THE INVESTIGATION OF THE ALEALOIDS OF DATURA STRAMONIUM CROWN IN KENYA

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Research project presented in partial fulfilment for the Derree of Bachelor of Pharmacy, University of Nairobi.

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TOPHENTS

DFD ICATION

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

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1.

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.

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The alkaloid was extracted from dried leaves and roots using the general method of extraction of alkaloide. Daturs 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.

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INTRODUCTION

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Alkaloids are usually alkali-like substances, obtained, mostly from plant material and containing one or more mitrogens 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 owate, dentante leaves, dichotomously branched with white flower at each fork (3) it is esually 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 feowers are solitary, axillary and short stalked. The corolls is funnel shaped. The ripe fruit seeds are dark brown or blackish in colour, remiform in outline and about 3mm long. The tests 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, Witui 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 Laturs 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).

Detura Stramonium contains hypersymmine and scopolamine (4). It has an anticholinelgic actions like that of Atropa Belladonna. It is used as powdeered stramonium for relief of asthma. It is intended to be burned and vapour inhaled. Originally, this asthma, gowder was sold on an overthe-counter basis until thrill-seekers began to imgest 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, Tsita 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 halluscination (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 is a result of accidental gathering of the young plant with other edible green vegetables.

Wright reports on accident in Kenya where green stuff vere 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 proprietory 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 1 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). cellab life contra. The soil was removed

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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 beeladonna (Asthmador). Besides the characteristic symptoms and signs of stropine poisoning, they experienced psychosis-like episodes with visual halluscinations. disorientations and impaired memory (15).

The total alkaloid of the leaves and roots vary from one species to the a hard the state of the second find one unread up and excitioned through the algorations. 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 persentage 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 hyoscysmine only. (20).

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CHAPTER I

EXPERIMENTAL

COLLECTION OF THE PLANT MATERIAL

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

The whole plant was picked together with its roots. The woil 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 polythyme 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 misroscope 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).

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RYSTAL LAYER IN SURFACE VIEW



CALCIUM CRYSTALS

LOWER EPIDERMIS LAYER

(b)



Anisocytic stomata

(c) Covering Trichome

CHAPTER II

		410.1
REAGENT	GRADE	BRAND
Acetone	Lab.Reagent	Riedel - De Haam AG Seelse - Hannover
amonium Carbonate	Lab.Reagent	Howse & McGeorge
Anhydrous Na SO4	Lab.Reagent	Howse & McGeorge
Atropine	Hospital	a experised on the last
Chloroform	Lab.Reagent	M&B
Diethylamine	Lab.Reagent	M&B
Ethyl Alcohol	Lab.Reagent	Howse & McGeorge
Mathyl Alcohol	11 11	H H
Sodium Hydroxide	H H	11 11
Methyl Red	VF 17	BDH
Silica Gel (GF 254)	na prog taxe distant Property	Marck
Ammonia Solution	HIL	Rowse & McGeorge
H2804	Challey and including spin	M & B
Feenalemine	Hospital	Francess of Astronomy 5

arouty from hardening before it was spread on the plates. The plates serve near it's utilizers thick. The layer was skilled for 10 minutes on aligning area. The plates one that dried is the over bot is electric at 10⁰ m. The antidit electricit for the antiference at elling gal, series allow all aligning is an article electricity modes emerially related to the separation of soldie and month anyoneds. It readily separates allohydes, between, stabilities and modes atigs (15).

the properties of the dried logars show encodingtion are made, are detersized by the combined officers of the coplifiery system between the eavily system with the grains and the surface chemical groups in his savity system (he). The chemistrylatus were buly solds to media

Townsightion of substitute advant systems of TLG analysia:

the signary and welcant.

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 supphuric acid for five minutes. The mixture was then filtered. To the liquid got 1ml.of 25X Ammonium hydroxide was added. The solution was filled with water to 10ml. The whole liquid was shaken with 10ml. of disthyl ether. The ether layer was dried over enhydrous 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 gell (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 20: NH 40H	ë dei Dabievies	r barly nab	lst SPOT	2nd SPOT
90 2713	11.5	68.2	11.0	68.0
Chloroform: Diethylamine 90 10	38.0	62.0	37.0	61.0
Ethanol: Ammonium hydroxi 99 : 1	de 35.0	58.5	34.0	60.0

Acetone: H.O: NH OH was found to give better separation of spots.

TLC ANALYSIS

and period to remain the deletate.

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 meighbouring 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.

artion was carelant mak for shows five neary (a bit of pryster

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 dragendorifis reagent followed by spraying with 0.1N H₂SO₄. Different spots were obtained as shown in Big.II and Fig.III.

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

and Hilberryd, in get rid of addies sulphane. The unter from summark was responsible to drymass under todacad processes in a rotary flash ecoperator. After distilling much of the chloroform, the comminder you trunsforred to a shallow apoin dian and the chloroform componented without the aid of air margane.

The realises was basked in an over for 15 minutes at 100°C.

CHAPTER III

- 6 -

QUANTETIVE ANALYS IS

EXTRACTION OF ALKALOIDS

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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.

is colderwis and use how titrated with these

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 Sohulet apparatus and its container washed with chloroform and washing transfered to Sohulet.

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 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 H₂SO₄ added.

This was cooled and excess sulphuric acid was back titrated with 0.05M MacH which was already standardised.

Mathyl red was used as the indicator. The mount of hyoseyamine was calculated. The calculation was based on the fact that each ml. of $0.95N H_2SO_4 = 0.01447$ g of alkaloids calculated as hyoseyamine (18). The above analysis was done for:-

- a) Dried powdered leaves.
- b) Dried powdered roots.

DIATESE OF ALKALOUDS IN COARD.

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

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~ <u>J.9 x (1) 0.909</u>] z f 3.9903 = l.f al. = 0 x f 0.9909

28,65,

- (3.35 - 1.00)

- bill min

1.0

E 0.01457g. athalude

0.01447 0.990

0.01447 × 0.15 = 0.9969 g of al cloid.

 $\Psi_{N} = \sum_{i=1}^{N} \left\{ \frac{1}{2} - \frac{1}{2} \right\}$

= 100 x 0.01447 x 3.15 x 0 9804

- C. 495% /w Accorptae calculated as the a

falculation our does for all the determinations; so the there complete

The needles are shown in Table 4.

RESULTS

PART OF THE PLANT	VOLUME OF 0.05N Nach use	d TO TITRATE EXCESS 0.05N B
à	Ist DETERMINATION	2nd DETERMINATION
LEAVES	3.9ml	3.45 ml
ROOTS	1.1ml	1.0 ml
The calculations were done	as illustrated below:	DD Press
Ist DETERMINATION OF ALKALO	DIDS IN LEAVES	Marge Services
3.9 ml. of 0.05N NaoH will that did not react with the	react with excess sulphus alkaloid.)	ric acid (i.e. the acid
E ₂ SO ₄ + 2Naoh 1 : 2		+ H ₂ 0
H_2 ^{SO} 4 (f = 0.9909) Nach (f = 0.999)	CALCULATIO AL AD	
Excess acid unreacted	$= \frac{3.9 \times (f) \ 0.999}{2 \times f \ 0.990}$ $= 1.8 \ ml.$	9
Acid used	= 5 x £ 0.9909	= 4.95 ml.
. Acid reacting with sod	ium hydroxide	= (4.95 - 1.80) ml = 3.5
Given: I ml. 0.5N H2804	= 0.01447g. alkal	oid
1 ml. 0.05N (f 0.9909)	= 0.01447 x 0.990	9
3.15ml. 0.05N(# 0.9909) H2	50. = 0.01447 x 3.15	x 0.9909 g of alkaloid.
Amount of powdered drug us	ed = 10 g	
X of Alkaloid	- 100 x 0.01447 x - 0.4552 V/w Atro alkaloid.	pine calculated as total

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

The results are shown in Table 2.



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RESUITS

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TLC EXAMINATION OF THE ALKALOIDS

MMOUND	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
pt 2	11.3 cm.	7.3	65	1.02	Orange

31.: 2

QUANTATIVI ANALYSIS OF ALKALOID

OF ALKALOID CALCULATED AS ATROPINE

T OF THE PLANE	1st DETERMINATION	2nd DETERMINATION
IVES	0.45	0.47
t	0.124	0.126





ALT 10 17

FIG. III

any may be comen in Tik TL, the Thi succeiventies revealed 2 spotssons rank showed the evide constinue to once of Atrophy Table L. The We spitze new found as in others L.

many must record and value advilor in that TC empelancies Table T. Tarks party strong of the party of and and a solar strong with Transaderill ranges handsoil Tiles stands offers services with 0.22 0.20 . It use he small middle Deture. Streponius, coursion, both acondianing land, and SOLVENT FRONT the sizes and intensity of the asots (Fig II) it can be concluded that According content is higher than that of scopolaning. is my be seen in Table 2, the Jefet leaves of Datura Stramonium contains 1.46 w/w of total alkaloids calculated as Atropine while the dried roots donitions about 0.125% w/w of apend alkaloids calculated as attropine. The Terre Pharmacoposia states that, The dried leaves and flowering tops of Thruta Stramonium and its variaties (solawaceae) contains not loss than cipti of alkeloids calculated as hypocyspine" (19). The percentage men rive up to 0.72 w/w (20). The alkaloid content obtained by different suthors varies considerably. This is due to the fact that alkaloid content of Data a Stramonium vary from one geographical region to the other. The alta wid content also varies from one part of the plant to the other. The leaves contain a higher percentage than the route. -BASELINE Timere is also veriation of alkaloid content from one Datura species to the

CHAPTER IV

DISCUSSION

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.

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CUONCLUSION

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

Renyatta 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 geopraphical 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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