THE INVESTIGATION OF THE ALKALOIDS OF DATURA STRAMONIUM GROWN IN KENYA

BY

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DEDICATION

This project has been dedicated to my beloved parents and my beloved sister Mrs. Kazau.

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ABSTRACT

THE ALKALOIDS OF DATURA STRAMONIUM GROWN IN KENYA

This project was carried out in order to study the alkaloids of Datura Stramonium grown in Kenya. Microscopical examination of the leaves was done to find out the diagnostic features of the leaves.

The alkaloid was extracted from dried leaves and roots using the general method of extraction of alkaloids. Datura Stramonium was found to contain two different alkaloids. These were scopolamine and Atropine. The method used for separation of the alkaloids was Thin Layer Chromatographic technique. The quantative analysis was carried out by volumetric analysis. The dried leaves contained 0.46% w/w of Atropine calculated as total alkaloid. The dried roots contained 0.125% w/w of Atropine calculated as total alkaloid.

INTRODUCTION

Alkaloids are usually alkali-like substances, obtained, mostly from plant material and containing one or more nitrogens in a heterocyclic ring. They are usually colourless, crystaline and have strong physiological action in man and animals, For this reason, most of them are used as therapeutic agents.

Datura Stramonium belong to the family solanaceous (2) 'Stramonium' means Stink weed from the french stramoine.

Datura Stramonium is an erect glabrous tree with ovate, dentante leaves, dichotomously branched with white flower at each fork (3) it is usually 1.5 metres high and having a whitish roots and numerous rootlets. The erect serial stem shows dichasial branching with leaf adnation. The stem and branches are round, smooth and green. The flowers are solitary, axillary and short stalked. The corolla is funnel shaped. The ripe fruit seeds are dark brown or blackish in colour, reniform in outline and about 3mm long. The testa is reticulated and finely rooted. (1)

Datula Stramonium was grown in England by Gerarde towards the end of the sixteenth century from seeds obtained from Constantinople. The use of drug is largely due to the experiments of Storck (1762). The genetic name, Datura, is derived from the name of the poison, dhat, which is prepared from Indian species and was used by the Thugs (1).

Datura Stramonium grow as weed in Kenya at Kisumu, Nakuru, Embu, Machakos, Kitui and Nairobi. It is indigenous to the region of Caspian Sea, naturalized in waste places in Europe, North America and is cultivated in Central Europe and South America (2).

Other species of Datura have been shown to be distributed along the coast in Kenya. The common one is Datura Meta L. This is similar to Datura Stramonium but is usually larger with cream-coloured flowers. It was once recorded in Thomson Falls (3) Datura Stramonium var-tatula is similar to D.Stramonium except that the stem and flowers are purplish (10).

Datura Stramonium contains hyoscyamine and scopolamine (4). It has an anticholinalgic actions like that of Atropa Belladonna. It is used as powdered stramonium for relief of asthma. It is intended to be burned and vapour inhaled. Originally, this asthma,powder was sold on an over-the-counter basis until thrill-seekers began to ingest them in order to get intoxicated. In 1968, the food and drug Adminstration placed stramonium containing asthma powders in the category of drugs which could be dispensed

only on prescription (7). In some parts of Kenya such as Kipsigis, Taita and Silulu, Stramonium is used in treating seriously aching ear and ring worm infection. In this, the mature green fruit is buried in hot ash until the inside of fruit gets very hot. It is then removed and a hole made and the fruit is left to cool. When it is cool, it is squeezed and the juice put in a seriously aching can. The leaves are used as poultices for rheumatism and other swellings. Seeds are mixed with leaves, dried and mixed with ghee for treatment of ringworms. The plant should be used with great care because of toxicity . (8).

Datura Stramonium is used officially for the control of salivation and muscular spasms in paralysis agitans and postencephalitic parkinsonism (9).

Datura Stramonium is considered as a toxic plant as it contains hyoscyamine and scopolamine. These alkaloids causes parasympatholytic effects on the eye. Hence causing mydriasis. The pupil may be dilated for even three days.

It also has CNS effect, it causes hallucination (4). Swine are the most affected with toxicity and exhibit gastro-intestinal effects, neuromuscular weakness, muscle twitching and occasional convulsions, paralysis and derilium which may lead to respiratory failure and death (4).

Poisoning by the seed is the most common, followed by that of the leaves. Poisoning by the leaves occurs as a result of accidental gathering of the young plant with other edible green vegetables.

Wright reports on accident in Kenya where green stuff were contaminated with Datura leaves and eaten as food (5). Hughes reports that the young plant, boiled as spinach, has produced symptoms of hilarity but no deaths (6).

In East Africa, Wright reports that stramonium poisoning is one of common causes of poisoning encountered in the Asian and African wards in Nairobi Civil Hospital (11).

Anderson reports that wholesale outbreaks of poisoning among soldiers have been reported in East Africa. Thus seventy recruits were poisoned by contaminated meal during October, 1943 and a pioneer group in Kenya on 20th November, 1933 produced 343 cases. The total number of casualties in the military units over nine months was 1524 African soldiers (12).

A proprietery preparation of Atropa Belladona and Datula Stramonium (Asthmador), supplied as a powder intended to be burnt and the smoke inhaled for the relief of asthma, was deliberately abused by 7 young psychiatric patients in order to exploit its psychic effects. They had been taking from 1½ to 4 teaspoonfuls by mouth and had all shown signs of atropine poisoning within an hour of ingestion. Mental symptoms included visual halluscination, disorientation, and impairment of memory and intellectual function (13).

An 18 year old student smoked 4 asthma cigarettes (asthmador) containing stramonium and belladonna. Three hours later he had slurred speech, ataxia gait, confusion, a red dry skin, dilated pupil, fever, swollen uvulla and palate and tachycardia. All the symptoms cleared after 36 hours (14).

Report of deliberate intoxication in 2 teenagers by the ingestion of a proprietary mixture of stramonium and belladonna (Asthmador). Besides the characteristic symptoms and signs of atropine poisoning, they experienced psychosis-like episodes with visual hallucinations, disorientations and impaired memory (15).

The total alkaloid of the leaves and roots vary from one species to the other. It also varies from one geographical region to the other.

Patel (1925) found in the leaves of Datura Stramonium 0.265 to 0.342% w/w of the total alkaloid. In the roots, 0.120% w/w was found. The leaves of Datura tatula Linne, was found to contain 0.318% w/w. The average alkaloid in commercial samples of Datura stramonium was found to be 0.22% w/w but the average may rise to 0.7% w/w.

By cutting off the flowers, Sievers (1921) got a high percentage of 1.825% w/w.

In South Africa leaves containing 0.54% w/w has been found and in Egypt 0.35% has been found. This contains hyoscyamine only. (20).

CHAPTER I

EXPERIMENTAL

COLLECTION OF THE PLANT MATERIAL

Datura Stramonium was collected in December, 1979 from Kenyatta National Hospital where it grows wildly in wasteplaces.

The whole plant was picked together with its roots. The soil was removed from the roots using hands and roots separated from the rest of the plant. The leaves were separated from the flowering parts of the plant. Both leaves and roots were dried in an oven at 60°C for over night. They were both stored in a polythyne paper to prevent them from moisture and stored in a cool place.

MICROSCOPICAL EXAMINATION OF THE LEAVES

Fresh leaves of Datura Stramonium were picked. 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 and examined through the microscope. The lower epidermis was also examined. The distinguishing features were drawn as shown in Fig.I.

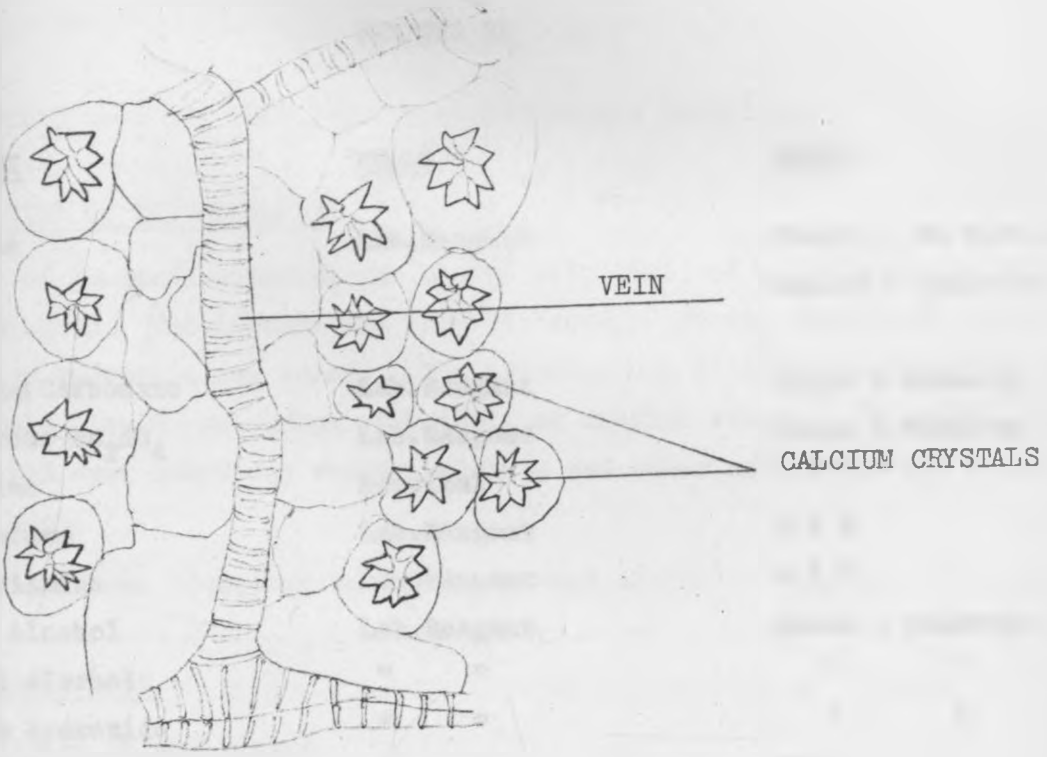
Fig. I shows that the leaves of Datura Stramonium consists of several characteristic features. They contain calcium oxalate crystals which are in clusters. A crystal layer is shown in surface view, on both sides of the vein (Fig.I). The leaves also contain different trichomes. The most common being the covering trichomes (Fig.IC).

On microscopical examination of the lower epidermis layer, it was observed that the leaves have Anisocytic type of stomata i.e. Each stoma is surrounded by three cells. (Fig. I b).

IG.I

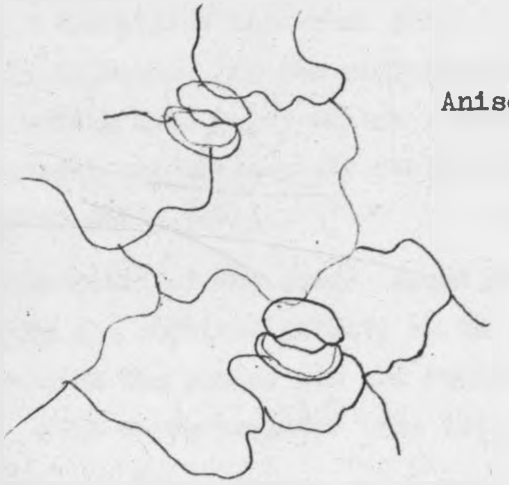
RYSTAL LAYER IN SURFACE VIEW

a)

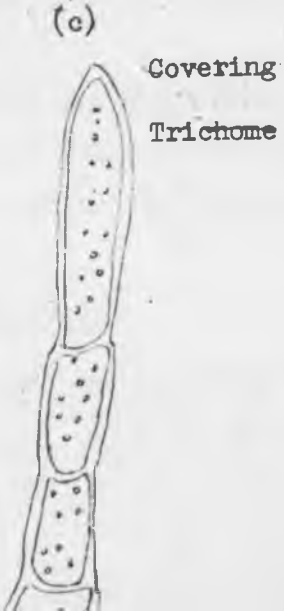


LOWER EPIDERMIS LAYER

(b)



(c)



CHAPTER II

REAGENT	GRADE	BRAND
Acetone	Lab.Reagent	Riedel - De Haem AG Seelze - Hannover
Ammonium Carbonate	Lab.Reagent	Howse & McGeorge
Anhydrous Na_2SO_4	Lab.Reagent	Howse & McGeorge
Atropine	Hospital	
Chloroform	Lab.Reagent	M & B
Diethylamine	Lab.Reagent	M & B
Ethyl Alcohol	Lab.Reagent	Howse & McGeorge
Methyl Alcohol	" "	" "
Sodium Hydroxide	" "	" "
Methyl Red	" "	BDH
Silica Gel (GF 254)	" "	Merck
Ammonia Solution	" "	Howse & McGeorge
H_2SO_4	" "	M & B
Scopolamine	Hospital	

QUALITATIVE ANALYSIS

The method used was thin layer chromatographic technique.

EXTRACTION OF THE ALKALOID

I gram of powdered material was shaken with 10ml. of 0.1N sulphuric acid for five minutes. The mixture was then filtered. To the liquid got 1ml.of 25% Ammonium hydroxide was added. The solution was filled with water to 10ml. The whole liquid was shaken with 10ml. of diethyl ether. The ether layer was dried over anhydrous sodium sulphate and ether was evaporated on the hot water bath.

The residue was dissolved in 0.25ml. Methyl alcohol.

TLC EXAMINATION

Preparation of the plates

The coating material used was slurry which was prepared by mixing 30g of silica gel (GF254) in 40ml. of water and then adding 20ml. of water and shaking vigorously. The slurry was immediately spread on the 20 x 20 cm chromatographic plates. The whole process took about 2 minutes to prevent slurry from hardening before it was spread on the plates. The plates were made 1.5 millimetre thick. The layer was dried for 10 minutes on aligning tray. The plates were then dried in the oven for 30 minutes at 110°C. This is important for the activation of silica gel. Activated silica gel is an acidic adsorptive medium especially suited to the separation of acidic and neutral compounds. It readily separates aldehydes, ketones, alkaloids and amino acids (15).

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 (16). The chromatoplates were left aside to cool.

Investigation of suitable solvent systems of TLC analysis:

Different solvent systems were used in order to choose the one that gave the highest hRf values.

The results got for different solvent systems are shown below:

SOLVENT SYSTEM	ATROPINE	SCOP	EXTRACT	
Acetone: H_2O: NH_4OH			1st SPOT	2nd SPOT
90 : 7 : 3	11.5	68.2	11.0	68.0
Chloroform: Diethylamine 90 10	38.0	62.0	37.0	61.0
Ethanol: Ammonium hydroxide 99 : 1	35.0	58.5	34.0	60.0

Acetone: H_2O: NH_4OH was found to give better separation of spots.

TLC ANALYSIS

Template was used in marking the start points which was 1.5cm from the lower edge and side of the plate. The distance between the neighbouring points was 1.5cm. It was necessary to use a template as this prevents the damaging of the rest parts of the plate during the application of the samples.

A centimetre scale was engraved along one edge of the transparent template, facilitating the marking of starting points at regular intervals. (17).

The chamber was allowed to saturate for 40 minutes. Capillary tubes were used for the application of the samples. The plates were run and sprayed with dragendorffs reagent followed by spraying with 0.1N H_2SO_4. Different spots were obtained as shown in Fig.II and Fig.III.

The hRf values and Rx values were calculated the results of which are shown in Table I.

CHAPTER III

QUANTATIVE ANALYSIS

EXTRACTION OF ALKALOIDS

Alkaloid extraction is based on inherent basic nature and ability to form salts with acids. The leaves are dried and powdered to increase the surface area and hence, increase the contact between the solvent and alkaloids possessing cells on tissues. Chloroform is used for extraction of most alkaloids except quaternary bases.

METHOD

10g powdered material was moistened with 10% Ammonium Carbonate. It was covered with chloroform and macerated for about one hour. The macerate was transfered to Sohxlet apparatus and its container washed with chloroform and washing transfered to Sohxlet.

Continuous extraction was carried out for about five hours (a bit of cotton wool was used to avoid subsequent filtration. The extract was free from particles). The filtrate was shaken with several portions of an aqueous acid (2% H_2SO_4). Three shakings were done each with 20ml 2% H_2SO_4 for every 100ml chloroform extract. Test for complete extraction was done using Dragendorff's Reagent. The non-alkaloidal and pigment impurity was removed from aqueous extract by shaking with portions of chloroform and discarding the chloroform layer. The aqueous acidic solution was made alkaline with 10% Ammonia.

Water was got rid of from the organic solvent by using 2g of anhydrous sodium sulphate for every 100ml. of the solvent. Sodium sulphate was removed by filtration using some cotton wool. Again 2g of anhydrous $NaSO_4$ was added and left overnight to extract the final traces of water. This was filtered to get rid of sodium sulphate. The water free extract was evaporated to dryness under reduced pressure in a rotary flask evaporator.

After distilling most of the chloroform, the remainder was transferred to a shallow open disc and the chloroform evaporated without the aid of air current.

The residue was heated in an oven for 15 minutes at 100°C.

VOLUMETRIC ANALYSIS

The dry residue got by extraction was dissolved in 2ml. chloroform and 5ml. of 0.05N H_2SO_4 added.

This was cooled and excess sulphuric acid was back titrated with 0.05N NaoH which was already standardised.

Methyl red was used as the indicator. The amount of hyoscyamine was calculated. The calculation was based on the fact that each ml. of 0.05N $H_2SO_4 \equiv$ 0.01447 g of alkaloids calculated as hyoscyamine (18).

The above analysis was done for:-

a) Dried powdered leaves.

b) Dried powdered roots.

Two determinations were done for each, and the average percentage calculated for each.

RESULTS

PART OF THE PLANT	VOLUME OF 0.05N Naoh used TO TITRATE EXCESS 0.05N H_2	
	1st DETERMINATION	2nd DETERMINATION
LEAVES	3.9ml	3.45 ml
ROOTS	1.1ml	1.0 ml

The calculations were done as illustrated below:

1st DETERMINATION OF ALKALOIDS IN LEAVES

3.9 ml. of 0.05N NaoH will react with excess sulphuric acid (i.e. the acid that did not react with the alkaloid.)

H_2SO_4 + 2Naoh $\rightarrow$ Na_2SO_4 + H_2O

1 : 2

H_2SO_4 (f = 0.9909)

NaoH (f= 0.999)

Excess acid unreacted = $\frac{3.9 \times (f)\ 0.999}{2 \times f\ 0.9909}$

= 1.8 ml.

Acid used = 5 x f 0.9909 = 4.95 ml.

∴ Acid reacting with sodium hydroxide = (4.95 - 1.80) ml = 3.15

Given: 1 ml. 0.5N H_2SO_4 = 0.01447g. alkaloid

∴ 1 ml. 0.05N (f 0.9909) = 0.01447 x 0.9909

3.15ml. 0.05N(f 0.9909) H_2SO_4 = 0.01447 x 3.15 x 0.9909 g of alkaloid.

Amount of powdered drug used = 10 g

∴ % of Alkaloid = $\frac{100 \times 0.01447 \times 3.15 \times 0.9909}{}$

= 0.455% $^{w}/_{w}$ Atropine calculated as total alkaloid.

Calculation was done for all the determinations, as the above example.

The results are shown in Table 2.

RESULTS

TLC EXAMINATION OF THE ALKALOIDS

OMPOUND	DISTANCE MOVED BY THE SOLVENT	DISTANCE MOVED BY REFERENCE	hRf	Rx	COLOUR AFTER SPRAY
opolamine	11.3 cm.	7.5	66		Orange
ropine	11.3 cm.	1.3	11.5		Orange
ot 1	11.3 cm.	1.2	10.6	1.02	Orange
ot 2	11.3 cm.	7.3	65	1.02	Orange

QUANTATIVE ANALYSIS OF ALKALOID

OF ALKALOID CALCULATED AS ATROPINE

T OF THE PLANT	1st DETERMINATION	2nd DETERMINATION
ives	0.45	0.47
t	0.124	0.126

TLC EXAMINATION OF THE ALKALOID IN LEAVES

FIG.II

ADSORBENT - Kieselgel 60 GF (Merck)

SOLVENT SYSTEM Acetone: Water : Ammonium Hydroxide

90 : 7 : 3

SPRAYING REAGENTS 1. Dragendorff

2. 0.1N H_2SO_4

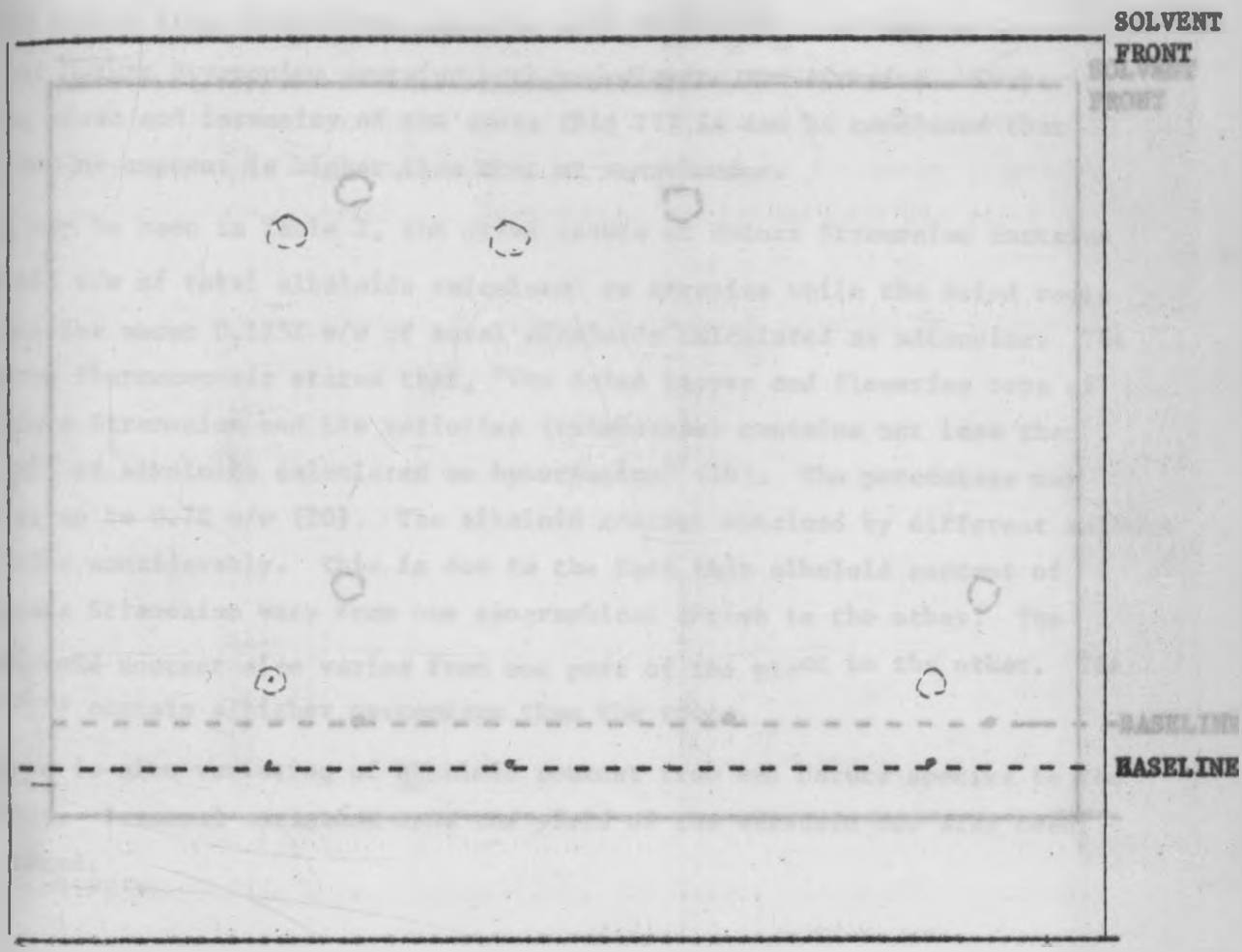
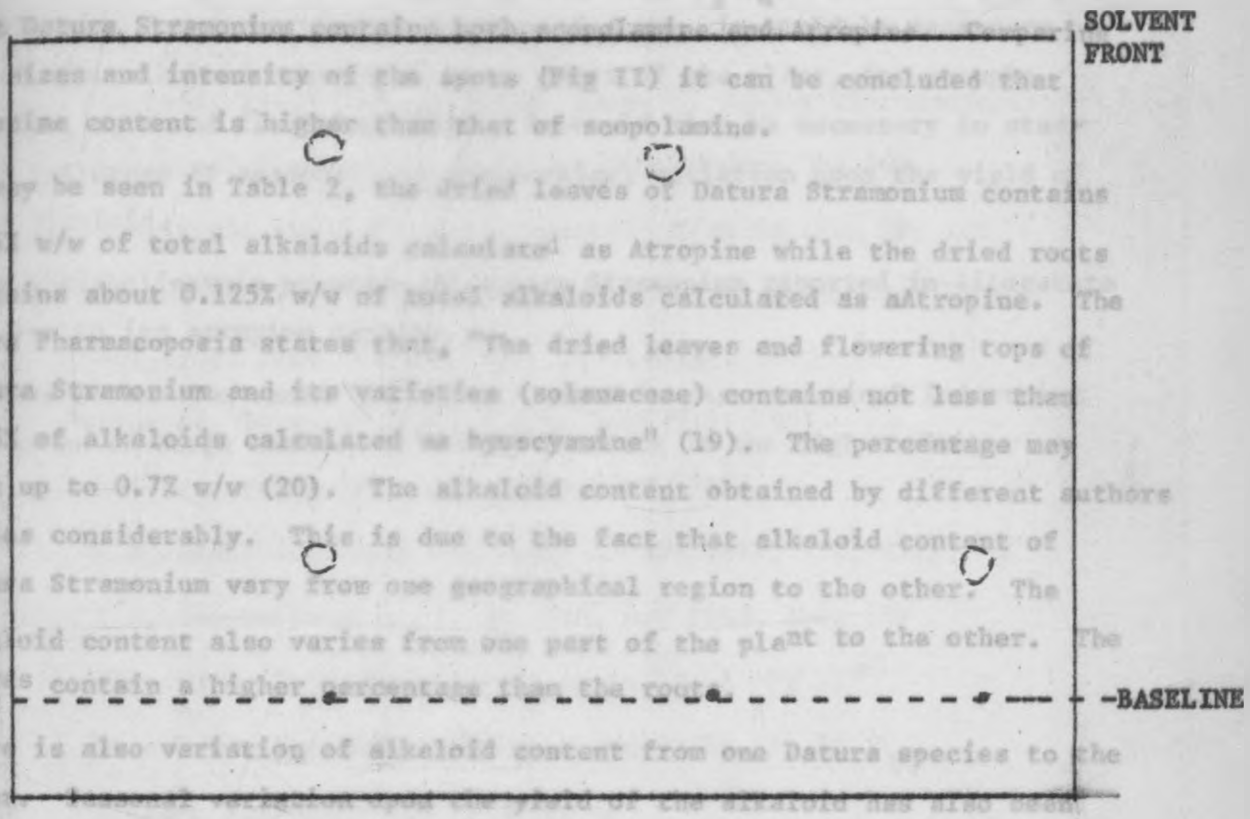


FIG. III



CHAPTER IV

D I S C U S S I O N

As may be seen in Fig II, the TLC examination revealed 2 spots.

Spot No.I showed hRf value similar to that of Atropine Table I. The Rx value was found to be about I.

Spot No.2 showed hRf value similar to that of scopolamine Table I. Both spots were deep orange in colour after spraying with Dragendorff reagent and looked like shade after spraying with 0.IN H_2SO_4. It can be concluded that Datura Stramonium contains both scopolamine and Atropine. Comparing the sizes and intensity of the spots (Fig II) it can be concluded that Atropine content is higher than that of scopolamine.

As may be seen in Table 2, the dried leaves of Datura Stramonium contains 0.46% w/w of total alkaloids calculated as Atropine while the dried roots contains about 0.125% w/w of total alkaloids calculated as aAtropine. The Extra Pharmacopoeia states that, "The dried leaves and flowering tops of Datura Stramonium and its varieties (solanaceae) contains not less than 0.25% of alkaloids calculated as hyoscyamine" (19). The percentage may rise up to 0.7% w/w (20). The alkaloid content obtained by different authors varies considerably. This is due to the fact that alkaloid content of Datura Stramonium vary from one geographical region to the other. The alkaloid content also varies from one part of the plant to the other. The leaves contain a higher percentage than the roots.

There is also variation of alkaloid content from one Datura species to the other. Seasonal variation upon the yield of the alkaloid has also been noticed.

CHAPTER V

CONCLUSION

The alkaloids from the leaves of Datura Stramonium grown in Kenya were found to be scopolamine and Atropine as shown by TLC examination.

The material used for this study was obtained from Nairobi around Kenyatta National Hospital. The yield of 0.46% w/w of total alkaloid calculated as Atropine in leaves is very promising. However, the roots seem to contain a relatively low yield of alkaloids about 0.125% of total alkaloid calculated as Atropine. Hence, before the caltivation of Datura stramonium for commercial production of drugs, it would be necessary to study the alkaloid content of other parts of the plant, for example fruits, stem and flowering parts. It would also be necessary to study the influence of seasonal and geographical variation upon the yield of the alkaloid.

The anticholinergic property of Datura Stramonium reported in literature is due to its atropine content.

Datura Stramonium would be ofgreat demand in pharmaceutical industries if grown in large scale. It's only drawback is the high toxicity.

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