PHARMACOGNOSTICAL EVALUATION OF THE LEAVES OF DATURA ARBOREA CULTIVATED IN KENYA

By

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A dissertation submitted in the partial fulfilment for the award of the Degree of Bachelor of Pharmacy of the University of Nairobi.

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June 1981

A C K N O W L E D G E M E N T S

In deep and sincere appreciation of Professor S. Talalaj whose guidance and criticism I found greatly invariable and without whom this project would have been impossible.

I am greatly indebted to Mr. Mwangi W, the Post-graduate student whose advise and directions in the practical part of the project was very helpful.

Much thanks to the technical staff, Mr. Mwaughu, Kamau, Thuranira and special mention of Mr. Muriithi for constantly bothering them for technical assistance.

Last but not least to Mrs Mdungu for typing the manuscript.

D E D I C A T I O N

This work is dedicated to my Mother and Father for

supporting me through many hardships and for their

Knowledge shall be immortal, a reward of the highest order

to all humble seekers after the truth.

J. H. 1981

D E D I C A T I O N

This work is dedicated to my Mother and Father for supporting me through many hardships and for their unfailing encouragement throughout my education.

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A B S T R A C T

The project was carried out as a pharmacognostical evaluation of the leaves of Datura arborea L. cultivated in Kenya. Macroscopical and microscopical examination of the plant material were carried out. Thin layer and gas liquid chromatographic studies showed the presence of hyosine and another alkaloid which was not identified. The total alkaloid content determined by means of potentiometric non-aqueous titration was 0.25% out of which 94.8% was hyosine and 5.2% was the unidentified alkaloid. Datura arborea L. cultivated in Kenya may be regarded as a source of hyosine.

INTRODUCTION

Datura arborea L. falls in the phylum Angiospermae, subphylum - Dicotyledons, family - Solanaceae. One Solanaceae family comprises about 90 genera and from 2000 to 3000 species and is found in tropical and temperate regions. It grows as a herb, shrub or small tree(2).

The genus Datura contains the tropane alkaloids which are found in different parts of the plant ie roots, stem, leaves, flowers and seeds. The main alkaloids of genus Datura are hyosine and hyoscyamine. Of the two alkaloids, the principal alkaloid varies from species to species and the proportion might change over the course of growth. The relative proportions of hyosine and hyoscyamine in a particular species not only vary with age of the plant but are susceptible to a number of other factors including day length, light intensity, general climatic conditions, chemical sprays, hormones, debudding and chemical races. The generic name Datura is derived from the poison dhat which is prepared from Indian species and was used by thugs (2).

Datura abrorea L. grown in Kenya is a perenial with leaves usually 10 - 30cm long and 4-17 cm wide. The flowers are large, solitary and short stacked with a sweet odour. The corolla is funnel shaped five lobed and white in colur. The plant reaches about 2½ metres in height and is cultivated in tropical countries. It is commonly grown in East African gardens as an ornamental.

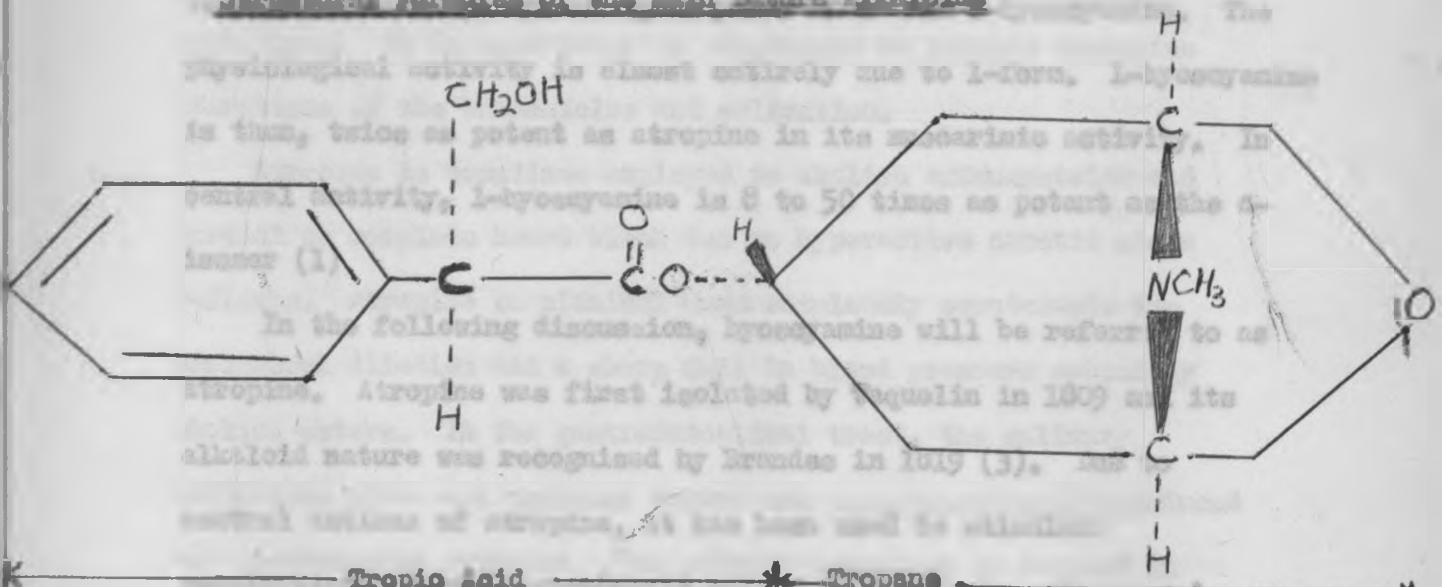
Chromatographic studies of Indian plants have shown that the alkaloid content is highest in flowers (0.427%) lower in leaves (0.217%) and lowest in stem (0.16%). In all parts a higher percentage of hyo cine was observed as compared with hyoscyamine. The ratio of the former to the latter was generally greater than 2:1. Aposcopolamine was presumed pre ent in the light petroleum fraction. Meteloidine was absent in the aerial parts. An unidentified alkaloid occured in the chloroform fraction(7)

In three varieties found in Peru, roots and leaves had the highest alkaloid content. The alkaloids found were atropine, duboisine and hyoscine. In Uruguay, no significant amounts of alkaloids could be isolated from thi plant. Alcoholic extracts of leaf, root and flower produced slight hypotensive effect in dogs. In Cuba, the dry leaves of Datura arborea has been found to be non toxic to rabbits. The herb has been used as a local application, both in Poultice and Ointment for relief of pain (4). According to Martines (4) the poultice of Datura leaves is used in Peru, Chile and other parts of South America to relieve pain. Even odour of the flower is said to relieve pain. In Bogota, the following results were obtained; in flower 0.49%, in leaves, 0.267% and in berries, 0.063%.75 - 80% of total alkaloid was composed of hyoscine(4).

Commercial Datura leaf consists of the dried leaves and flowering tops of Datura innoxia and Datura metel, obtained principally from India.(2). Members of the British trade expressed interest in obtaining supplies of the plants and other

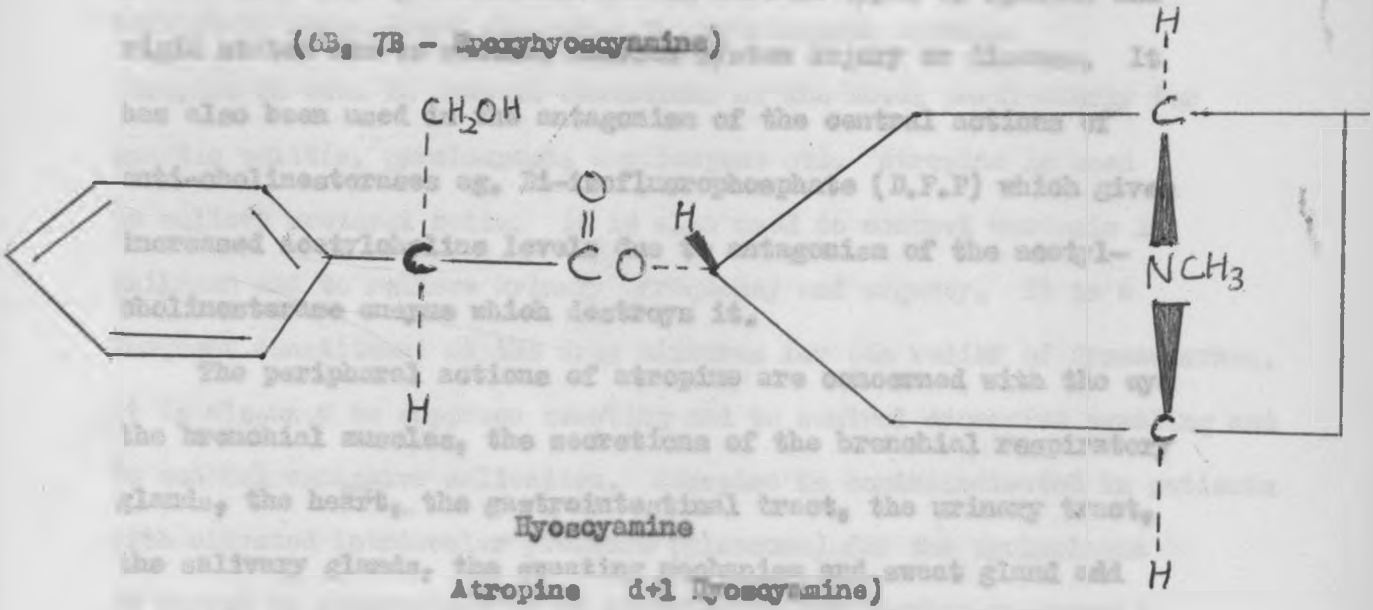
species with a high alkaloid content. In England, demand for plant material for isolation of hyoscine is of the order of 70-100 tons per year. There is a demand for plant material with alkaloid content between 0.3 and 0.5% (5)

Structural Formulae of the main Datura Alkaloids



L-Hyoscine (Scopolamine)

(6β, 7β - Epoxyhyoscyamine)



Hyoscyamine

Atropine d+l Hyoscyamine)

The presence of an asymmetrical carbon atom in tropic acid (boldface C in the formulas above) allows for optical activity and stereoisomerism. Hyosine (scopolamine) occurs in two forms, ie laevorotatory (l-hyosine) and dextrorotatory (d-hyosine) forms. L-hyosine is more active than d-hyosine. Atropine is racemic ie a mixture of equal parts of d- and l-hyoscyamine. The physiological activity is almost entirely due to l-form. L-hyoscyamine is thus, twice as potent as atropine in its muscarinic activity. In central activity, l-hyoscyamine is 8 to 50 times as potent as the d-isomer (1)

In the following discussion, hyoscyamine will be referred to as atropine. Atropine was first isolated by Vaquelin in 1809 and its alkaloid nature was recognised by Brandes in 1819 (3). Due to central actions of atropine, it has been used to stimulate respiration, to give symptomatic relief in paralysis agitans and postencephalitic parkinsonism and in certain types of spastic and rigid states due to central nervous system injury or disease. It has also been used in the antagonism of the central actions of anti-cholinesterases eg. Di-isofluorophosphate (D.F.P) which gives increased acetylcholine levels due to antagonism of the acetyl-cholinesterase enzyme which destroys it.

The peripheral actions of atropine are concerned with the eye the bronchial muscles, the secretions of the bronchial respiratory glands, the heart, the gastrointestinal tract, the urinary tract, the salivary glands, the sweating mechanism and sweat gland and the uterus.

In ophthalmology, atropine is used to dilate the pupil and to paralyse accomodation (Cycloplegia). The ocular effects lasts for 7 to 12 days.

In bronchial asthma, atropine causes relaxation of the bronchial muscles and drying up of bronchial secretion . It is used in hay fever and rhinitis to dry up the annoying excessive secretions. It is used prior to anesthesia to inhibit excessive secretions of the bronchioles and salivation.

Atropine is sometimes employed to abolish extrasystoles and partial or complete heart block due to hyperactive carotid sinus reflexes. Atropine in clinical doses completely counteracts the peripheral dilation and a sharp fall in blood pressure caused by choline esters. In the gastrointestinal tract, the salivary secretions which are copious, watery and parasympathetically induced are abolished by atropine. The gastric secretion is reduced in volume and total acid content. This reduction is only notable when relatively large doses are given to experimental animals. Atropine is used in spastic conditions of the bowel particularly for spastic colitis, pyrolospasm, cardiospasm etc. Atropine is used to relieve ureteral colic. It is also used to control enuresis in children and to relieve urinary frequency and urgency. It is a frequent constituent of the drug mixtures for the relief of dysmenorrhea. It is alsoused to suppress sweating and to control excessive sweating and to control excessive salivation. Atropine is contraindicated in patients with elevated intraocular pressure (glaucoma) for the cycloplegia ~~is~~ caused is accompanied by an increase in intraocular pressure(3)

Hyoscine (scopolamine) on the other hand, has similar antimuscarimic

actions to atropine except in its therapeutic doses it is a sedative and a tranquilising depressant to the central nervous system. Hyoscine is a stronger blocking agent for the iris, ciliary body, salivary bronchial and sweat glands. Hyoscine is weaker than atropine in its action on the heart, the intestinal tract and bronchial musculature (1). Hyoscine is employed as a sedative. It is frequently given as a pre-anaesthetic medicament for both its sedative-tranquilising and anti-secretory actions. It is an effective antiemetic and was once used to prevent motion sickness. It is used in maniacal states in delirium tremens and in obstetrics. It is also used in post-encephalilic parkinsonism and in certain spastic states due to nervous system injury. It is used in ophalmocology as a mydriatic and cycloplegic agent. Mydriasis is of shorter duration than atropine (3 - 7 days) and intraocular pressure is affected less markedly than in atropine (3).

Poisoning by these alkaloids containing plants is very common. Datura species and other plants containing tropane alkaloids are the common source of poisoning. During famines, people are willing to experiment on new vegetables and these plants are taken as vegetables and hence poisoning. They have also been mistaken for vegetables in darkness. These plants are occationally used for homicidal and suicidal cases. Several homicidal cases using Datura arborea and other related species have been reported in India. The leaves or the alkaloidal extracts are usually mixed with food and given to unsuspecting victims in homicidal cases (8).

The symptoms and signs of poisoning develop promptly after ingestion of the drug. The mouth becomes dry and burns, swallowing and talking are difficult or impossible. The voice becomes hoarse. There is marked thirst, the vision is blurrred and photophobia is prominent. The skin is hot, dry and flushed. A rash may appear especially over the face neck and upper part of the trunk. Rash is more likely to occur in children. The pulse is weak and very rapid in children. Palpitation is prominent and blood pressure is elevated. Urinary urgency and difficulty in micturation are sometimes noted.

The patient is restless, excited and confused and exhibits weakness, giddiness and muscular inco-ordination. Gait and speech are disturbed. Nausea and vomiting occur at times. Memory is disturbed, orientation is faulty, visual hallucinations, mania and delirium are not unusual. Atropine and hyoscine give similar symptoms but hyoscine sometimes give idiosyncratic responses. The syndrome often lasts 48 hours or longer. In severe intoxication, depression and circulatory collapse occur. Death is due to respiratory paralysis.

In Kenya no research has been carried out on the possibility of exploiting the Datura arborea plant as a source of tropane alkaloids. The aim of the project was, therefore to investigate the alkaloid content of the leaves of locally grown plants.

Collection and Preparation of Plant Material

The leaves of Datura arborea L. for preliminary work were collected from a private garden in Nairobi area in December 1980. The plant material for total alkaloid determination were collected in February 1981 from the same location during the flowering season. The plant was about 2 years old at the time of collection.

The leaves were dried in the oven at 65°C for 5 hours. Prior to alkaloids extractions the leaves were powdered.

Macroscopical and Microscopical examination of Plant material

Macroscopical Characters

The leaf has a greyish green colour. It has an entire margin and the apex is acuminate. The veins are more prominent on the lower surface and they leave the midrib at an acute angle afterwards branching and anastomosing repeatedly. The leaves are ovulate to broadly ovate with asymmetrical bases. They are 10-30 cm long and 4-17 cm wide. They have a slight odour and a bitter taste.

Microscopical characters:

A transverse section of the midrib of the leaf, which had been cleared by boiling in chloral hydrate solution, shows a bifacial structure and collateral vascular bundle. Cluster crystals of calcium oxalate are clearly visible and occur in the spongy mesophyll. Crystals are absent from cells adjacent to the veins.

Few prisms of calcium oxalate can be seen in the spongy mesophyll too (Fig 2). There are covering and glandular trichomes on the upper and lower surface of leaf. The covering trichomes are uniseriate and composed of four to five cells. The glandular trichomes have a short stalk and an ovoid to pyriform multicellular head (fig 1).

The parenchyma of the midrib is composed of cells which are elongated longitudinally and have slightly thickened walls. The xylem takes the form of a curved arc. The stomata are anisocytic with a higher number occuring on the lower epidermis than the upper one (Fig. 3)

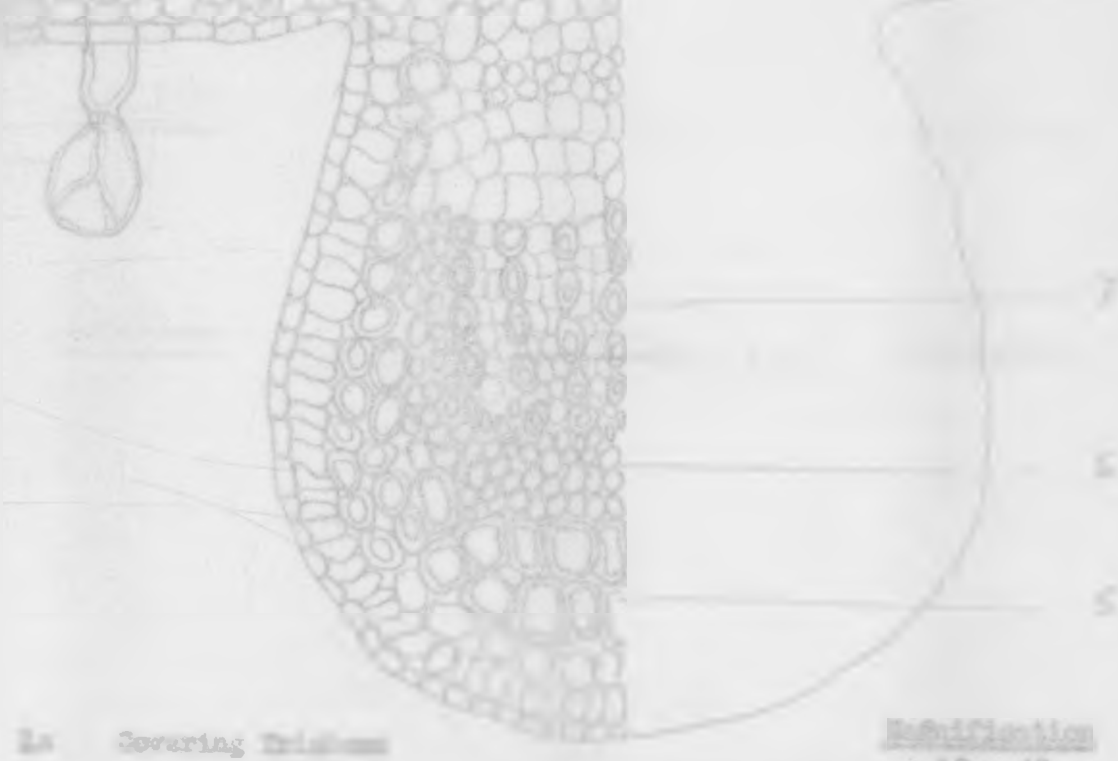
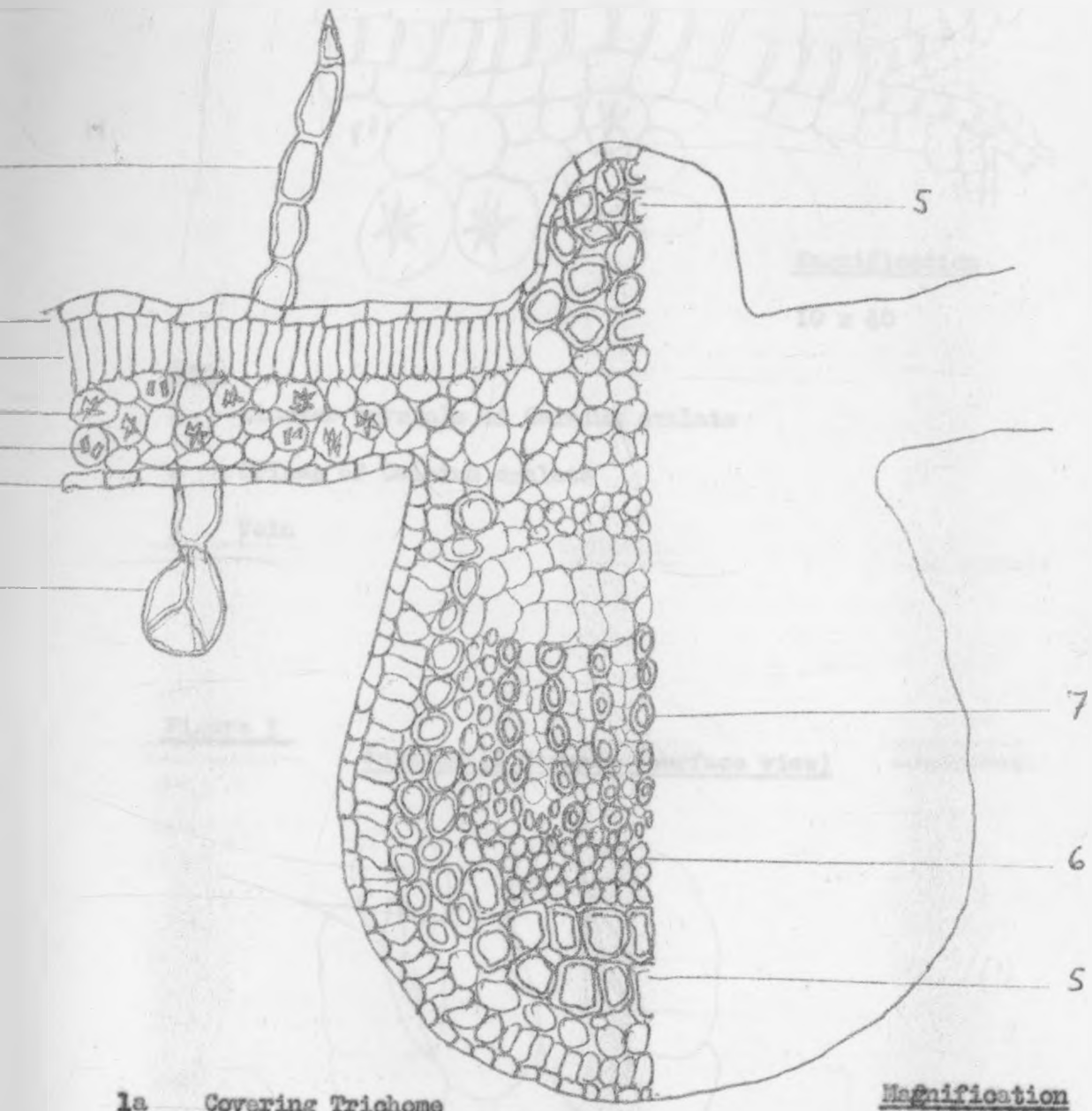


Fig 1.

Transverse Section of the Midrib of Datura Arborea L. Leaf



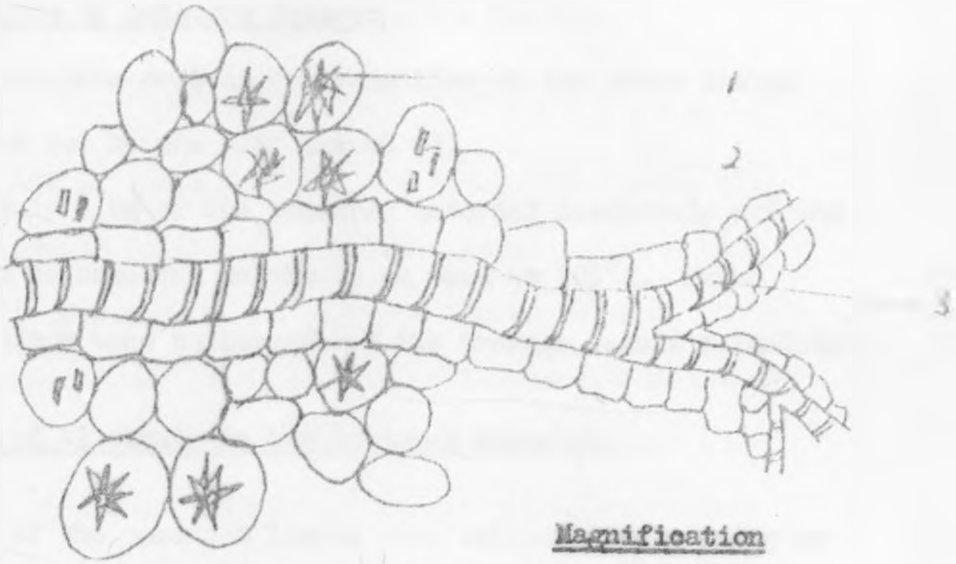
Magnification
10 x 40

1a	Covering Trichome		
1b	Glandular trichome	5	Collenchyma
2a	Upper epidermis	6	Phloem
2b	Lower epidermis	7	Xylem vessels.
3	Palisade layer		
4a	Cluster crystals of Calcium oxalate		
4b	Prisms of Calcium oxalate		

Figure 2

Part of a vein with Adjacent cells having no Calcium Oxalate crystals

(Surface view)



Magnification

10 x 40

Key

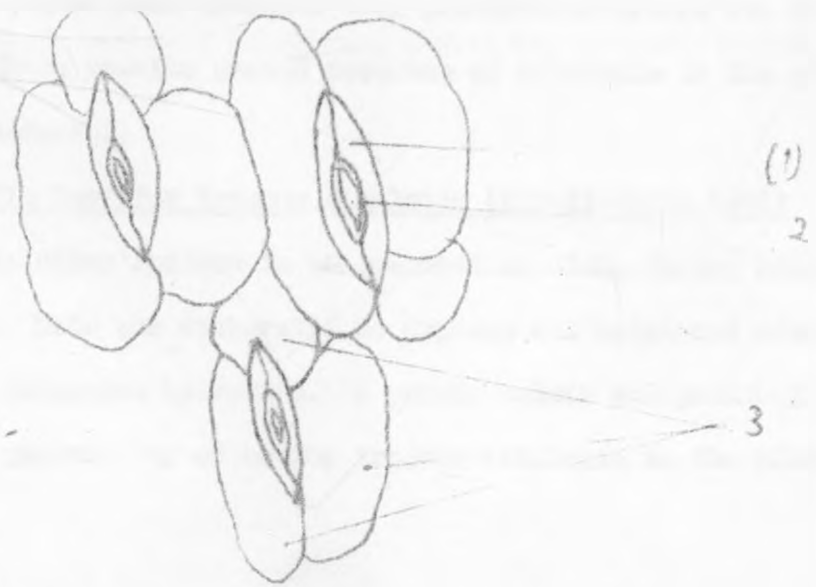
1 Cluster crystals of Calcium oxalate

2 Prisms of Calcium oxalate

3 Vein

Figure 3

Anisocytic Stomata (surface view)



Key

1 Guard Cell

2 Stomata

3 Three Subsidiary cells with one smaller than the others

Determination of Moisture Content

The moisture content determination of the dried leaves was carried out by the B.P. method (9)
Approximately 1.0g of the powdered material accurately weighed was heated to constant weight in an oven at 105°C. Three determinations were performed and the average result calculated.

Detection of Alkaloids in the Powdered Material

1.0g of the powdered leaves were extracted by warming on a water bath with 2ml 1% sulphuric acid for two minutes. The filtrate was made alkaline with dilute ammonia solution. Extraction was carried out with 2ml chloroform. The chloroform layer was separated and washed with a little amount of water. Filtration was performed using a small plug of cotton wool and the filtrate was divided into two equal portions which were evaporated to dryness.

To one portion of the residue, 0.2ml 1% sulphuric acid was added and to each 0.1ml portion of the solution was added:

i) Mayers reagent, this produced a white precipitate

ii) Dragendorffs reagent; this produced an orange red precipitate.

These results showed presence of alkaloids in the plant material.

Semispecific Test for Tropane Alkaloids (Vitali-Morin Test)

To the other residue in an evaporating dish, fuming nitric acid was added. This was evaporated to dryness and moistened with ~~dish~~ alcoholic potassium hydroxide. A purple colour was produced. This indicated probability of having tropane alkaloids in the plant

material, but the test is not specific for tropane.

Extraction of Alkaloids for Chromatographic Studies

10g. of the powdered material was made into a paste with 5% sodium carbonate solution. This was transfered to a flask and 50ml. chloroform added and refluxed for 20 minutes, after which it was cooled and filtered. The filtrate transferred to a separating funnel and 25ml of 5% sodium carbonate solution was added and this was agitated gently for 5 minutes. The chloroform layer was removed and reduced to about 5ml. in volume by evaporation using a rotary evaporator. 25ml of 1% sulphuric acid was added and extraction was carried out using 20ml. volumes of chloroform five times, The aqueous phase was separated and made alkaline with ammonium hydroxide solution. This was then extracted with five 10ml. portions of chloroform. The chloroform layers were combined and washed with 5ml. by evaporation. The conventrated chloroform extract was transferred to a crystallising dish and the remainder of the chloroform evaporated to dryness in an oven at 105°C. The residue was dissolved in 2ml. of absolute ethanol and this solution was used for chromatographic studies (10).

Thin Layer Chromatographic Separation of Alkaloids

T.L.C separation was carried out by the method described by Stahl. (11). One way ascending technique was applied using 20 X 20 cm. glass plates. Silica gel tO G.F 254 was used as adsorbent. After preliminary work with microscope slides using different solvent system, methanol: Acetone: Triethanolamine: (50:50:15) solvent system was found to give the best separation The separation was carried out at room temperature (25°C). Visualisation of the spots was first performed using ultra violet light followed by spraying with Dragendorffs reagent Thickness of layers was 250 microns and the length of run was 10cm. 1% solutions of hyoscine, l-hyoscyamine and Atropine in absolute ethanol were used as reference substances (Figure 3).

Gas Liquid Chromatographic Study of Alkaloids

G.L.C study of the alkaloids was carried out using PYE-UNICAM chromatograph (Series 104) with FLAME IONISATION DETECTOR. A. 1.5m glass column with internal diameter 4mm was used. The stationary phase was silicone oil (5% S.E.30) on Diatomite(CQ) solid support of 80-100 mesh. Nitrogen was used as carrier gas with a flow rate of 30ml/minute. The column temperature was 236°C while the detector oven was 300°C. Attenuation was 5 x 10^4 and the backing off range wa x100. Chart speed was lcm/minute (16).

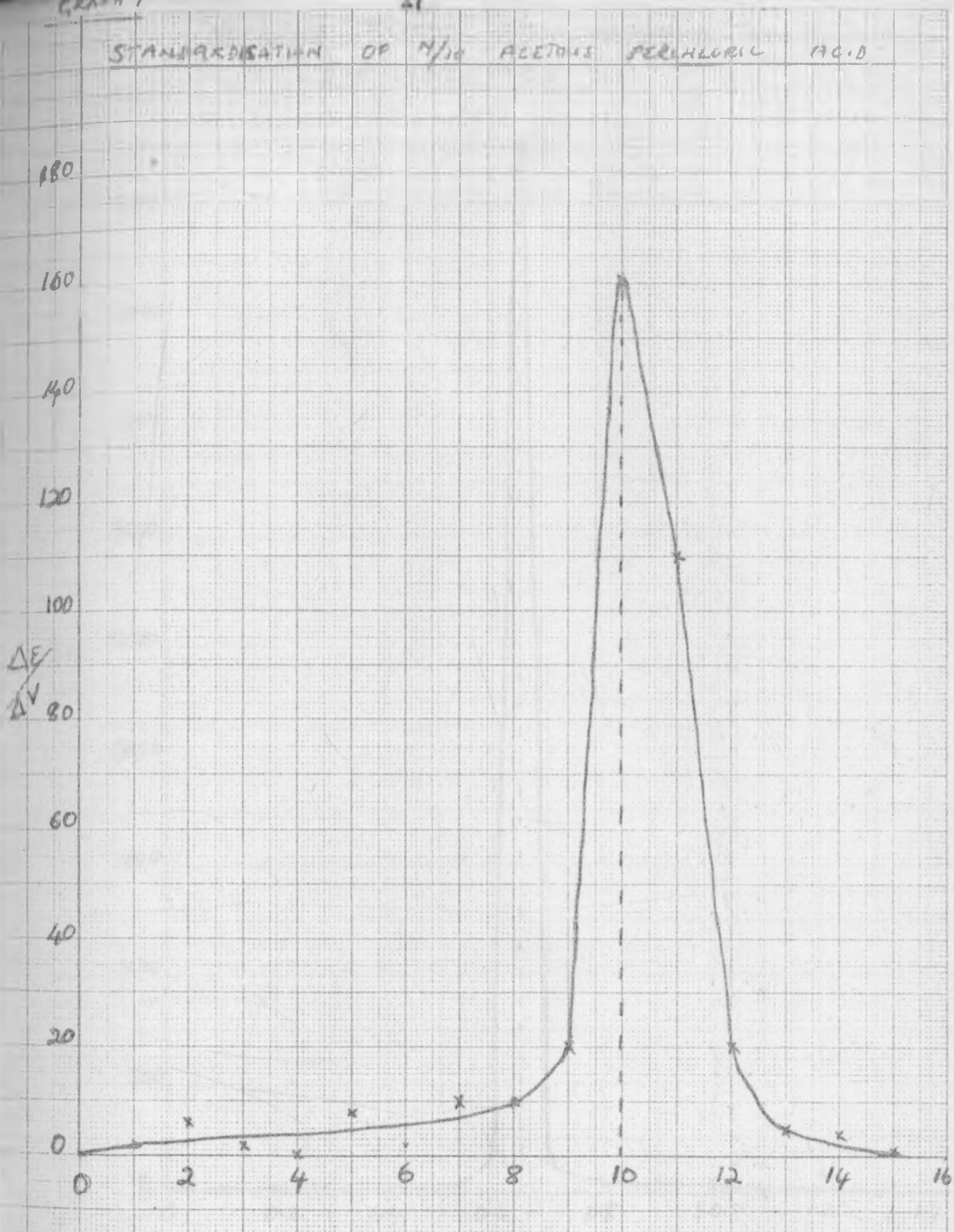
The sample (0.2ul and 0.4ul) dissolved in absolute ethanol was introduced by means of Hamilton syringe. Identification of the alkaloids was carried out by enhancement technique using hyosine and hyoscyamine as references. (Figures 4,5,6 and 7).

Determination of Total Alkaloid Content

About 10g. of the powdered material was accurately weighed and made into a paste with 5% sodium carbonate solution. This was transferred to a Soxhlet apparatus and after addition of 70ml. chloroform, extraction was continued for 5 hours on a boiling water bath. The solution was transferred to a separating funnel and 25ml of 5% sodium carbonate solution was added and the content was agitated gently for 5 minutes. The chloroform layer was removed and reduced to about 5ml. in volume by evaporation. Using a rotary evaporator 25ml. of 1% sulphuric acid was added and extraction with 20ml. volumes of chloroform carried out five times. The aqueous phase was separated and made alkaline with ammonium hydroxide solution. This was then extracted with five ml. portions of chloroform. The chloroform layers were combined and washed with 5ml. water. The volume was reduced to about 5ml by evaporation. The concentrated chloroform extract was transferred to a crystallising dish and the remainder of the chloroform evaporated to dryness in an oven at 105°C. The residue was dissolved in 50ml glacial acetic acid and potentiometric non-aqueous titration was carried out by the method described in B.P. (9) (Graph 1). Standardisation of N/10 acetous perchloric acid used for the above titration was performed as outlined in the B.P. (9) using Potassium hydrogen phthalate (Graph 2).

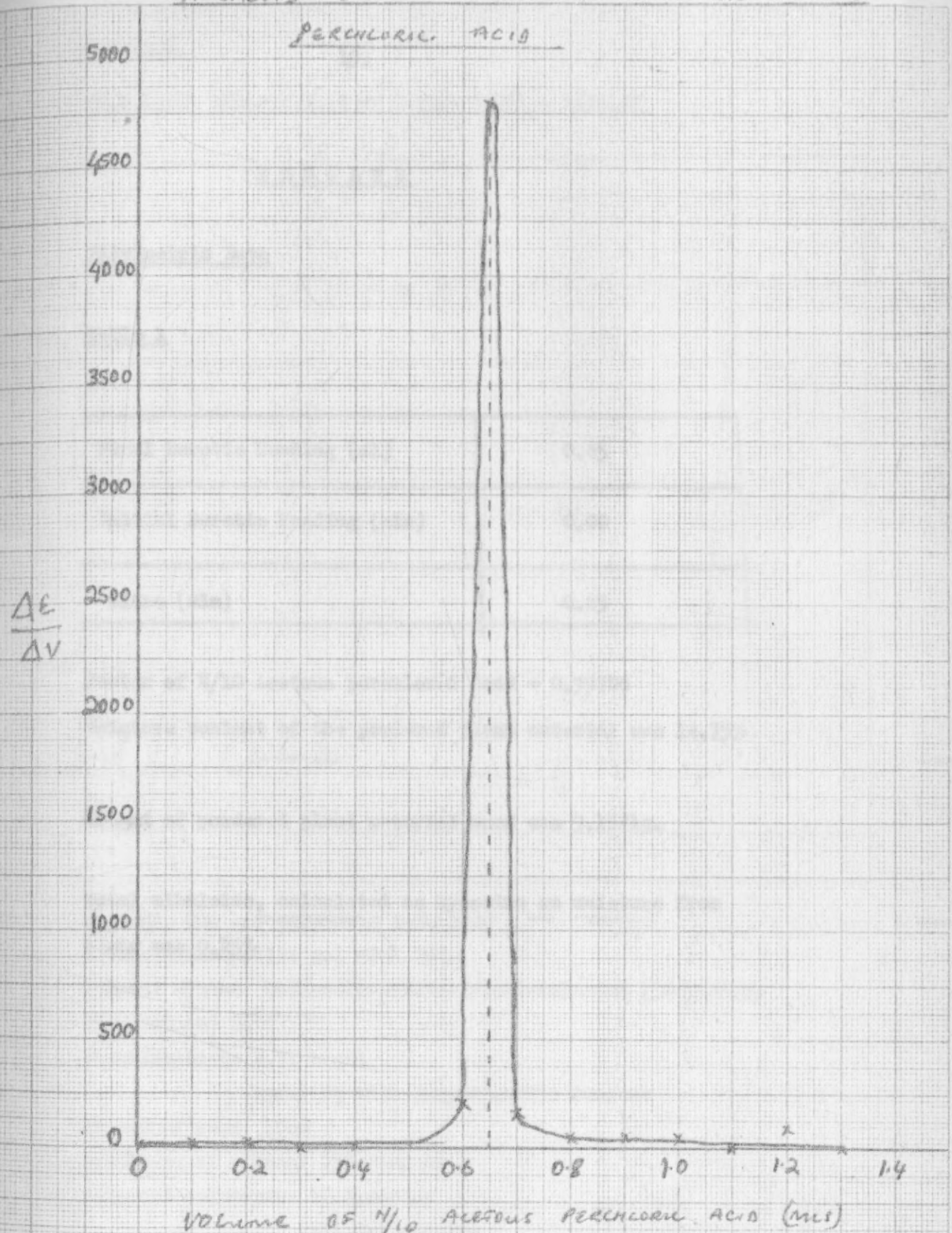
GRAPH 1

STANDARDISATION OF N/10 ACETOUS PERCHLORIC ACID



GRAPH 2

ALKALOID EXTRACT TITRATION WITH N/10 ACETOUS PERCHLORIC ACID



RESULTS

Titrimetric Data

Table 1

Final Burette Reading (ml)	0.65
Initial Burette Reading (mls)	0.00
Titre (mls)	0.65

Factor of M/10 Acetous perchloric Acid = 0.99706

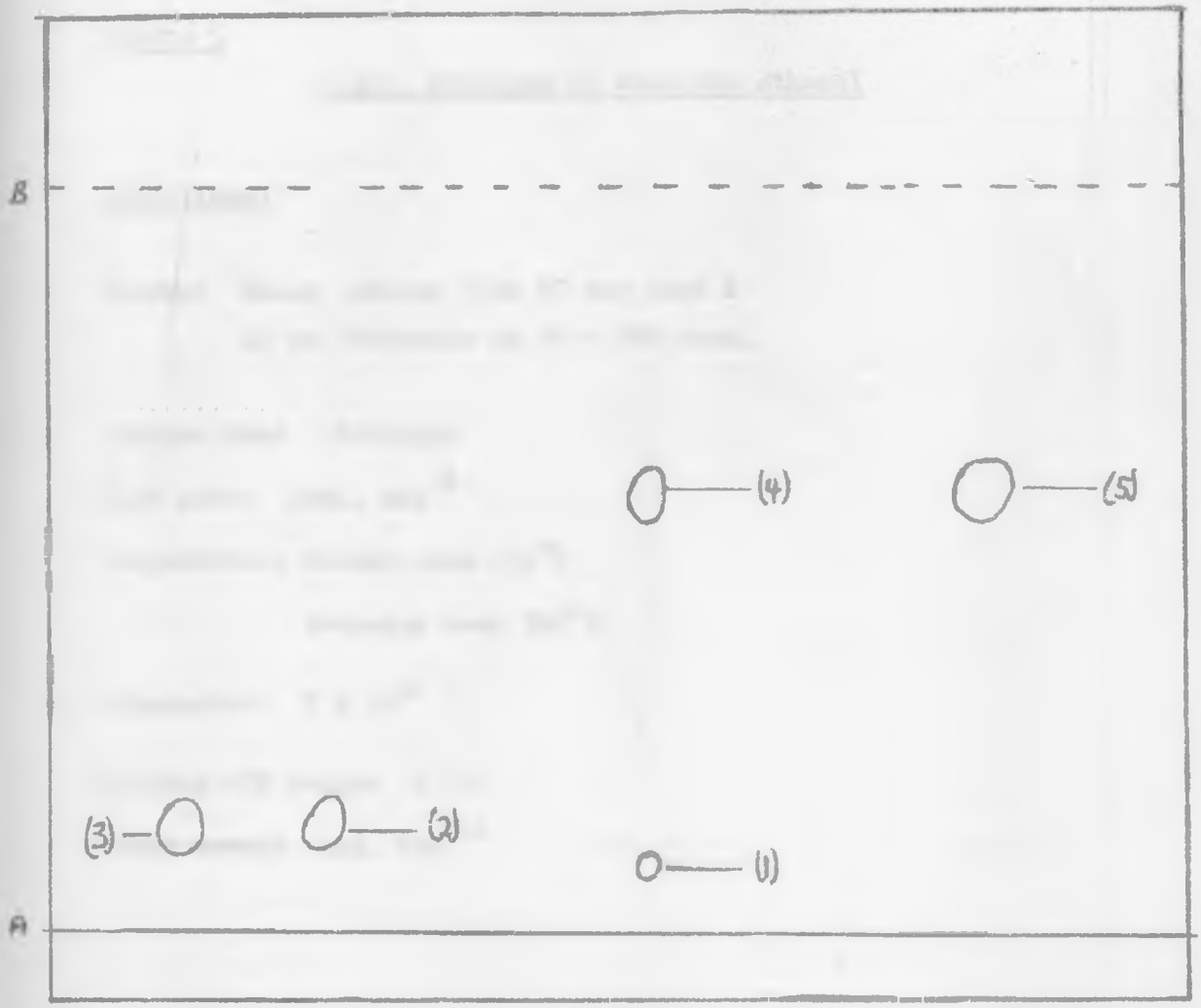
Moisture content of the powdered plant material was 14.75%

Weight of powdered plant material used was 9.1941g.

Total alkaloids, calculated as hyoscine on moisture free basis was 0.25%

Figure 4

THIN LAYER CHROMATOGRAM OF DATURA ARBOREA EXTRACT



Method: One way ascending technique

Adsorbent: Silica gel 60GF 254.

Solvent sytem: Methanol:Acetone:Triethanolamine (50:50:1.5)

Temperature: 25°C

Visualisation: U.V. light

spraying with Dragendorff's reagent

Length of run: 10cm

Thickness of plates: 250 microns

Standard solutions: 1% Hyoscine

1% l-Hyoscyamine

1% Atropine

GAS LIQUID CHROMATOGRAMS

Figure 5

0.4ul. Hyoscine in Absolute Ethanol

Conditions:

Column: Glass column with 50 per cent S
30 in Diatomite Ca 80 - 100 mesh.

Carrier Gas: Nitrogen

Flow Rate: 30ml. Min^{-1}

Temperature: Column oven 236°C

Detector oven 300°C

Attenuator: 5×10^4

Backing off Range: x 100

Chart speed: 1cm. min^{-1}

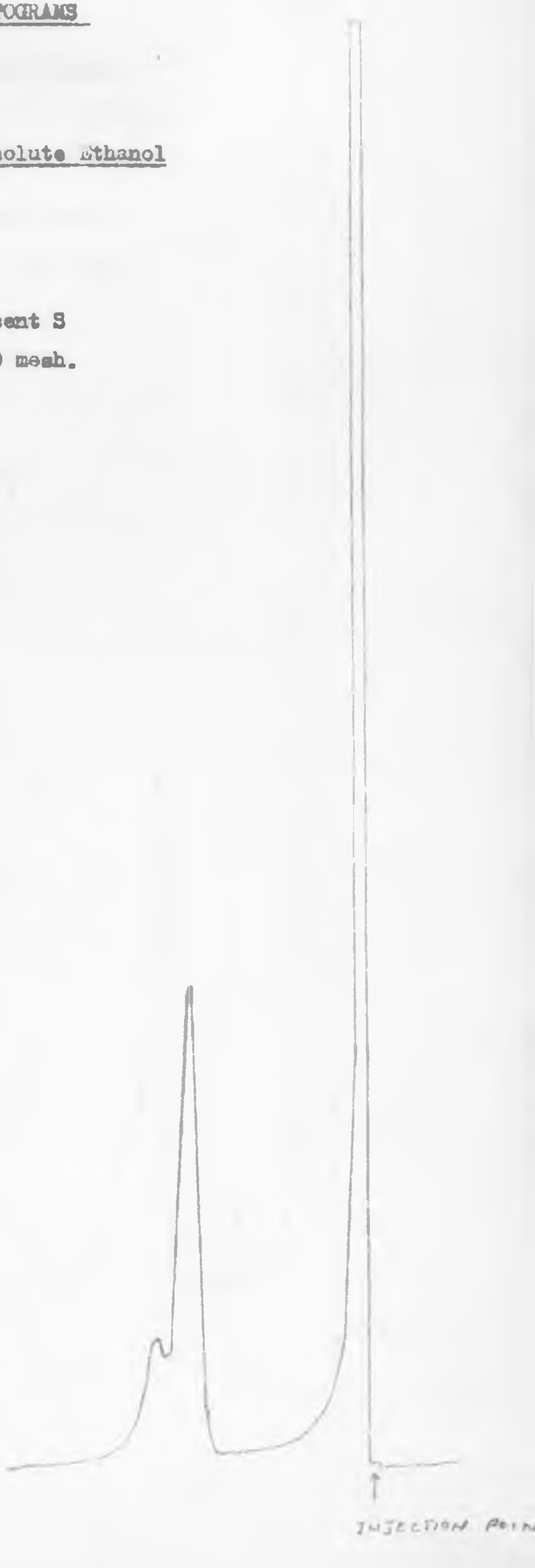


Figure 6

0.4 ul Alkaloid Extract in Ethanol

Conditions

Column: Glass Column with 50 per cent SE 30 in Diatomite CQ 80 - 100 mesh.

Carrier Gas: Nitrogen

Flow Rate: 30ml. min^{-1}

Temperature: Columnoven 236°C

Detector oven 300°C

Attenuator: 5×10^4

Backing off Range: X 100

Chart speed: 1cm min^{-1}

Volume injected: 0.4ul.

Figure 7

PEAK ENHANCEMENT

0.4 ul. Alkaloid Extract + 0.4ul. Hyoscine

Conditions:

Column: Glass column with 50 per cent SE 30 in Diatomite CQ 80 - 100 mesh.

Carrier Gas: Nitrogen

Flow rate: 30ml min^{-1}

Temperature: Column oven 236°C

Detector oven 300°C.

Attenuator: 5×10^4

Backing off Range: X100

Chart Speed: 1cm min^{-1}

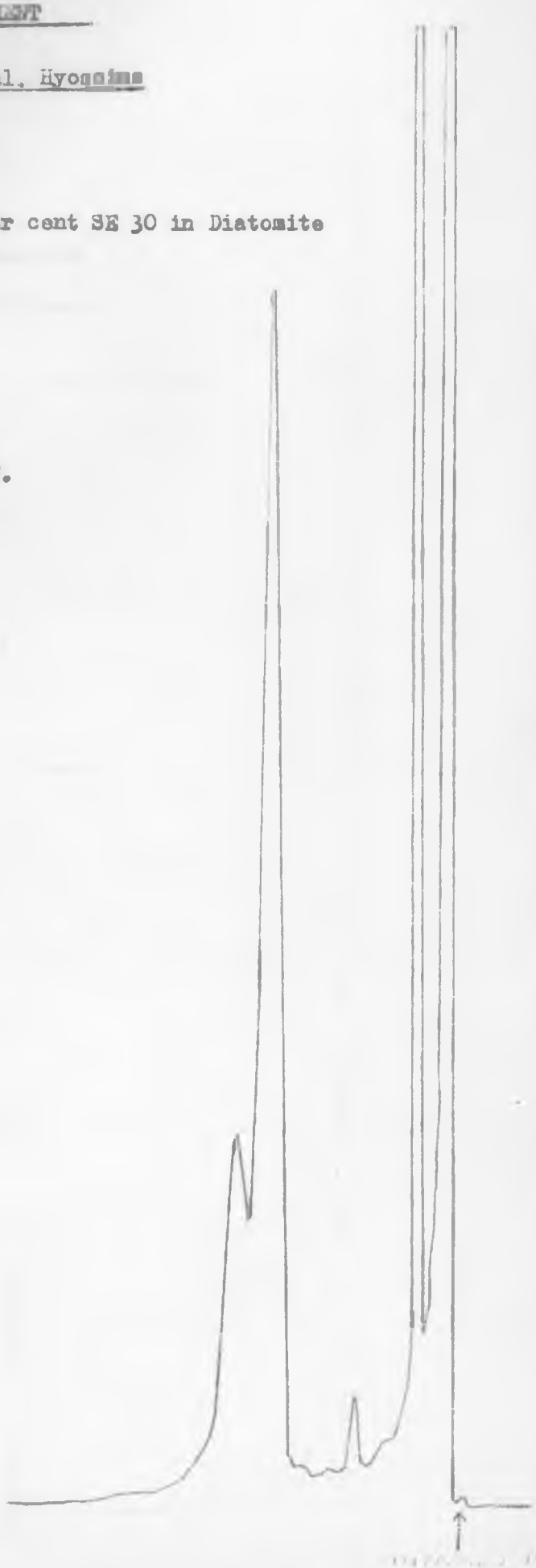


Figure 8

0.2 ul. Absolute Ethanol (Solvent)

Conditions

Column : Glass column with 50 percent SE

30 in Diatomite Ca 80 - 100 mesh

Carrier Gas: Nitrogen

Flow Rate: 30ml. min^{-1}

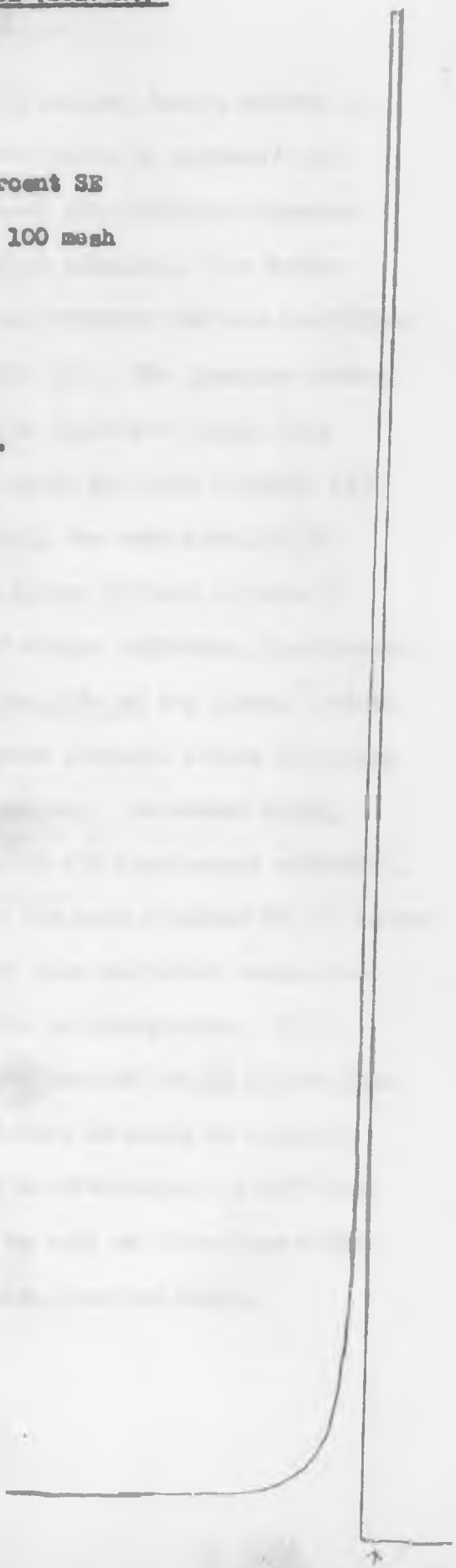
Temperature: Column oven 236°C

Detector oven 300°C.

Attenuator: 5×10^4

Backing of Range: X 100

Chart speed: 1cm min^{-1}



DISCUSSION

From the results obtained (See tables) Datura arborea L. leaves cultivated in Kenya have been shown to contain 0.25% of alkaloids. About 95% of the total alkaloids was hyoscine and about 5% was another unidentified alkaloid. The latter might be the same alkaloid which was detected but not identified from D. arborea L. leaves from India (7). The hyoscine content of D. arborea L. leaves collected in Kenya was higher than that obtained from the same plant grown in India (0.217%) (7). It should be taken into account that, the main alkaloid in Solanum species sometimes changes during various periods of plant growth, eg. in the leaves of Atropa belladona, hyoscyamine is the main alkaloid throughuout the life of the plant. Datura stramonium has hyoscyamine as the main alkaloid before flowering after which hyoscine becomes predominant. In Datura ferox, the principal alkaloid is hyoscine at all development stages(2).

Hyoscine has been shown to be the main alkaloid in the leaves of Datura arborea L. grown in Kenya when collected during the flowering stage. The plant contains no hyoscyamine. It is important that extensive studies are carried out to see whether the plant can be commercially exploited in Kenya as a source of hyoscine. The alkaloids should be determined at different developmental stages of the plant as well as in various other parts of the plant ie flowers, roots, stem and seeds.

REFERENCES

1. Remingtons Pharmaceutical Sciences (1975)
15th Edition pp 436, 839 - 842.
Mack Publishing Company
Easton, Pennsylvania.

2. Trease, E.D and Evans W.L. (1978)
'Pharmacognosy'
11th Edition pp 534 - 550
Bailliere, Tindall. London.

3. Goodman, L. S and Gilman, A.
'The Pharmacological Bais of Therapeutics (1970)
4th Edition pp 523 - 535
MacMillan Publishing Co., Inc. New York.

4. Mitchel, W., Brayer-Brandwijk M. G. (1962)
'The Medicinal and Poisonous Plant os Southern and
Eastern Africa'
2nd Edition. pp 951 - 956.
E & As Livingstone Ltd. London

5. Markets for Selected Medicinal Plant and their Derivatives
(1974) pp 66 - 67
International Trade Centre UNCTAD/GATT Geneva

6. Clarke E.G.C 1969
'Isolation And Identification of Drugs'
Volume II 376, 378.

14. Kirkland J.J
'Introduction to Modern Liquid Chromatography'
436 - 439
Wiley Interscience Publications
New York U.S.A

15. Bernard, V. and Trump E.C. (1969)
Common Poisonous Plant of East Africa'
p. 169. Collins St. James Place, London.