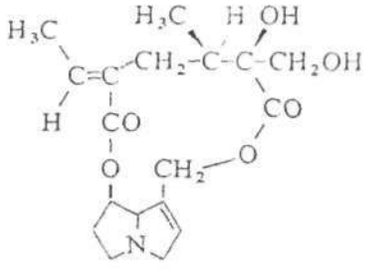
101. Trichodesmine
(retronecine, trichodesmic acid)



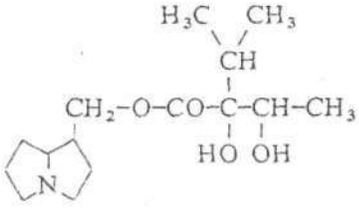
102. Turneforcine (?)
(turneforcidine, angelic acid)



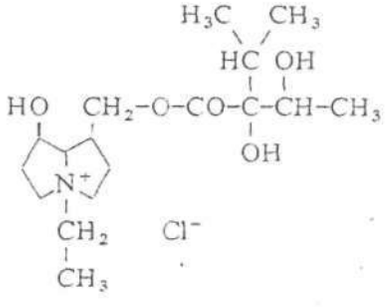
103. Turkestanine
(unknown aminoalcohol, viridifloric acid)



104. Usaramine
(retronecine, retronecic acid)



105. Viridiflorine
(trachelanthamidine, viridifloric acid)



106. *Lindelofia* base
(hastanecine etho-salt, trachelanthic acid)

TABLE 18: PHYSICAL PROPERTIES OF PYRROLIZIDINE ALKALOIDS.

Alkaloid	Empirical formula	B.p. (°C/mm)	M.p. (°C)	$[\alpha]_D$ (°, solvent)	R_T* (min/°C)	R_F (paper)**	R_F (TLC)†
7β-Acetoxy-1-methoxymethyl-1,2-dehydropyrrolizidine	$C_{11}H_{17}O_3N$	61/0.03	—	+15.2, EtOH	15.0/140	0.54	0.30
Acetylindicine	$C_{17}H_{27}O_6N$	—	—	—	—	—	—
O-Acetylsenkirkine	$C_{21}H_{29}O_7N$	—	195–196	−34, MeOH	—	0.64	—
Amabiline	$C_{15}H_{25}O_4N$	—	—	−7.1, EtOH	8.7/205	0.40	0.10
Anacrotine	$C_{18}H_{25}O_6N$	—	191–192	+29.8, EtOH	33.2/205	0.46	0.43
7-Angelylheliotridine	$C_{13}H_{19}O_3N$	—	116–117	+10.8, EtOH	4.8/205	0.52	0.45
7-Angelylheliotridine trachelanthate	$C_{20}H_{31}O_6N$	—	—	—	39.9/205	0.64	0.55
7-Angelylheliotridine viridiflorate	$C_{20}H_{31}O_6N$	—	—	—	39.9/205	0.64	0.55
7-Angelylretronecine	$C_{13}H_{19}O_3N$	—	76–77	+49, EtOH	4.2/205	0.49	0.33
Angularine	$C_{18}H_{25}O_6N$	—	200–201	−98, EtOH	—	—	—
Campestrine	$C_{13}H_{19}O_3N$	—	93	—	—	—	—
Carategine	—	—	200–201	—	—	—	—
Cassipourine	$C_{14}H_{22}N_2S_4$	—	212	−11.8, $CHCl_3$	—	—	—
Crispatine	$C_{16}H_{23}O_5N$	—	137–138	+40.7, EtOH	15.6/205	0.39	0.29
Crosemperine	$C_{19}H_{29}O_6N$	—	113–115	+2.2, EtOH	28.8/205	0.52	0.25
Cynaustine	$C_{15}H_{25}O_4N$	—	picrate, 135–136	+13.2, EtOH	8.7/205	0.40	0.10
Cynaustraline	$C_{15}H_{27}O_4N$	—	picrolonate, 149–150	+48.0, EtOH	9.5/205	0.40	0.03
Decorticasine	$C_{10}H_{16}O_2N_2$	—	—	+26.1, EtOH	—	—	—
Dicrotaline	$C_{14}H_{19}O_5N$	—	170 (dec); hydrochloride, 258–260	hydrochloride, +25.7, H_2O	—	—	—
Echimidine	$C_{20}H_{31}O_7N$	—	picrate, 142–143	+13.4, EtOH	47.6/205	0.57	0.45
Echinatine	$C_{15}H_{25}O_5N$	—	109–110	+12.8, EtOH	15.6/205	0.36	0.30
Echiumine	$C_{20}H_{31}O_6N$	—	99–100	+14.4, EtOH	35.6/205	0.65	0.47
1-Ethoxycarbonylpyrrolizidine	$C_{10}H_{17}O_2N$	—	—	—	—	—	—
Europine	$C_{16}H_{27}O_6N$	—	N-oxide, 171	+10.9, EtOH	18.2/205	0.34	0.29

Alkaloid	Empirical formula	B.p. (°C/mm)	M.p. (°C)	$[\alpha]_D$ (°, solvent)	R_T* (min/°C)	R_F (paper)**	R_F (TLC)†
Festucine	$C_8H_{14}ON_2$	—	dihydrochloride 237–242 (dec)	dihydrochloride, +4.6, H_2O	—	—	—
Fuchsisenecionine	$C_{12}H_{21}O_3N$	—	hydrochloride, 225–227	—	—	—	—
Fulvine	$C_{16}H_{23}O_5N$	—	213.5–214	−1.5, EtOH	15.5/205	0.43	0.33
Grantaline	$C_{18}H_{25}O_6N$	—	219.5–220	+101, EtOH	23.6/205	0.38	0.32
Grantianine	$C_{18}H_{23}O_7N$	—	209–209.5	+64, EtOH	57.0/205	0.33	0.31
Hastacine	$C_{18}H_{27}O_5N$	—	170–171	−72.3	32.5/205	0.57	0.38
Heleurine	$C_{16}H_{27}O_4N$	—	67–68	−12.0, EtOH	8.0/205	0.50	0.11
Heliosupine	$C_{20}H_{31}O_7N$	—	picrate, 103–106	−4.3, EtOH	50.6/205	0.51	0.53
Heliotrine	$C_{16}H_{27}O_5N$	—	128	+17.6 EtOH	12.4/205	0.43	0.30
7α-Hydroxy-1-methoxymethyl-1,2-dehydropyrrolizidine	$C_9H_{15}O_2N$	90/0.3	54	+24.8, EtOH	12.1/140	0.32	0.17
7β-Hydroxy-1-methoxymethyl-1,2-dehydropyrrolizidine	$C_9H_{15}O_2N$	77/0.4	36–38	+38, EtOH	9.0/140	0.33	0.12
7β-Hydroxy-1-methylene-8α-pyrrolizidine	$C_8H_{13}ON$	41/0.1	35–36	−150, EtOH	3.3/140	0.27	0.07
7β-Hydroxy-1-methylene-8β-pyrrolizidine	$C_8H_{13}ON$	62/0.03	34–36	+36, EtOH	4.4/140	0.28	0.14
1-Hydroxymethyl-1β,2β-epoxy-pyrrolizidine	$C_8H_{13}O_2N$	80/0.04	65–66	−49.5, EtOH	8.3/140	0.20	0.18
Hygrophylline	$C_{18}H_{27}O_6N$	—	173–174	−67.3, EtOH	40.7/205	0.42	0.12
Incanine	$C_{18}H_{27}O_5N$	—	97	−38.8, EtOH	—	—	—
Indicine	$C_{15}H_{25}O_5N$	—	97–98	+22.3, EtOH	14.3/205	0.38	0.19
Integerrimine	$C_{18}H_{25}O_5N$	—	172.5	+4.3, $CHCl_3$	24.3/205	0.57	0.39
Intermedine	$C_{15}H_{25}O_5N$	—	picrate, 146.5–147	+4.7, EtOH	14.3/205	0.38	0.19
(+)-Isoretronecanol	$C_8H_{15}ON$	90/0.15	39–40	+76, EtOH	7.4/140	0.24	0.02
Jacobine	$C_{18}H_{25}O_6N$	—	228	−46.3, $CHCl_3$	34.0/205	0.36	0.38
Jacoline	$C_{18}H_{27}O_7N$	—	221	+48, $CHCl_3$	36.6/205	0.23	0.37

Alkaloid	Empirical formula	B.p. (°C/mm)	M.p. (°C)	$[\alpha]_D$ (°, solvent)	R_T* (min/°C)	R_F (paper)**	R_F (TLC)†
Paucicaline	$C_{18}H_{27}O_8N$	—	184	—	—	—	—
Planchonelline	$C_{12}H_{19}O_2NS$	—	picrate, 127–128	+9, EtOH	11.3/205	0.23	0.03
Platyphylline	$C_{18}H_{27}O_5N$	—	129	−59, EtOH	24.0/205	0.50	0.18
Renardine (see senkirkine)	$C_{19}H_{27}O_6N$	—	—	—	—	—	—
Retronecine	$C_8H_{13}O_2N$	—	117–118	+50, EtOH	15.0/140	0.20	0.07
Retronecine-sceleratinic ester							
Retrorsine	$C_{18}H_{25}O_6N$	—	216–216.5	−18, EtOH; −62, $CHCl_3$	40.7/205	0.36	0.35
Retusamine	$C_{19}H_{25}O_7N$	—	174.5	+13, EtOH	50.3/205	0.51	0.30
Retusine	$C_{16}H_{25}O_5N$	—	174–175	+16.2, EtOH	13.9/205	0.44	0.16
Riddelliine	$C_{18}H_{23}O_6N$	—	198	−109.5, $CHCl_3$	41.9/205	0.28	0.32
Rinderine	$C_{15}H_{25}O_5N$	—	100–101	+24.6, EtOH	15.6/205	0.34	0.29
Rosmarinine	$C_{18}H_{27}O_7N$	—	209	−94, EtOH	37.1/205	0.40	0.35
Sarracine	$C_{18}H_{27}O_5N$	—	45–46	−121, EtOH	29.1/205	0.56	0.16
Sceleratine	$C_{18}H_{27}O_7N$	—	178	+54, EtOH	34.6/205	0.39	0.34
Senecionine	$C_{18}H_{25}O_5N$	—	245	−56, $CHCl_3$	20.6/205	0.56	0.40
Seneciphylline	$C_{18}H_{23}O_5N$	—	217	−139, $CHCl_3$	21.1/205	0.50	0.38
Senkirkine	$C_{19}H_{27}O_6N$	—	198	−2, $CHCl_3$	31.6/205	0.47	0.29
Silvasenecine	$C_{12}H_{21}O_4N$	—	—	—	—	—	—
Sincamidine	$C_{16}H_{27}O_5N$	—	—	—	—	0.43	—
Spartioidine	$C_{18}H_{23}O_5N$	—	178	−83.7, EtOH	—	—	—
Spectabiline	$C_{18}H_{25}O_7N$	—	185.5–186	+143, EtOH	27.2/205	0.43	0.34
Strigosine	$C_{14}H_{25}O_4N$	—	hydrochloride, 137.5	−19.3, EtOH	—	—	—
Supinine	$C_{15}H_{25}O_4N$	—	148–149	−12.1, EtOH	8.8/205	0.40	0.10
Thelepogidine	$C_{18}H_{29}O_2N$	—	254–255	—	41.3/205	0.73	0.07
Thelepogine	$C_{20}H_{31}ON$	—	184–185	—	47.4/205	0.73	0.10
Thesine	$C_{34}H_{42}O_6N_2$	—	254–256	—	—	—	—
Thesinicine	$C_{10}H_{11}O_2N$	—	124–125	—	—	—	—
Thesinine	$C_{17}H_{21}O_3N$	—	38–40	—	—	—	—

Alkaloid	Empirical formula	B.p. (°C/mm)	M.p. (°C)	$[\alpha]_D$ (°, solvent)	R_T* (min/°C)	(R_F (paper)**	R_F (TLC)†
Jaconine	$C_{18}H_{26}O_6NCl$	—	147	+52.5, $CHCl_3$	40.4/205	0.47	0.47
Jacozine	$C_{18}H_{23}O_6N$	—	228	−140, EtOH	35.5/205	0.32	0.37
Junceine	$C_{18}H_{27}O_7N$	—	191–192	−3, pyridine	52.0/205	0.31	0.30
Laburnamine	$C_{12}H_{22}O_2N$	—	125–127				
Laburnine	$C_8H_{15}ON$	80–90/0.01	—	+15.5, EtOH	7.4/140	0.24	0.02
Laburnine tiglate	$C_{13}H_{21}O_2N$	—	—	—	3.7/205	0.55	0.03
Laburnine benzoate	$C_{15}H_{19}O_2N$	—	hydrochloride, 202–204	—	8.8/205	0.63	0.03
Lasiocarpine	$C_{21}H_{33}O_7N$	—	96.5–97	−3.0, EtOH	46.4/205	0.59	0.54
Latifoline	$C_{20}H_{27}O_7N$	—	102–103	+57, EtOH	55.0/205	0.54	0.53
Lindelofamine	$C_{20}H_{33}O_5N$	—	88	—	—	—	—
Lindelofine	$C_{15}H_{27}O_4N$	—	106–107	+50, EtOH	—	—	—
Lofine	$C_8H_{14}ON_2$	103/5	dihydrochloride, 256–257	dihydrochloride, +6.2, H_2O	—	—	—
Lolinine	$C_{10}H_{16}O_2N_2$	—	73	+51, acetone	—	—	—
Lycopsamine	$C_{15}H_{25}O_5N$	—	—	+3.3, EtOH	14.3/205	0.38	0.19
Macrophylline	$C_{13}H_{21}O_3N$	—	42–44	+34.5, EtOH	4.6/205	0.54	0.05
Macrotomine	$C_{15}H_{27}O_5N$	—	95–97	−6.9, EtOH	—	—	—
Madurensine	$C_{18}H_{25}O_6N$	—	175–176	+55.7, EtOH	38.1/205	0.47	0.43
1-Methoxycarbonylpyrrolizidine	$C_9H_{15}O_2N$	—	—	+63.5	—	—	—
1-Methoxymethyl-1,2-dehydro-pyrrolizidine	$C_9H_{15}ON$	100/10	—	−24, EtOH	5.4/140	0.36	0.08
1-Methoxymethyl-1*β*,2*β*-epoxy pyrrolizidine	$C_9H_{15}O_2N$	53/0.1	—	−63, EtOH	6.4/140	0.32	0.23
1-Methylenepyrrolizidine	$C_8H_{13}N$	115/150	—	−100, EtOH	1.6/140	0.39	0.05
Monocrotaline	$C_{16}H_{23}O_6N$	—	202–203	−55, $CHCl_3$; −15, EtOH	19.5/205	0.29	0.29
Neoplatyphylline	$C_{18}H_{27}O_5N$	—	131–133	+1.95, $CHCl_3$	26.2/205	0.53	0.14
Norloline	$C_7H_{12}ON_2$	94–95/5	dihydrochloride, 309–311	+15.1, MeOH	—	—	—
Onetine	$C_{19}H_{29}O_8N$	—	192–193	+73.0, $CHCl_3$	—	0.47	—
Otosenine	$C_{19}H_{27}O_7N$	—	221	+20.8, $CHCl_3$	51.0/205	0.34	0.23

Alkaloid	Empirical formula	B.p. (°C/mm)	M.p. (°C)	$[\alpha]_D$ (°, solvent)	R_T* (min/°C)	R_F (paper)**	R_F (TLC)†
Trachelanthamidine	$C_8H_{15}ON$	114–115/3	hydrochloride, 114–115	−13.8, EtOH	—	—	—
Trachelanthamine	$C_{15}H_{27}O_4N$	—	92–93	−18.1, H_2O	—	—	—
Trichodesmine	$C_{18}H_{29}O_6N$	—	160–161	+38, EtOH	26.6/205	0.49	0.34
Turneforcine	$C_{13}H_{21}O_3N$	—	hydrochloride, 169–170	−58.6, H_2O	—	—	—
Usaramine	$C_{18}H_{25}O_6N$	—	182.5–183.5	+7.1, EtOH	48.7/205	0.40	0.35
Viridiflorine	$C_{15}H_{27}O_4N$	—	102.5–103.5	−11.7, EtOH	—	—	—
Unnamed base ex *Lindelofia*	$C_{17}H_{32}O_5NCl$	—	149–151	+20.3, MeOH	—	0.27	—

* Retention times are measured with the apparatus of Chalmers et al. (1965), using a 6 ft. glass column, 6 mm. i.d., packed with Gas Chrom. P. carrying 4% S.E. 30, operated at 205°C with inlet pressure 960 mm, or at 140 °C with inlet pressure 650 mm.

** Solvent is the upper layer resulting from shaking butanol with an equal volume of 5% acetic acid. Measured by ascending flow.

† Plates, prepared from a slurry of silica gel (Merck 'Silicagel G', 30 g) and sodium hydroxide (N/10, 60 ml), were kept for at least one day before use and developed with methanol.

Table 19 : Median Lethal Dose of Pyrrolizidine Alkaloids for Rats (Intraperitoneal injection; based on survivors at 72 hr)

AMINO – ALCOHOL	ALKALOID	SEX	LD_{50} mg/kg	Confidence Limits, 95%
Heliotridine	Heliotrine	M	330	270-330
		F	340*	316-364
	Lasiocarpine	M	72	66-79
		F	79	75-83
	Lasiocarpine-N-oxide	M	547	495-741
		F	181	141-201
Platynecine	Platyphylline	M	252*	240-267
		F	160*	153-167
	Sarracine	M	--	--
		F	218*	207-230
Retronecine	Jacobine	M	--	--
		F	138	117-149
	Jaconine	M	--	--
		F	168	128-208
	Monocrotaline	M	175	160-195
		F	189	163-202
	Seneciphylline	M	77	71-86
		F	83	77-90
Supinidine	Supinine	M	450*	410-490
		F	--	--

* All death occurred within 1 hr.

APPENDIX II

SAMPLES OF QUESTIONNAIRES USED IN THE ETHNOMEDICAL STUDY ON CROTALARIA SPECIES AND TYPICAL ANSWERED COPIES

The following are samples of the questionnaires used in the ethnomedical study of the Crotalaria species. Four types of questionnaires were administered to the following:

- Herbalists
- Herdsmen
- Medical doctors
- Veterinary doctors

Samples of typically completed copies of the questionnaires are also attached.

AHMADU BELLO UNIVERSITY, ZARIA.

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT

RESEARCH ON COMPARATIVE PHARMACOGNOSTIC INVESTIGATION OF CROTALARIA SPECIES IN ZARIA

QUESTIONNAIRE EXERCISE

A - HERBALISTS

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1:

Name by which plant is locally known in Hausa [please tick as appropriate]:

a) Bi rana
b) Bi ranar awaki
c) Bakar bi rana
d) farar bi rana
e) biya rana
f) other:.....

QUESTION 2:

Types of species known:

a) Ire-iren bi rana nawa aka sani? .....
.....

b) Shin minene banbancin su? .....
.....

QUESTION 3:

Uses in herbal medicine (if any) or other applications.

a) Wane anfani akeyi da bi rana?

i) Ga mutane .....
.....

ii) Ga dabbobi .....
.....

iii) Wasu anfanin .....
.....

b) Wane irin magunguna akeyi da bi rana? .....
.....

c) Wajen maganin, mi ake anfani da shi [ganyen/ "ya"yan] ?
.....
.....

d) Yaya ake yin hadin? .....
.....

QUESTION 4:

Any other information.

Ko akwai wani karin bayani .....
.....
.....

Mun gode kwarai.

Suna da adireshin malamin [idan akwai bukatar haka]: .....
.....Kwananwata.....

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RESEARCH ON COMPARATIVE PHARMACOGNOSTIC INVESTIGATION OF CROTALARIA SPECIES IN ZARIA

QUESTIONNAIRE EXERCISE

B - HERDSMEN

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1:

Name by which plant is locally known in Hausa [please tick as appropriate]:

a) Bi rana
b) Bi ranar awaki
c) Bakar bi rana
d) farar bi rana
e) biya rana
f) other:.....

QUESTION 2:

Types of species known:

a) Ire-iren bi rana nawa aka sani? .....
.....

b) Shin minene banbancin su? .....
.....

QUESTION 3:

Uses to animals or other applications.

a) Shin dabbobi suna cin bi rana?

.....

.....

b) Wadanne irin dabbobi, tumaki ko shanu ko awaki?

.....

.....

c) Akwai wadanda suka fi so ko wadanda basa so?

.....

.....

d) Wane sashe ake basu: ganye, yayan, ko duka?

.....

.....

QUESTION 4:

Human Uses of Crotalaria species.

Shin mutane suna amfani da bi rana? Wane irin amfani ne?

.....

.....

.....

QUESTION 5:

Any other information.

Ko akwai wani karin bayani .....

.....

.....

Mun gode kwarai.

Suna da adireshin malamin [idan akwai bukatar haka]: .....

.....Kwananwata.....

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RESEARCH ON COMPARATIVE PHARMACOGNOSTIC INVESTIGATION OF CROTALARIA SPECIES IN ZARIA

QUESTIONNAIRE EXERCISE

C - VETERINARY MEDICAL PRACTITIONERS

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1: What are the Crotalaria species you are aware of in the Zaria environment? .....
.....

QUESTION 2: Do animals feed on Crotalaria? .....
.....

QUESTION 3: Are there any adverse effects after livestock feed on Crotalaria ? .....
.....
.....

QUESTION 4: What are the usual syptoms of these effects? .....
.....
.....

QUESTION 5: Which of the Crotalaria species is/are associated with these effects? .....
.....

QUESTION 6: What treatment do you provide to handle this kind of cases? .....
.....
.....

QUESTION 7: What traditional treatment is given by herdsmen [if any is known] and how effective is it? .....
.....
.....

QUESTION 8: Is there any other information you wish to provide [including literature references, etc] on this problem?.....
.....
.....
.....
.....
.....

Thanks.

NAME & ADDRESS [OPTIONAL] .....
.....

Date:.....

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AHMADU BELLO UNIVERSITY, ZARIA.

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT

RESEARCH ON COMPARATIVE PHARMACOGNOSTIC INVESTIGATION OF CROTALARIA SPECIES IN ZARIA

QUESTIONNAIRE EXERCISE

D - MEDICAL DOCTORS {DEALING WITH LIVER DISEASES}.

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1: . Are there any patients with liver diseases that could be traced to consumption of herbal medicines?
.....
.....
.....

QUESTION 2: Are these patients few or many? .....
.....

QUESTION 3: Do you consider Crotalaria species [which produce Pyrollizidine alkaloids] as one of the causes of liver diseases? .....
.....
.....
.....

1

QUESTION 4: Can you state the symptoms associated with liver malfunction due to herbal intake? .....
.....
.....
.....

QUESTION 5: What treatment do you normally give to handle such cases? .....
.....
.....

QUESTION 6: What comment do you have on herbal consumption in Nigeria and liver diseases? .....
.....
.....

QUESTION 7: Any other information on this subject? .....
.....
.....
.....

Thanks.

NAME & ADDRESS [OPTIONAL] .....
.....

Date:.....

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RESEARCH ON COMPARATIVE PHARMACOGNOSTIC INVESTIGATION OF CROTALARIA SPECIES IN ZARIA

QUESTIONNAIRE EXERCISE

A - HERBALISTS

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1:

Name by which plant is locally known in Hausa [please tick as appropriate]:

a) Bi rana ✓
b) Bi ranar awaki
c) Bakar bi rana
d) farar bi rana
e) biya rana
f) other:.....

QUESTION 2:

Types of species known:

a) Ire-iren bi rana nawa aka sani? ....4.....
.....

b) Shin minene banbancin su? ..Wasu farare ne
....wasu ...baki ne.....

QUESTION 3:

Uses in herbal medicine (if any) or other applications.

a) Wane anfani akeyi da bi rana?

i) Ga mutane ...Maganin fari...

ii) Ga dabbobi ...Sukari...

iii) Wasu anfanin ...Ciwon ji...

b) Wane irin magunguna akeyi da bi rana? ...Suna da...

...Hanta...

c) Wajen maganin, mi ake anfani da shi [ganyen/ "ya"yan] ?

...Mukan yi...da ganyen kuma...

...mukan yi...da 'ya'yan...

d) Yaya ake yin hadin? ....Wannan...kuma.....

...a sirinmu...ta...baki...ko.

QUESTION 4:

Any other information.

Ko akwai wani karin bayani .....Ni'l.....

Mun gode kwarai.

Suna da adireshin malamin [idan akwai bukatar haka]: Malam San...

Mai magani...Sakin...T/V...T/W...Zaria Kwananwata 29/12/96.

AHMADU BELLO UNIVERSITY, ZARIA.

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT

RESEARCH ON COMPARATIVE PHARMACOGNOSTIC INVESTIGATION OF CROTALARIA SPECIES IN ZARIA

QUESTIONNAIRE EXERCISE

B - HERDSMEN

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1:

Name by which plant is locally known in Hausa [please tick as appropriate]:

a) Bi rana
b) Bi ranar awaki ✓
c) Bakar bi rana
d) farar bi rana
e) biya rana
f) other:.....

QUESTION 2:

Types of species known:

a) Ire-iren bi rana nawa aka sani? .....2.....
.....

b) Shin minene banbancin su? .Akwai fari
.....akwai baki.....

QUESTION 3:

Uses to animals or other applications.

a) Shin dabbobi suna cin bi rana?

.....Suna...ci...wani...lokaci....

.....

b) Wadanne irin dabbobi, tumaki ko shanu ko awaki?

.....Shanu...da...tumaki.....

.....

c) Akwai wadanda suka fi so ko wadanda basa so?

.....Sun.fi.so...mai...yado.....

.....

d) Wane sashe ake basu: ganye, yayan, ko duka?

.....Ganye.....

.....

QUESTION 4:

Human Uses of Crotalaria species.

Shin mutane suna amfani da bi rana? Wane irin amfani ne?

..Mu...idan...dabba...tana...ciwon...hanta
..Sai...mu...kirba...mu...bata...ruwan

.....

QUESTION 5:

Any other information.

Ko akwai wani karin bayani .....

.....Nil.....

.....

Mun gode kwarai.

Suna da adireshin malamin [idan akwai bukatar haka]: Bello

Na...bizara...mai...Shanu.....Kwananwata 29/10/96

AHMADU BELLO UNIVERSITY, ZARIA.

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QUESTIONNAIRE EXERCISE

C - VETERINARY MEDICAL PRACTITIONERS

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1: What are the Crotalaria species you are aware of in the Zaria environment? Crotalaria retusa

QUESTION 2: Do animals feed on Crotalaria? Goats on most occasions are seen nibbling dry leaves of this plant.

QUESTION 3: Are there any adverse effects after livestock feed on Crotalaria ? If feeds are cut for intensively managed animals such may be included and nervous and gastro-intestinal problems have been observed (fresh leaves)

QUESTION 4: What are the usual syptoms of these effects? foamy discharges from mouth, dyspnea, muscle tremors, congested mucous membranes, diarrhoea,

QUESTION 5: Which of the crotalaria species is/are associated with these effects? Crotalaria retusa

QUESTION 6: What treatment do you provide to handle this kind of cases? Those cases attended to : placed on mineral oil (groundnut or palm oil) to reduce further absorption from GIT ; placed on Atropine sulphate, Anti-diarrhoea agents given in severe cases

QUESTION 7: What traditional treatment is given by herdsmen [if any is known] and how effective is it? Give local herbs to reduce the diarrhoea (though not get shown the herbs)

QUESTION 8: Is there any other information you wish to provide [including literature references, etc] on this problem? Literature on this problem is very scanty or non available This is because animals rarely feed on this plant so most they happen are rarely reported to the relevent authorities by the livestock owners for documentation

Thanks.

NAME & ADDRESS [OPTIONAL] D.K. SACKEY, VET SURG. & MEDICINE DEPT

Date: 5-11-96

AHMADU BELLO UNIVERSITY, ZARIA.

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT

RESEARCH ON COMPARATIVE PHARMACOGNOSTIC INVESTIGATION OF CROTALARIA SPECIES IN ZARIA

QUESTIONNAIRE EXERCISE

D - MEDICAL DOCTORS {DEALING WITH LIVER DISEASES}.

INTRODUCTION:

A postgraduate research on Crotalaria species in Zaria is being undertaken in the above department. It would be appreciated if you could kindly complete the questionnaire below as it relates to your profession. Information supplied shall be treated as confidential and used only for the purpose of the research.

QUESTION 1: Are there any patients with liver diseases that could be traced to consumption of herbal medicines?

Very difficult to be positive about

QUESTION 2: Are these patients few or many? Not Known

QUESTION 3: Do you consider Crotalaria species [which produce Pyrollizidine alkaloids] as one of the causes of liver diseases?

No idea

1

QUESTION 4: Can you state the symptoms associated with liver malfunction due to herbal intake? It is unlikely to differ from those from other causes.

QUESTION 5: What treatment do you normally give to handle such cases? If one can trace the disease to herbs, one would advise the stop of herbs and apply the usual treatment to liver diseases.

QUESTION 6: What comment do you have on herbal consumption in Nigeria and liver diseases? It is possible the liver disease ~~to be~~ related to herbs but nobody has categorically known cause and effect in Nigeria.

QUESTION 7: Any other information on this subject? N/A

Thanks.

NAME & ADDRESS [OPTIONAL] .....

.....

Date: 29/10/96

APPENDIX III

SYNOPSIS OF NAPRALERT SEARCH ON CROTALARIA SPECIES

NAPRALERT is an acronym for NAtural PRoducts ALERT. It is a dynamic database which is updated periodically. The data in NAPRALERT represents a synthesis of information from more than 150,000 scientific journal articles, books, abstracts and patents, collected systematically from the global literature, since 1975. Information covered concerns mainly ethnomedical data on plants, biological effects reported for extracts of living organisms and the occurrence of secondary chemical metabolites in living organisms. Biological effects reported for chemical compounds from natural sources of defined chemical structure are also included in the database. NAPRALERT is currently maintained by the Program for Collaborative Research in the Pharmaceutical Sciences, within the Department of Medicinal Chemistry and Pharmacognosy, in the College of Pharmacy of the University of Illinois at Chicago, USA.

NAPRALERT search is essential in assessing the extent of work done on Crotalaria species globally. This synopsis has been prepared from a 134-page literature obtained from NAPRALERT via the Internet Service. The search carried out was extensive and it was updated to cover up to December 1, 1997 on all aspects of research on the genus Crotalaria.

There are two major information presented here:

- Crotalaria species reported (number and names of individual species).
- Literature on Crotalaria species under this investigation. These are :
  - (a) C. microcarpa
  - (b) C. naragutensis
  - (c) C. retusa

III.1 CROTALARIA SPECIES REPORTED IN NAPRALERT SEARCH.

The total number of Crotalaria species found in the NAPRALERT Data Base was ninety five (95) as at December 1, 1997. These are listed alphabetically in Table 20.

TABLE 20 : THE INDIVIDUAL CROTALARIA SPECIES REPORTED IN THE NAPRALERT.

1.	*Crotalaria aegyptiaca*
2.	*Crotalaria agathifolia*
3.	*Crotalaria alata*
4.	*Crotalaria albida*
5.	*Crotalaria anagyroides*
6.	*Crotalaria angulata*
7.	*Crotalaria argyrolobioides*
8.	*Crotalaria aridicola*
9.	*Crotalaria assamica*
10	*Crotalaria axillaris*
11	*Crotalaria barbata*
12	*Crotalaria brevidens*
13	*Crotalaria brevifolia*
14	*Crotalaria brownei*
15	*Crotalaria burhia*
16	*Crotalaria candicans*
17	*Crotalaria cannabina*
18	*Crotalaria capensis*
19	*Crotalaria cephalotes*
20	*Crotalaria courosa*
21	*Crotalaria crispata*
22	*Crotalaria cunninghamii*
23	*Crotalaria damarensis*
24	*Crotalaria deserticola*
25	*Crotalaria distans*
26	*Crotalaria dura*

27	*Crotalaria emarginata*
28	*Crotalaria falcata*
29	*Crotalaria filipes*
30	*Crotalaria fulva*
31	*Crotalaria gatiflora*
32	*Crotalaria globifera*
33	*Crotalaria goreensis*
34	*Crotalaria grahamiana*
35	*Crotalaria heyneana*
36	*Crotalaria incana*
37	*Crotalaria intermedia*
38	*Crotalaria juncea*
39	*Crotalaria laburnifolia*
40	*Crotalaria lachnosema*
41	*Crotalaria lanceolata*
42	*Crotalaria leioloba*
43	*Crotalaria leschenaultii*
44	*Crotalaria linifolia*
45	*Crotalaria longirostrata*
46	*Crotalaria madurensis*
47	*Crotalaria maritima.*
48	*Crotalaria mauensis*
49	*Crotalaria maxillaris.*
50	*Crotalaria maypurensis*
51	*Crotalaria medicaginea*
52	*Crotalaria mesopontica*
53	*Crotalaria mitchellii*

54	*Crotalaria mucronata*
55	*Crotalaria nana*
56	*Crotalaria naragutensis*
57	*Crotalaria nitens*
58	*Crotalaria obovata*
59	*Crotalaria ochroleuca*
60	*Crotalaria othonnae*
61	*Crotalaria pallida*
62	*Crotalaria paulina*
63	*Crotalaria petitiana*
64	*Crotalaria polysperma*
65	*Crotalaria prolongata*
66	*Crotalaria prostrata*
67	*Crotalaria pumila*
68	*Crotalaria quinquefolia*
69	*Crotalaria ramosissima*
70	*Crotalaria recta*
71	*Crotalaria retusa*
72	*Crotalaria rosenii*
73	*Crotalaria rubiginosa*
74	*Crotalaria sagittalis*
75	*Crotalaria saltiana*
76	*Crotalaria scassellatii*
77	*Crotalaria semperflorens*
78	*Crotalaria senegalensis*
79	*Crotalaria sericea*
80	*Crotalaria sessiliflora*
81	*Crotalaria spartiodes*

82	*Crotalaria spectabilis*
83	*Crotalaria striata*
84	*Crotalaria tetragona*
85	*Crotalaria thebaica*
86	*Crotalaria timorensis*
87	*Crotalaria trifoliastrum*
88	*Crotalaria triquetra*
89	*Crotalaria usaramoensis*
90	*Crotalaria verrucosa*
91	*Crotalaria vestita*
92	*Crotalaria virgulata*
93	*Crotalaria walkeri*
94	*Crotalaria wightiana*
95	*Crotalaria zanzibarica*

III.2 LITERATURE ON THE CROTALARIA UNDER THIS STUDY.

III.2.1 C. microcarpa

The NAPRALERT Data Base does not contain any literature or information directly or indirectly on Crotalaria microcarpa.

III.2.2 C. naragutensis

Detection of the alkaloids of the seeds of C. naragutensis has been reported by Nwude and Mattocks (1988). These alkaloids were integerrimine, nilgrine, acetyl integerrimine and usaramine. No report of alkaloids detected in leaves.

III.2.3 C. retusa

The NAPRALERT search has revealed an appreciable amount of work done on C. retusa on the following aspects:

1. Occurence of high monocrotaline yielding strain of C. retusa (Kumari et al, 1966).
2. A novel cardenolide from seeds of C. retusa (Yadava and Thakur, 1995).
3. Clastogenic effects of extracts obtained from C. retusa and C. mucronata on mouse bone marrow cells (Ribeiro et al, 1993). C. retusa extracts were found to have induced chromosome aberrations.

4. Unusual fatty acids of C. retusa seed oil (Daulatabad, et al, 1989). Linoleic acid was detected in C. retusa.
5. Effects of feeding diets containing seeds of C. retusa (Ross, A. J., 1977). C. retusa was found to be toxic to pigs.
6. Screening of Sudanese plants for mollusicidal activity and identification of leaves of Tacca leontopetaloides as a potential new exploitable resource (Abdelaziz et al, 1990).
7. Plants in Kano ethnomedicine: screening for antimicrobial activity and alkaloids (Hussain and Deeni, 1991).
8. Studies on the identification of alkaloids of Crotalaria species, isolation and identification of alkaloids of C. retusa (Han, et al, 1981).
9. Vitexin 4-0-galactoside from C. retusa (Srinivasen and Subramanian, 1983).
10. Investigation of molluscidal activity of certain Sudanese Plants used in Folk Medicine (Ahmad, et al, 1984).
11. Inventory of Plants used in traditional medicine in Somalia: Plants of families lauraceae – papilionaceae (Samuelson, et al, 1992). Fresh roots of C. retusa were reported to be used in the treatment of heart diseases in Somalia.
12. Budd-Chiary syndrome as a result of consumption of Bush tea in young Guadaloupians (Bonnet and Loret, 1988). Infusion of C. retusa orally used proved toxic to human child with symptoms of veno occlusive disease.
13. Traditional practices affecting the health of Women and Children in Africa (Kargbo, T. K., 1984). C. retusa used in the treatment of constipation in pregnancy.

14. Veno Occulusive disease of the liver (VOD) (Stuart and Bras, 1951). VOD resulting from the use of C. retusa for woophing cough in Jamaica where poisoning is associated with VOD symptoms.

15. Alkaloid bearing plants and their contained alkaloids (William and Schubert, 1961). Occurence of isatinecine, monocrotaline–N--oxide in leaf and seed.

16. A survey of the plants of Bhagalpur and Santal Pargana for Saponins, flavonoids and alkaloids (Sinha and Dogra, 1985). Alkaloids were found to be absent in shoots of C. retusa but present in roots and seeds, flavonoids present in shoot, root and seeds, while saponins absent in roots but present in shoots and seeds.

17. Analysis of Amino acids in the seeds of certain Crotalaria species (Ramakrishna et al, 1981). Amino acids were found in the seeds of C. retusa .

18. Identification of the flavonoids in Papilionaceae flowers using paper chromatography (Ogbeide and Parvez, 1992). Astragalin and genistein were detected in the flowers of C. retusa .

19. Pyrrolizidine alkaloids: Biosynthesis of monocrotalic acid, the necic acid component of monocrotaline (Robins et al, 1974). Monocrotaline was detected in the entire plant of C. retusa.

20. Alkaloids of C. retusa (Culvenor and Smith, 1957). Retusamine and its N-oxide were detected in the seeds.

21. Attraction of Ethyl ether extracts of 232 botanicals to oriental fruit flies, melon flies and mediteranian flies (Keiser et al, 1975). C. retusa extract was a positive sex attractant {signalling}.

22. The crystal structure of spectabiline (Wang and Chen, 1984).
23. Orthine decarboxylase, polyamines and pyrrolizidine alkaloids in Senecio and Crotalaria (Birecta et al, 1988). Detection of retusine in seeds of C. retusa was reported.
24. Occurence, concentration and toxicity of pyrrolizidine alkaloids in Crotalaria seeds (William and Molyneux, 1987). Alkaloids of C. retusa were found to be fatal to chicks.
25. Screening of Indian Plants for Biological activity (Dhawan, et al, 1977). C. retusa extracts were found to be negative in analgesic screening but positive for semen coagulation.

The above represents information directly on Crotalaria from NAPRALERT. By far, C. retusa is the most extensively studied among the three species in this investigation.

APPENDIX IV

DETAILS OF RESULTS

AND SAMPLE CALCULATIONS

The results of the various determinations carried out in the evaluation of the Crotalaria species are presented in Chapter 3. The following are details that are important in arriving at those results. Sample calculations are also illustrated.

IV.1 PHYSICAL CONSTANTS.

Each physical constant was calculated for the three Crotalaria species as illustrated below. The sample calculations are for C. microcarpa.

(a) Moisture Content.

Weight of drug used	=	3.0 g
Constant weight of empty crucible	=	27.21 g
Weight of crucible + drug	=	30.21 g
Constant weight of Crucible + drug after drying	=	30.04 g

Weight of moisture : 0.165 g, 0.168 g, 0.169 g,

Average weight being = 0.0168g

$$\% \text{ Moisture} = \frac{0.168}{3} \times \frac{100}{1}$$

$$= \underline{5.6\ \%.}$$

(b) Ash Value.

Weight of drug used	=	2.0 g
Constant weight of empty crucible	=	27.20 g
Weight of crucible + drug	=	29.20 g
Constant weight of Crucible + drug after drying	=	28.80 g

Weight of moisture : 0.40 g, 0.43 g, 0.38 g,

Average weight being = 0.40 g

$$\% \text{ Ash} = \frac{0.40}{2} \times \frac{100}{1}$$

$$= \underline{20\ \%.}$$

(c) Acid Insoluble Value.

Origional weight of drug used	=	2.0 g
Constant weight of nickel crucible	=	27.31 g
Weight of crucible + ash	=	27.71 g
Weight of Crucible + residual ash	=	27.41 g
Weight of acid insoluble ash	=	0.30g

$$\% \text{ Acid insoluble Ash} = \frac{0.3}{2} \times \frac{100}{1}$$

$$= \underline{15\ \%.}$$

(d) Alcohol Soluble Extractive Value.

Weight of drug used	=	5.0 g
Constant weight of empty crucible	=	27.31 g
Weight of crucible + drug	=	32.31 g
Constant weight of Crucible + residue	=	28.02 g
Weight of residue	=	0.75 g,

$$\% \text{ Alcohol Soluble Extractive} = \frac{0.75}{5} \times \frac{100}{1}$$

$$= \underline{15\ \%.}$$

IV.2 CALCULATION OF PERCENTAGE ALKALOIDS.

The average molecular weight of the possible pyrrolizidine alkaloids in the species under study is 330.

N/200 Sulphuric acid was used in the titration.

1 liter of the acid contains 0.005 equivalent weight.

1 ml of the acid contains 5×10^{-6} equiv. weight.

Equivalent weight of the P.A.'s is 330 gm.

Since 1 equiv wt of acid would neutralise 1 equiv wt of alkaloid,

1 ml of acid is equivalent to $5 \times 10^{-6} \times 330$ g = **1.65×10^{-3}g**.

This factor was used to determine the amount of alkaloid in gramme and the percentage was subsequently calculated.



The End

