THE INVESTIGATION OF THE ALKALOIDS OF STRYCHNOS DECUSSATA

BY

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D E D I C A T I O N

This project is dedicated to my parents for their sacrifice to see me through my education.

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A B S I R A C I

This project was carried out in order to study the alkaloids of Strychnos decussata growing in Tsavo National Parkin Kenya.

Macroscopical and microscopical examination was carried out on the leaves to find out the diagnostic features of the leaves which include:-

1) Leaves are ovate in shape
2) Dimensions of the leaves are 1.6cm - 2.3cm. long and 0.9cm - 1.3c.m wide.
3) The leaves have a smooth surface, free from hairs with a thick layer of cuticle on both sides.
4) The apex is bluntly rounded (obtuse)
5) The leaves are petiolated and equal at the base.
6) The leaves show isobilateral arrangement of palisade layer and epidermis layer.
7) The stomata were peculiar in that they had 6-7 neighbouring cells Fig III.
8) Arrangement of vascular bundles is Hydrocentric.
9) Xylem was lignified

Extraction of the alkaloids was carried out from the powdered leaves and powdered bark using the method that was used by Rolfsen W.N.A, Olaniyi A.A; Sanberg. F, and Maick A.N. (1980) in the Journal of Acta - Pharmaceutica Suecica 17, 105 - 111.

Thin Layer Chromatographic technique was used for the separation of the alkaloids. The alkaloids appeared as one spot.

Quantitative analysis was carried out by Gravimetric method to give:-

1) Dried le-ves contained 0.303% w/w of Alkaloids calculated as decussine

2) The dried bark contains 0.664% w/v of alkaloids calculated as decusaine.

Pharmacological study on the crude aqueous extract of the leaves of Strychnos decuscate to test for muscle relaxant activity was done on a rabbit's ileum using 100ug/ml of adrenaline as the standard.

The crude extract was found to be a potent muscle.

I N T R O D U C T I O N

The genus Strychnos L. is a member of the tribe Strychneae of the family Loganiaceae. Strychnos is pantropical in distribution, occuring in central and south America, Africa and Asia as forest trees or savannah trees. Some forest trees are S. mitis, S. elaeocarpa, S. malladora, S. henningsii, while savannah trees are S. cocculoides, S. decussata, S.henningsii, S. potatorum, S. madagascariensis, S. pungens, S. Spinosa etc

Some of the widely used herbal remedies were derived from strychnos species which contain alkaloids among other constituents. Some species that are used medicinally has different parts of the plant employed as shown below:

Part	%
Fruit(pulp and/or seeds)	14
Leaves	24
Stem bark	14
Roots (whole, bark or wood)	35
Not specified	21

This gives over 100% since a number of remedies call for the use of more than one plant part. The following table indicates some major categories into which the uses and ailments treated can be divided and the number of reports for each category.

Emetic	7
Purgative	5
Snake bite	10
Stomach, abnominal and intestinal complaints	14
Worms and parasites	9

Cicatrization of wounds	8
Febrifuge (and malaria)	10
Analgesic (and rheumatism)	9
Eye trouble	8
Ear, nose, and throat troubles	6
Chest and lung complaints	11
Venereal disease: sexual complaints; aphrodisiac, abortifacient	19
Epilepsy and insanity	4
Miscellaneous	15

It is therefore clear that African strychnos species have acquired widespread use either as a source of medicinal remedies or edible fruit. By 1970 over 75 strychnos species found in Africa had been screened for alkaloids. Strychnos species has been found to contain alkaloids with convulsine and muscle relaxant effects. The firt isolation of a convulsine alkaloid from African strychnos species was achieved by continued Pharmacological screening for convulsine and muscle relaxant effects by E.A.S. Icaja Daoli (Sandberg et al) (1959) leading also to the detection of 4 hydroxy strychnine. As a result of further screening new tertiary indole alkaloids with pronounced muscle relaxant and producing chronic convulsions in high doses were found in other species.

Strychnos decussata is the subject of this project. Distribution: East and South Africa and Madagascar.

The plant is a shrub or a small tree with a trunk up to 45 cm in diameter; the wood is hard. It is found in woodlands, often near rocks and sometimes near river banks and in Madagascar in dry forests and in bushes on limestone.

In Kenya it is found in the Eastern region especially in Nzue and Kitui districts. It bears an orange fruit which is edible. The wood which is red-brown is used for shafts of assegais and rural utensils.

Work done by Wencha N.A, Rofsen, Ajibola, A. Olaniyi, and F. Sandberg (1980) isolated decussine, a tertiary indole alkaloid from the stem bark of the tree. It was found that decussine although a tertiary amine had a pronounced muscle relaxant effect both in vivo and in vitro. Muscle relaxant activity of decussine is in line with activity of strychnine derivatives, there is also the possibility that decussine acts as an inhibitor of choline uptake.

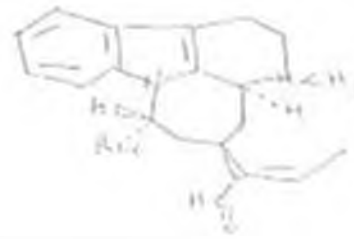
In the same year (1980) Rofsen W.N.K, Olaniyi and Hylands P.J isolated five tertiary indole alkaloids from the stem of strychnos decussata

L. Rivier and J.G. Brulin while investigating on medicinal plants in tropical West Africa acting on nervous system found out that from the stem bark of Strychnos decussata a tertiary indole alkaloid was isolated and found to have muscle relaxant activity. (Invivo and invitro). The blocking effect of this alkaloid was not antagonised by eynstigmine (Bisset and Phillipson 1970, 1973).

Alkaloids of strychnos decussata

Some of the already isolated alkaloids are:-

1. Decussine



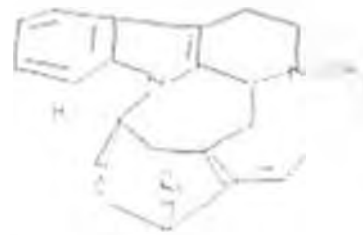
H H for Akagerine

for 17-O-methyl-akagerine

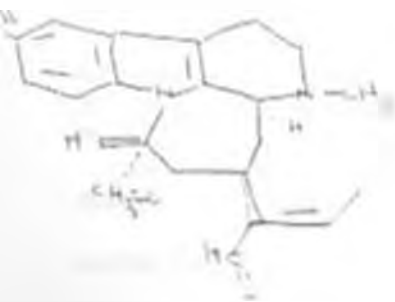
$R_1 = OCH_3$, R_2=H for 10-hydroxy-21-O methyl Kribine

R_1 = H, $R_2 = OCH_3$

for 10-hydroxy-epi-21-O-methyl kribine



10-hydroxy-17-O methyl akagerine



R_1 - OCH_3 R=H, for 21-O-methyl Kribine

R_1 = H, R_2=OCH_3 for -epi 21-O methyl kribine

EXPERIMENTAL

COLLECTION OF PLANT MATERIAL

The material used was collected from Taavo National Park in October [illegible]. Stems were cut which contained leaves and seeds. The bark was crapped from the stem while fresh using a knife and the scrapplings dried at room temperature before grinding into powder.

The leaves were picked, dried seperately at room temperature and then ground into pouder. The powders were then kept seporately in well closed glass containers. Some of the leaves were [illegible]ved in 70% Ethanol.

MACROSCOPICAL EXAMINATION OF THE LEAVES

The whole leaves are ovate - between 1.6 - 2.3 cm long and 0.9 - 1.3cm wide. The surface is quite smooth and entirely free from hairs. (Glabrous). The margin is entire and apex is bluntly rounded (obtuse). The leaves are also petioled and equal at the base (symmetric). (Fig 1).

MICROSCOPIC EXAMINATION OF THE LEAVES

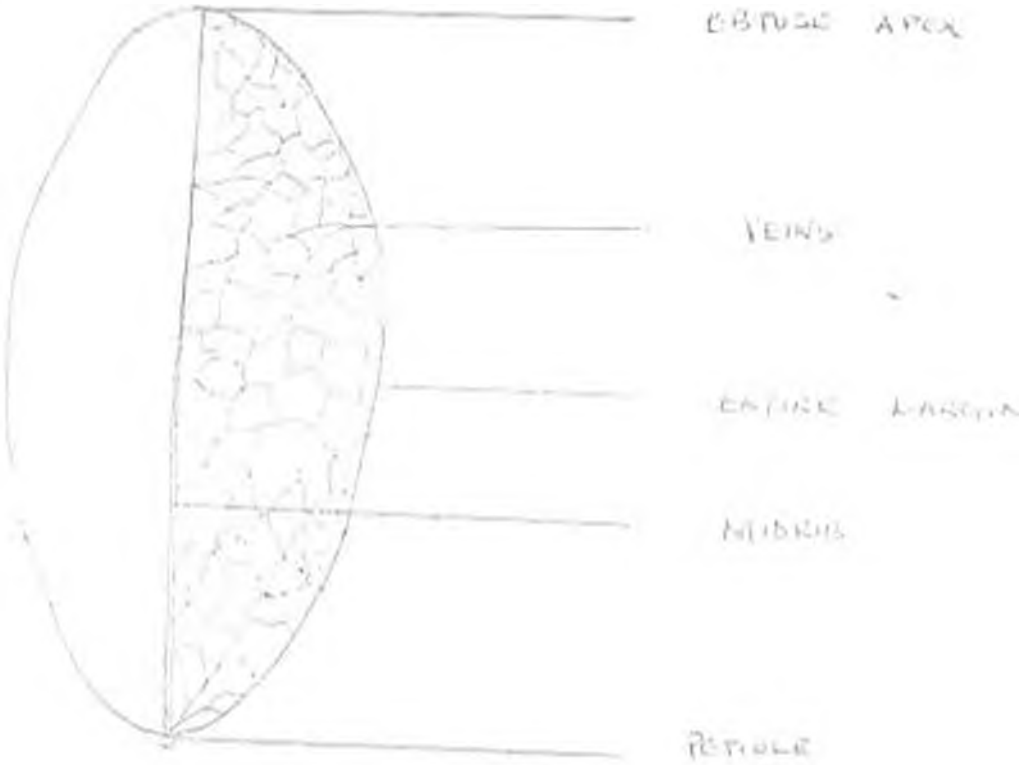
Preserved leaves of Strychnos decussata were used. A transverse section through the midrib was cut and the section mounted on the microscope slide and a few drops of chlorohydrate added and cover slip put in position. This was warmed up then examined through the microscope. The lower epicermis was also

examined and distinguishing features were drawn as shown in Fig II and Fig III respectively.

Figure II shows that the leaves have an isobilateral structure. The epidermal cells have straight walls. The epidermal cells have straight walls. The vascular bundles show Hydrocentric arrangement. Phloroglucinol reagent was used to test for lignification and iodine for starch.

acopical EXAMINATION OF THE LEAF

E I

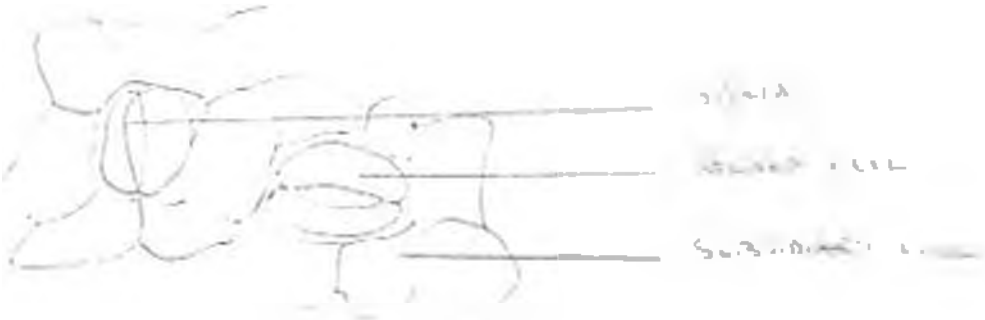


x 5

MICROSCOPICAL EXAMINATION OF LOWER EPIDERMIS

FIGURE I

STOMATA



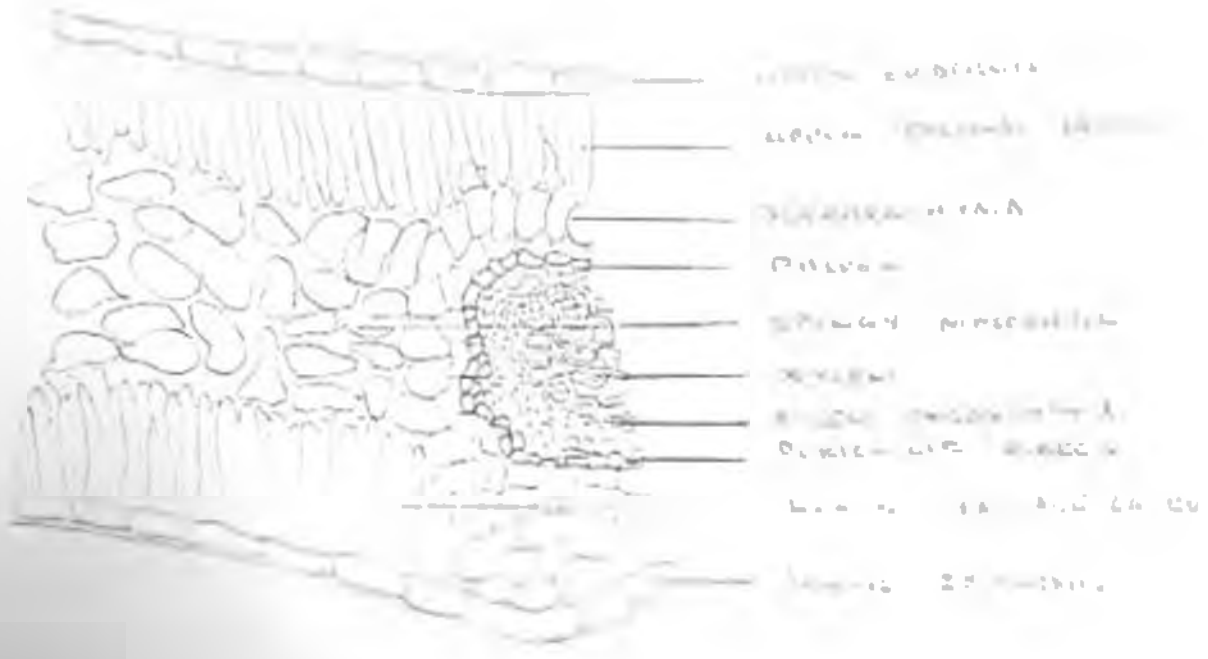
R E S U L T S

Table 1

Test	Observation	Inference
For Lignification with phloroglucinol	Pink coloration on [illegible] vessels	Xylem is lignified
For starch with Iodine	Tiny blue/black spots in spongy mesophyll	Starch granules [illegible] present

MICROSCOPICAL EXAMINATION OF T.S OF THE LEAF

FIGURE III



REAGENTS USED

REAGENT	GRADE	BRAND
Acetic acid	Lab.Reagent	M & B
Amberlite	" "	BDH Ltd
Ammonia solution	" "	Howse & McGeorge
Anhydrous Na_2SO_4	" "	" "
Chloroform	" "	M & B
Diethylamine	" "	"
Diethylether	" "	BDH
Ethanol	" "	M & B
Hydrochloric acid	" "	M & B
Methanol	" "	Koch-Light Ltd
Silica gel (GF254)	" "	Merk
Sulphuric acid	" "	M & B

SCREENING OF ALKALOIDS FROM THE LEAVES OF STRYCHNOS DECUSSATA:

1.0g of powdered leaf of Strychnos decussata was extracted by warming on a water bath with 2ml 1% sulphuric acid for 2 minutes. The solution was filtered and tested by adding 1-2 drops of Meyers Reagent so that a white to buff precipitate was formed. The rest of the extract was made distinctly alkaline with dilute ammonia solution and extracted with a little amount of water. The chloroform layer was filtered through a plug of cotton wool and the filtrate divided into two equal portion each of which was evaporated to dryness. The test was then repeated using powdered bark.

TEST FOR ALKALOIDS

The residue was dissolved in 0.2ml 1% sulphuric acid and to 0.1ml of the solution was added:-

1. One drop Meyers Reagent
2. One drop Drangendoffs' Reagent

TABLE II

REAGENT	LEAVES	BARK
Meyers Reagent	White to buff Precipitate formed	White to buff precipitate formed
Drangendoffs reagent	Orange/Red precipitate formed	Orange/Red precipitate formed
Inference	Alkaloids were Present	Alkaloids were present

QUALITATIVE ANALYSIS OF THE LEAVES OF STRYCHNOS DECUSSATA

EXTRACTION OF THE ALKALOIDS

The method used by Rolfsen W.N.A Olunlyi A.A. Sanberg P. and Kolck A.N. (1980) was utilised. (Journal of Acta - Pharmaceutica Succica 17, 105 - 111).

METHOD:

The ground stem bark (2.0g) was extracted twice with 10% acetic acid in ethanol by macerating the material for 48 hours. The combined filtrates was acidified to pH-2 with 5% Hydrochloric acid and Mayers reagent was added until no more precipitate formed. The solution was centrifuged and the precipitate was dissolved in a mixture of acetone:Methanol:water (6:2:1) respectively. The mixture was passed through an anion exchange resin (Amberlite LAA - 400, Cl^- form) and same solvent was used to elute the alkaloid chlorides. The solution containing the alkaloid chloride was evaporated until removal of acetone and methanol was complete. The remaining aqueous solution was basified with 10% ammonia and extracted with chloroform (5 X 50ml). The chloroform layer was evaporated to dryness. The chloroform fraction was dissolved in chloroform. This solution was used to spot on the TLC plates. The same was repeated for ground leaves material.

THIN LAYER CHROMATOGRAPHIC EXAMINATION OF ALKALOIDS EXTRACT OF STRYCHNUS DECUSSATA

PREPARATION OF THE PLATES

The coating material used was slurry which was prepared by mixing 60g of Silica Gel GF_{254} and 120ml of water by shaking vigourously. The slurry was immediately spread on clean 20X20cm. chromatographic plates. The whole process took less than 2 minutes to prevent slurry hardening before it was spread on the plates. The plates were made 1.5mm thick. The layer was allowed to dry without disturbance for 15 minutes on aligning tray. The plates were then dried in the oven for 30 minutes at $110^{0}C$. This is important for the activation of Silica gel.

The properties of the dried layers when chromatograms are made, are determined by the combined effects of the capillary system between the cavity system with the grains and the surface chemical groups in the cavity system. The plates were left aside to cool.

INVESTIGATION OF THE BEST SOLVENT SYSTEM FOR T.L.C ANALYSIS

Different solvent systems were tried in order to choose the one that gave the highest Rf values.

TABLE III

Solvent Syotem	Rf Values
1. Methanol : Conc. NH_3 200 : 3	0.1
2. Chloroform: diethylamine 9 1	0.4
3. Diethylether:Ethanol:diethylamine 90 : 4 : 6	0.75
4. Chlorofom : Methanol 8 : 2	0.2
5. Cychlohexane:chloroform:diethylamine 90 : 3 : 7	0.1
6. Benzene:ethylacetate:diethylamine 7 2 1	0
7. Benzene : Chloroform 1 : 1	0.1

$$Rf = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by solvent}}$$

The best solvent system was taken as Diethylether:Ethanol: diethylamine (90:4:6).

THIN LAYER CHROMATOGRAPHY

Conditions

Technique : One way ascending

Adsorbent: Silica gel GF$_{254}$

Solvent system: Diethylether:Ethanol:diethylamine

Temperature: 25°C

Visualisation: U.V. light, spray with Drangendoffs followed by sulphuric acid (0.1N)

Distance travelled: 15 cm.

Plates: 20 X 20cm.

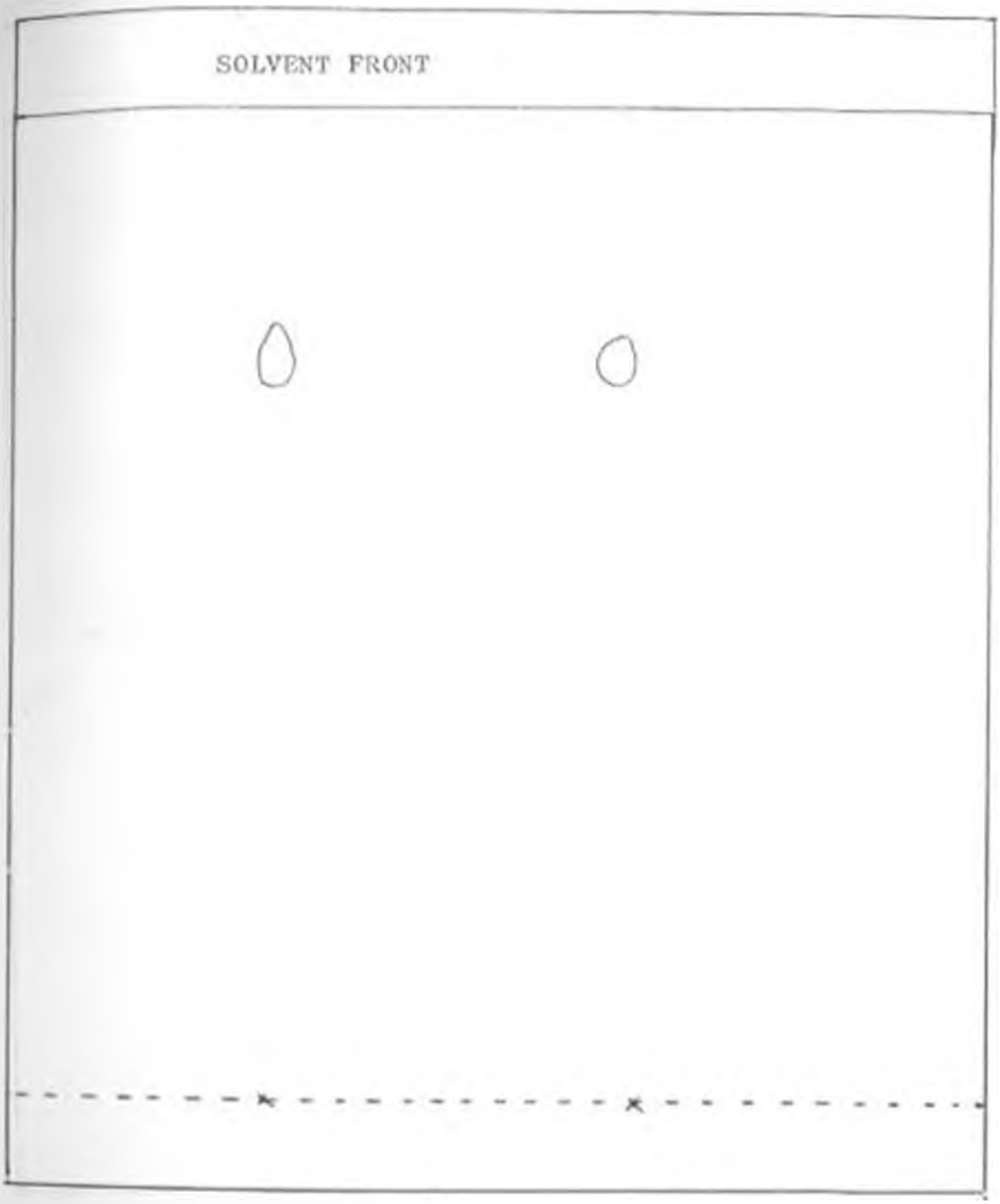
METHOD

Template was used in making the start points from lower edge and side of the plate. It was necessary to use the template as this prevents the damaging of the rest parts of the plate during the application of the samples. Capillary tubes were used for the application of the samples. The chamber was allowed to saturate for 40 minutes before the plates were developed. The plates were run for a distance of 15cm then removed allowed to dry and observed under UV light then sprayed with Drangendoffs reagent followed by spraying with 0.1N sulphuric acid. The spots obtained are as shown in Figure IV and V. The Rf values were then calculated as shown in Table 1.

TLC OF THE BARK EXTRACT

TLC OF THE BARK EXTRACT

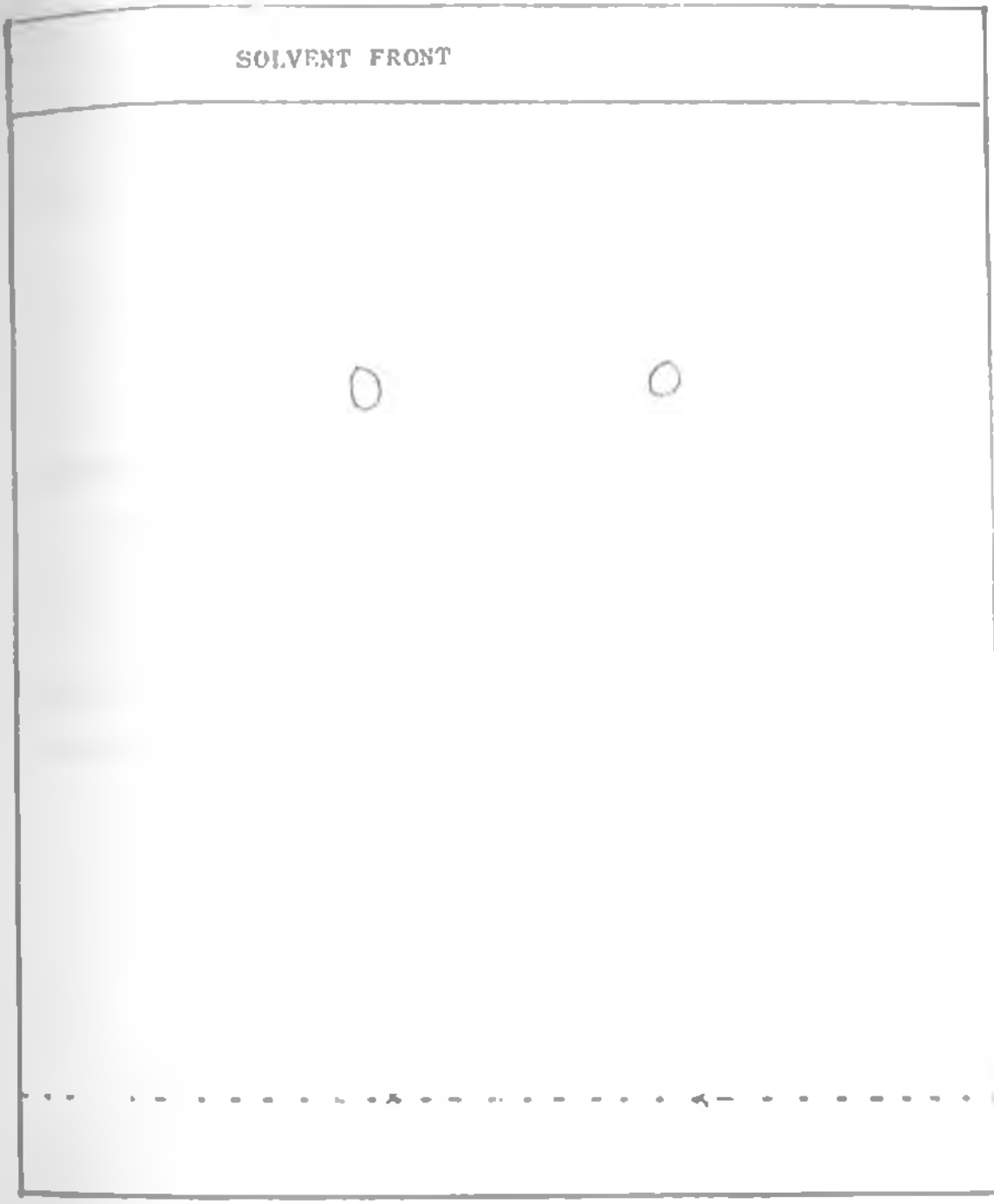
FIGURE IV



$$Rf = \frac{11.25}{15} = 0.75$$

TLC OF THE LEAVES EXTRACT

FIGURE V



$$Rf = \frac{10.8}{15} = 0.72$$

Table IV

	Distance moved by solvent	Distance moved by substance	Rf
Bark	15.0cm.	11.25cm	0.75
Leaves	15.0cm	10.2cm	0.72

QUANTITATIVE ANALYSIS

Extraction of Alkaloids

Alkaloid extraction is based on inherent basic nature and ability to form salts with acids. The leaves and bark are dried and powdered to increase the surface area and hence, increase the contact between the solvent and alkaloids possessing cells or tissue.

DETERMINATION OF TOTAL ALKALOIDAL CONTENT OF STEM BARK AND LEAVES OF STRYCHNUS DECUSSATA: BY GRAVIMETRIC METHOD

10g. of ground bark material was continuously extracted for about 48 hours in a Soxhlet apparatus using 1% acetic acid in ethanol. The filtrate was reduced to half volume by evaporating some solvent in a rotary evaporator. The filtrate was shaken with several portions of an aqueous acid, 2% sulphuric acid. Three shakings were done, each with 20ml 2% sulphuric acid for every 100ml 1% acetic acid in ethanol extract. Test for complete extraction was done with Drangendorff's reagent.

The non alkaloidal and pigment impurity was removed from aqueous extract by shaking with portions of chloroform and discarding chloroform layer. The aqueous acidic solution was made alkaline with 10% ammonia and alkaloid extracted with (50 X 5) chloroform. Water was removed from organic solvent using 2g anhydrous sodium sulphate for every 100ml solvent. Sodium sulphate was removed by filtration using some cotton wool. Again 2g anhydrous sodium sulphate was added and left overnight to extract the final traces of water. This was filtered to get rid of the sodium sulphate. The water free extract was evaporated to dryness. The residue was dissolved in minimum amount of chloroform and solution transfered into a preweighed petri dish and left on the bench for the chloroform to evaporate.

The residue obtained was recrystallised from methanol and after drying for 15 minutes at 100^{o}C the weight of petri dish and crystals was taken. The same was repeated for powdered leaves.

RESULTS

Table V

	Bark	Leaves
Weight of petri dish + residue	41.4180	37.0900
Wt. of empty petri dish	41.3516	38.1927
Wt. of crystals	0.0664	0.03027
% w/w of alkaloid	0.664	0.303

Calculations

10g. of powdered material was used

Wt. of crystals formed = 0.0664 (bark)

$$\% \text{ w/w} = \frac{0.0664 \times 100}{10}$$

% w/w Yield = 0.664% w/w

Calculation was done similarly for the leaves yield

PHARMACOLOGICAL STUDY OF THE CRUD AQUEOUS EXTRACT OF THE LEAVES OF STRYCHNOS DECUSSATA

INTRODUCTION

Pieces of intestine of any small animal will continue to give responses for many hours if kept in suitable physiological solution such as Tyrode solution. Rabbits ileum exhibit regular pendular movements and is usually used to study effects of drugs on motility and tone of the intestine. The movements of the intestine are of 3 types:

1. Regular contractions of longitudinal muscle
2. Localised contractions of circular muscle
3. Localised contractions of circular muscle propagated along the muscle under influence of Auerbachs' plexus.

AIM

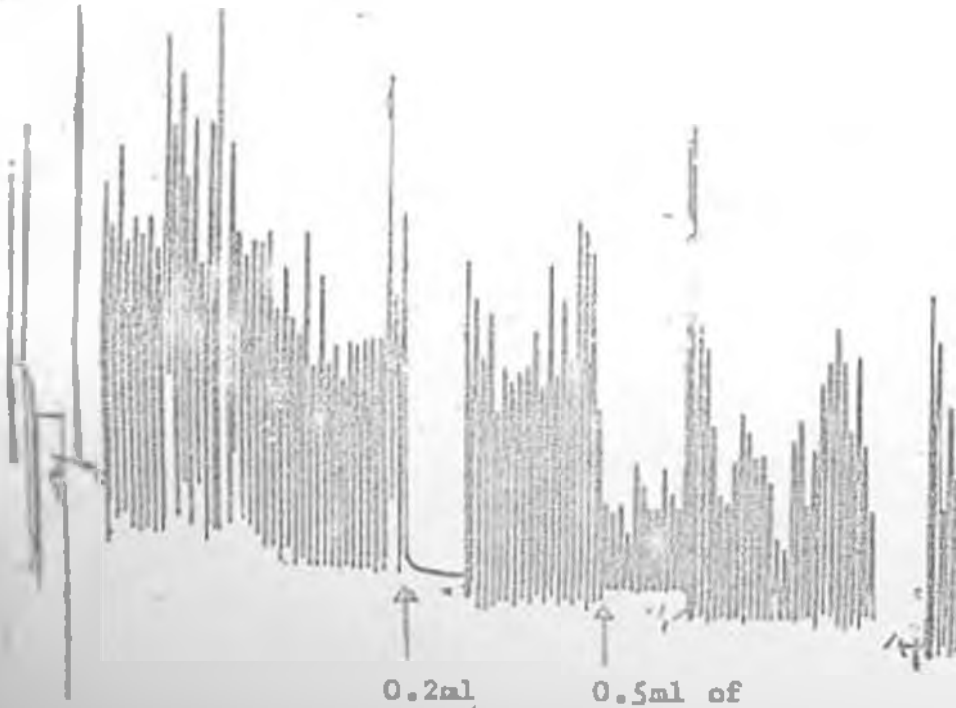
To investigate if the crude aqueous extract of Strychnos decussata has any effect on motility and tone of the intestine.

METHOD:

A piece of jejenum was taken from a rabbit soon after killing it by a blow at the back of the head. The preparation was then set up in Tyrode solution. The requisite tone of the piece of jejenum was allowed to develop by leaving the preparation in the bath for some time.

After allowing for normal tracing 0.2ml of 100ug/ml of adrenaline was added to the bath. Adrenaline was used as a standard. Adrenaline was then washed out thoroughly after acting for about one minute. The jejenum was allowed to recover after which 0.5ml of crude aqueous extract was added. The effect was traced out and then washed out throughly. After allowing recovery, the experiment was repeated using 1ml of crude extract.

RESULTS ON EFFECT OF AQUEOUS CRUDE EXTRACT

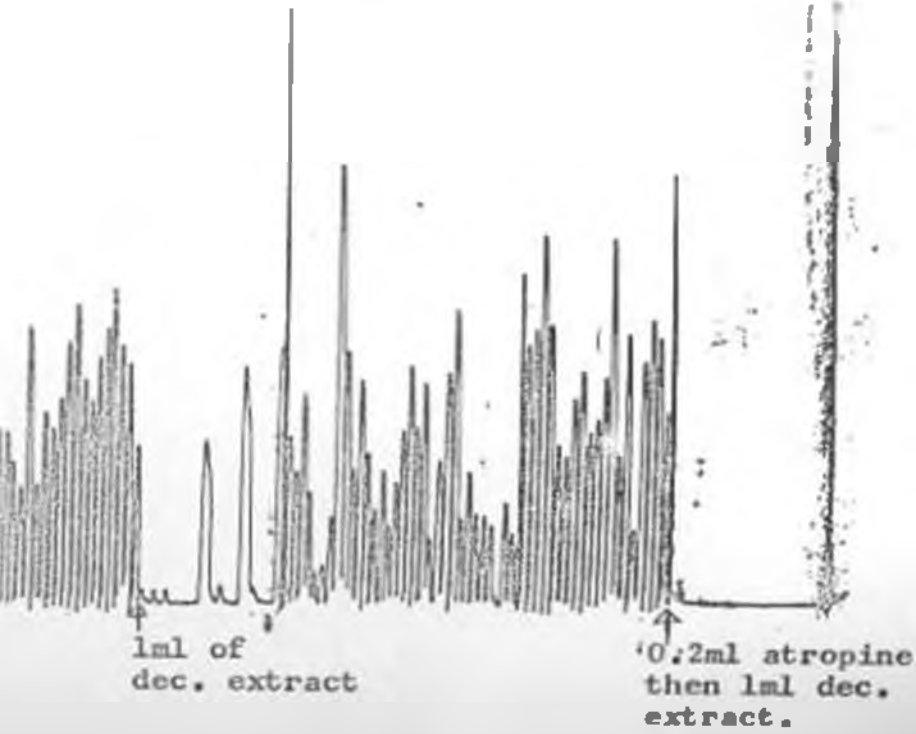


OF STRYCHNOS NUCUSSATA ON RABBIT ILEUM

Volume of organ bath used - 20ml

Temp. of Tyrode solution in organ bath - 36° - 37°C

Aerating gas - 95% O_2 + 5% CO_2 mixture



This was again followed by 0.2ml of Atropine then 1ml of extract in same bath.

RESULTS: As on the tracing.

DISCUSSION: The crude extract has smooth muscle relaxant activity. The extract does not abolish all the contractions and the contractions that were still present were blocked by Atropine since addition of atropine followed by the extract gave no contractions.

CONCLUSION:

The crude extract has sympathomimetic activity since it shows muscle relaxant activity on the G.I.T muscle. The crude extract can be said to have no effect or very little effect on muscarinic sites. The extract is therefore most probably acting on nicotinic sites to cause muscle relaxation. The extract however seems to have no or very little effect on the tone of G.I.T smooth muscle.

DISCUSSION

As shown in Figures IV and V, TLC examination revealed that both bark and leaves of Strychnos decussata contain one alkaloid.

The Rf values of the alkaloid in the bark and that in the leaves is about the same which shows that the alkaloid is the same. Comparing the sizes and intenses of the spots of figures IV and V it can be concluded that the alkaloid content in bark is more than in the leaves.

The alkaloid content was found to be 0.664% w/w for the bark and 0.303% w/w for the leaves.

The pharmacological study showed that the crude extract has muscle relaxant activity on smooth muscle of the G.I.T. Further investigation showed that the activity in the gut is nicotinic. The extract however does not seem to affect the tone of the G.I.T smooth muscle.

C O N C L U S I O N

The yield shown that the bark contains [illegible] w/w of total alkaloid calculated as decussive while that of leaves was [illegible].303% w/w.

Its therefore clear that the bark has about twice the amount of alkaloid as is found in the leaves.

The pharmacological effect was shown to be of smooth muscle relaxation without significant effect on the tone of the G.I.T muscles.

The muscle relaxant effect can be exploited by including decoction from the bark of this plant in therapy for ulcers and diarrhoea

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