COMPARATIVE STUDY OF CAPE ALOE AND ALOE SECUNDIFLORA, USING CHEMICAL PHYSICAL AND INSTRUMENTAL METHODS

By

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A dissertation submitted in partial fulfilment for the award of the Degree of Pharmacy (B.Pharm) of the University of Nairobi.

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MAY 1984

D E D I C A T I O N

This work is dedicated to my parents, Mr. and Mrs. Phillip Auma whose efforts have brought me this far in education and to my friends and relatives whose encourengements during troubled moments have been comfort:

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

I owe great debt of gratitude to Mr. A.K. Getuma, who offered constant assistance, guidance and critically read the original manuscript.

To the Technical staff for their co-operation, particularly Mr. R.G. Mwalughu and Mr. I.G. Mureithi.

To Mrs. Rose I. Muturi for the part she played in typing out the manuscript.

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ABSTRACT

The aim of this work was to determine the different anthraquinones in Aloe Secundiflora, an indigenous species of Aloe growing around Pharmacy Department (Kenyatta National Hospital). This was compared with the anthraquininespresent in cape Aloe species which is mainly produced from Cape Province(South Africa)

First, chemical method was used to determine the quantities of the anthraquinones by usually comparing the intesities of colour produced in the two species.

Chemical methods showed that the Cape species had more anthraquinone than the Secundiflora.

The Anthraquinones were then seperated using thin layer chromatography method, both species being run on the same plate using same developing solvent that had previously been selected by trying different combination of solvents on glass slides. The Rf values were comparrable; the conditions having been reasonably the same in the Developing chamber.

The spots on T.L.C. were recovered using chloroform, which anthraquinones are soluble in. The chloroform portion was filtered and then run on Infra-red machine to try and determine any structural differences that resulted in the separation into spots of different heights.

CHAPTER ONE:

INTRODUCTION:

Aloe is from Arabic word alloeh or the Hebrew halal, meaning a shinny, bitter substance.

There are about 150 species of Aloe known, most of which are indigenous to Africa. The Aloes are typical xerophytic plants with fleshy leaves, usually having spines at the margins, and resemble to some extent the agave or century plant (12).

Aloe secundiflora studied is a perennial herb having a rosette of about 20 leaves from 30 to 40 cm long and from 5 to 7 cm in diameter at the base.

The Genus Aloe include a wide range of plants, herbs, shrubs. Aloe is the dried juice of the leaves of :

(1) Aloe-peryl Baker known in commerce as secotrine Aloe.

(2) Aloe Barbadensis miller (aloe vera) known in commerce as curacao Aloe or of Aloe ferox.

(ii) Aloe - Africana
(iii) Aloe-Spicata
(iv) Aloe-Capa
(v) Aloe - Secundiflora

The species studied in this project grows abudantly in dry rocky places, having been indentified in Machakos, Thika, Nyanza and Nairobi area. Depending on the species, Aloe contains, socoloin (7.5 - 10 per cent.

- Barbaloin 5-30%
- Capaloin 4.5-9%
- A pale yellow volatile oil,
- resinous material 16-63%

Apart from the Pale Yellow Volatile Oil and the resinous material, all are glycosides. These glycosides have also been called Aloinosides. Aloe - contains enough free anthraquinones to give positive Borntrager test.

The Aloe Resins consists of resinotannols, combined with cinnamic or p-hydroxycinnamic)(p.coumaric acids.

Long before their structures were elucidated. It was realised that Rhubarb, Trangulla Senna, Cascara had purgative action on the intestines. Their Alaysis proved that they have basic Anthraquinone derivatives within them and it is the Anthraquinone, responsible for the purgation. The anthraquinones are present both in free form and as glycosides. (1c)

The derivatives of anthraquinones present in these plants are (a) dihydroxy phenols (b) trihydroxyphenols, like in emodine and even tetrahydroxyphenols such as carminic acid (1c).

When the Anthraquinones exist as glycosides the sugar may be attached in different positions within the ring system.

Aloe is classified as a pharmaceutical necessity for compound Benzoin tincture, it possesses carthetic properties acting chiefly on the large intestines when used for this purpose the does given normally is in the region of 250 mg (16).

The fresh mucilaginous juice of the leaves of Aloe vera has been used for centuries in the treatment of burns, abrasions, and other skin irritation by the natives of the countries in which this plant grows (12).

The Seminole Indians are known to split the aloe leaves and apply them directly on injuries and wounds to promote healing. In 1953 the juice was recommended in treating third-degree X-ray burns and more, recently it has been advocated in treating atomic radiation; burns.

At the present time the application of fresh gel is popular in cosmetic industry. For a long time the variable nature of this mucilaginous juice made it extremely difficult to be incorporated into a stabilized type of preparation (7).

The mucilaginous juice has been found to contain a substance called Lectin which has been proved to be involved in the resolution of inflamation in wounds and burns.

Lectin is a group of haemaglutinating proteins found primarily in plant seeds, which bind specifically to the branching sugar molecules of glycoproteins and glycolipids on the surface of cells (8).

The mucilaginous substances when inco-operated in some food staff is known to aid in digestion, soothing effects of ulcers (9).

When cut cross-sectionally, the fluid that oozes out can chemically be devided into (1) The Raw gel (mucilage). This comes from the parenchymal tissue found in the internal portion of leaves and (2) The Latex; This is the bitter Yellow Liquid contained in the pericyclic tubules and it is the latex that contains Alnin, Aloe-emodin, responsible for cathartic action.

The cellular composition of the leaf necessitates the puncture of each cell containing the mucilage. The gel must be forcefully removed from the leaf tissue and, to remove cellular impurities, must be passed through stainless steel, strainers; because of the acid pH of the juice. The Extracted gel is then blended with special Lanolin base. Theointment is recommended for the treatment of sunburns, of deep thermal burns and in radiation burns. It affords relief from pain and itching and tends to minimise Keratosis and ulceration. Thus retarding and possibly preventing any changes toward malignancy (12).

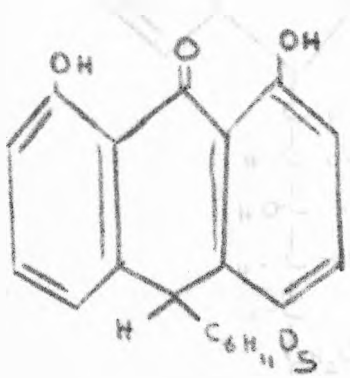
HISTOLOGY

CAPE: The leaves of all species of the genus Aloe are of succilent, xerophytic centric type. The outer walls of the epidermal cells are strongly cutinized. Beneath the epidermise is the mesophyl which is differentiated into an outer cortical and inner central zone.

Cape Aloe comes to the market in Olice-black or dusky to dark-brown masses frequently covered with yellowish powder. It fractures sharply exhibiting pieces with a smooth and vitrous broken surfece It has a distinct sour odour and a nauseous bitter taste ((1b)

Cape Aloe containe a variable mixture of bitter pentosides which are hydrolyzed in the presence of alkalies into anthraquinone derivatives like.

(1) Barbaloin ($C_{6}H_{20}O_{9}$). This is a pale yellow anthraquinone glycoside (pentoside) with the strucutre below.



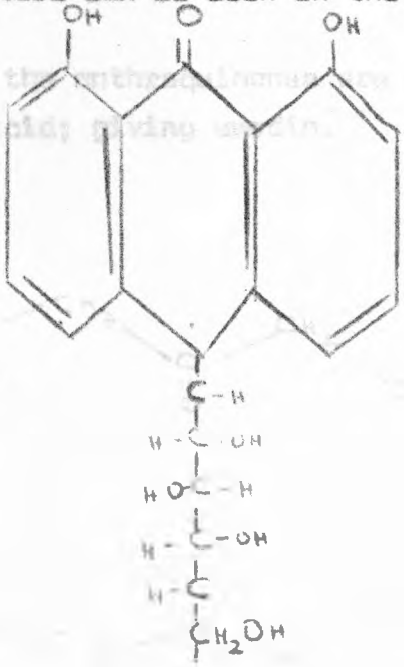
(1c)

BARBALOIN

- Aloe emodin (bitter resin)
- Isobarbaloin
- B-barbaloin - Are all present.

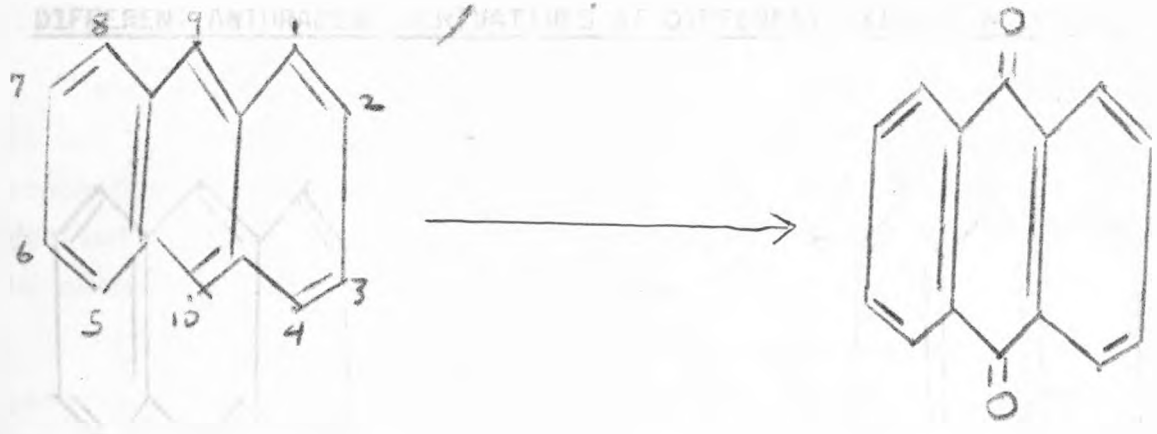
When Barbaloin is boiled with Alcohol and then acidifiedwith Hydrochloric acid, emodin ($C_{15}H_{10}O_5$) is yielded. All these are Anthraquinone derivatives and when released in-vivo from the plant extract are responsible for the powerful irritant action. Often they affect the uterus and thus has a bontificient effect (4).

As glycosides then Anthraquinones can either be C or O-glycosides depedning on the atom linking the anthraquinone to the free sugar and the Aglycone portion. The ease with which the bond is broken depends on the atom linking the two portions. The C-glycosides structure can be seen in the case of barbaloin below:



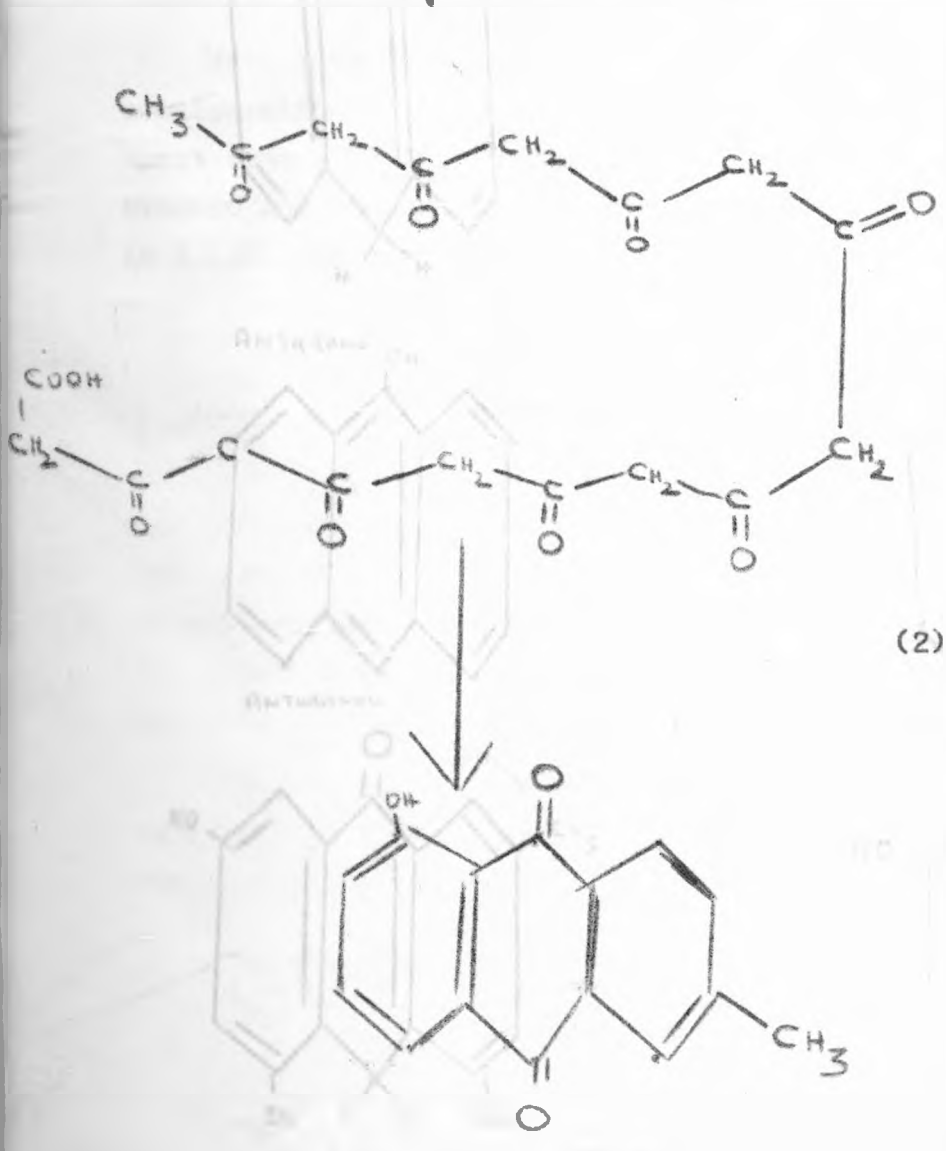
The sugar moety in the glycoside can be attached at various positions. The structure above is a C-glycoside being a 10-glucopyranosyl derivative of Aloe-emodin anthraquinone (9).

The anthraquinones are chemically derived from Anthracenes.



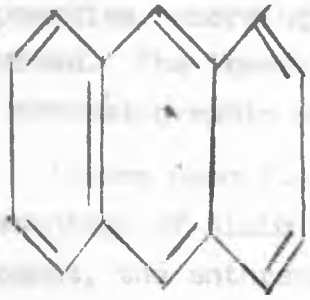
Anthracene Anthraquinone

However in plants the anthraquinones are synthesised from poly-β-Ketomethylenic acid; giving emodin.

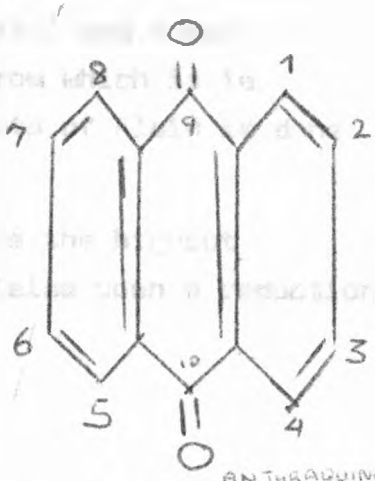


(2)

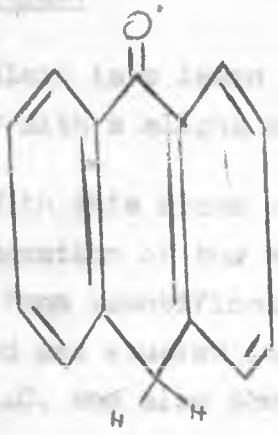
DIFFERENT ANTHRACENE DERIVATIVES AT DIFFERENT OXIDATION LEVELS



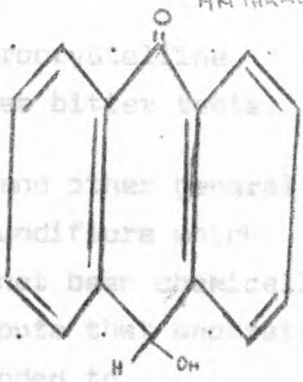
ANTHRACENE



ANTHRAQUINONE

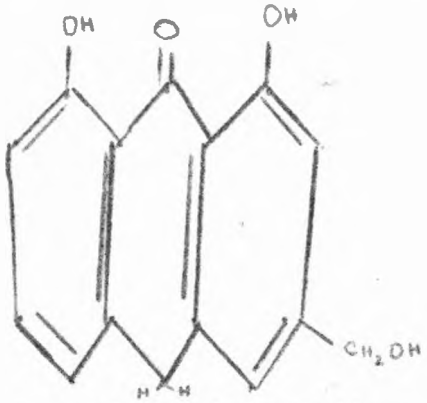


ANTHRONE

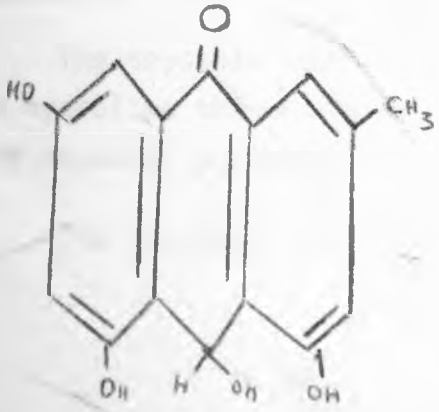


OXANTHRONE

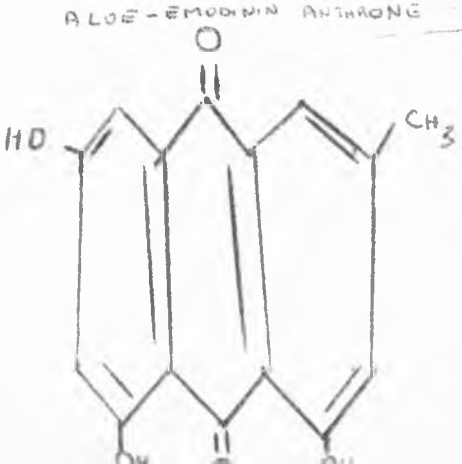
ANTHRANOL



ALOE-EMODINANTHRONE



EMODIN



ALOIN:

This is a mixture of active principles obtained from Aloe. It varies in chemical composition and in physical and chemical properties according to the variety of Aloe from which it is derived. The identification of the constituents of Aloin is done by chromatographic method.

It has been found that curacao Aloe yields the highest percentage of Aloin (barbaloin). Barbaloin yields upon a reduction product, the anthranol of Aloe-emodin (9).

Properties:

Aloin is a lemon Yellow to dark Yellow microcrystelline powder with a slight odour of Aloe and an intense bitter taste.

With this broad view of a structure, uses and other general consideration of the Aloes in general; Aloe-secundiflora which apart from identification by early workers had not been chemically studied was studied looking for the number of spots that separated in T.L.C. and also the colour reaction it responded to.

CHAPTER TWO

COLLECTION OF PLANT MATERIALS:

Aloe-secundiflora studied in this project grows around the Pharmacy Department wildly although some flower gardens have it. The leaves of the plant was collected and carried to the Department.

For collection of the juice that was used for the chemical tests, the leaves were cut transversely near the base and arranged around wide beaker. The leaves were arranged so that the cut ends overlap and drawn freely into the beaker. The leaves were allowed to drain for 6 hours after which they were removed and replaced with a new set of leaves. The juice is dark brown as it oozes out. On standing, there is evaporation, leaving behind brown-black crystals. (The juice has faint odour with intense bitter taste).

Another set of leaves were dried and ground into fine powder. From this powder Anthraquinones were extracted and the colour int intesities from this powder compared to that from crystals.

EXTRACTION FROM POWDERED DRUG:

One gram of the powdered drug was weighed and mixed with 7 mls of methanol and 10 mls of water and allowed to stand for 2 minutes. 3 ml of freshly prepared 25% aqueous ferric chloride solution was added. Followed with 6 ml of conc. Hcl, the mixture was refluxed in a water-bath for 30 minutes for hydrolysis tc occur. The solution was cooled and shaken with chloroform (15 ml). The chloroform layer was filtered and then everporated to dryness. The crystals were then taken in 1.5 ml of chloroform and 0.5 ml methanol. This layer contains the different Anthraquinones (11).

The crystals were also reacted with Ferric chloride 25% as above to hydrolise the anthraquione glycosides to free anthraquione for chemical analysis and for chromatographic work.

The Chemical Test done were:

	REAGENT	GRADE	BRAND
1.	Methanol	Lab. Reagent	May and Baker
2.	Ferric chloride Soln. 25%	"	"
3.	Cone Hydrochloric acid	"	"
4.	Chloroform	"	"
5.	Dilute Ammonia	"	Howse and MacGeorge
6.	Carbon Tetrachloride	Lab. Chemical	Kobian (Kenya) Ltd
7.	Dilute Nitric Acid	"	
8.	Bromine Solution	"	
9.	Copper Sulphate Soln.	"	E.T. Monks
10.	Sodium Chloride	"	E.T. Monks
11.	Silica Gel	Lab. Reagent	Merck

CHEMICAL TEST FOR IDENTIFICATION OF FREE ANTHRAQUINONE

This is a test designed to find if there are any free anthraquinone in the Aloe-secundiflora plant. The test is similar to the previously done one in that it depends on colour change. Like in the original tests of this kind the major draw back is description of colour which varies from individual since there is no colour standard reference.

FREE (a) 1 gram of the powdered drug was extracted with 10 ml of Hot water for 5 minutes and filtered when hot. The filtrate was cooled and extracted with 10 ml carbon Tetrachloride. The Carbon Tetrachloride layer was separated and washed with 5 ml water. This layer was then shaken with 5 ml of dilute ammonia.

Free Anthraquinone yields cherry-red colour in the ammoniacal layer.

THE CLICOSIDES (b) 1 gram of powdered drug was mixed with 5 ml of ferric chloride solution; 25% freshly prepared. 5 ml of Hydrochloric acid was added, The clycode was hydrolysed by heating on a water bath for 10 mins. The solution was filtered when hot and the filtrate cooled. To this wasadded dilute ammonia. A more intense pink or red colouration indicates presence of anthraquinone glycoside.

TABLE 2

SPECIES OF ALOE	TEST FOR FREE ANTHRAQUINONE	TEST FOR ANTHRAQUINONE GLUCOSIDES
CAPE	CHERRY-RED COLOUR (INTENSE)	MOST-INTENSE
SECUNDIFLORA	CHERRY-RED	MORE INTENSE

THE TESTS

1. SCHONTETENS REACTION:

To 5 ml of solution of Aloes 0.2 grams of Aloes was added and then heated until dissolved. A few drugs of this solution was added to a tube containing water and examined in strong sunlight, and colour noted.

2. BROMINE TEST

To 2 ml of solution of Aloes, 2 ml of freshly prepared solution of bromine was added and the colour noted.

3. NITRIC ACID TEST:

To 2 ml nitric acid, 5 ml sample of the extract was added and colour noted.

4. KLUNGE'S ISOBARBALION TEST:

To 20 ml of an aqueious 1 in 200 solution of Aloes a drop of copper sulphate 5% w/v was added, followed by 1 gram of sodium chloride and 10 ml of 90% alcohol, warmed gently and colour checked.

5. MODIFIED BORNTRAGER TEST:

200 mg of powdered Aloe was shaken with 100 ml of ferric chloride solution, mixed with 5 ml of hydrochloric acid and immersed in a water bath for 10 minutes, the solution was filtered and cooled, this was extracted with 10 ml carbon Tetrachloride. Then carbon tetrachloride layer was washed with 5 ml of water and shaken with 5 ml of dilute ammonia and colour noted (9).

COLOUR TEST RESULTS

	TEST	SECUNDIFLORA	CAPE	INFERENCE
1.	Schontetens	Brown-Green	Greenish-Yellow	Presence of Anthranols
2.	Bromine	Light Brown coloured Turning dark	Pale Yellow	Due to formation of tetrabromaloin (Aloin)
3.	Nitric Acid	No colour Test	Brownish colour, changing to Green	
4.	Klunge's Isobarbalion	No-colour Change	Light red colour, fading to yellow	Oxidation of Isobarbaloin gives violet colour. Transient in Cape
5.	Borntragers Test	Rose-Pink Cherry Red Less Intense	Rose-Pink to Cherry red More intense	Presence of Anthrequinones
6.	Nitrous Acid Test	No-colour change	Light pink colour	Test for Isobarbalion
7.	Borax Sol. Test	Greenish Colour	Intense Green Colour	Presence of Anthranols from anthrones

THIN LAYER CHROMATOGRAPHY

In thin layer chromatography a number of developing solvents were tried to find out the best developing solvents. The Pilot work was done on thin layer chromatograms using glass slides.

The solvent combination tried were:

1.	BENZENE	ETHYL FORMATE	FORMIC ACID	(6)
	75	24	1	

With 1% alcoholic potassium hydroxide solution used for visualisation. The anthracene derivatives Fluoresecence mainly yellow, orange or red in long-wave light.

2.	DIETHYL - ETHER,	METHANOL,	WATER
	75	24	1

3.	BENZENE	AMYLALCOHOL	FORMIC ACID
	75	15	10

The choice of developing solvent being based on the fact that Anthracene derivatives have high organic solvent solubility. The major complication was however the appearance of tailing even after 2 hours of development.

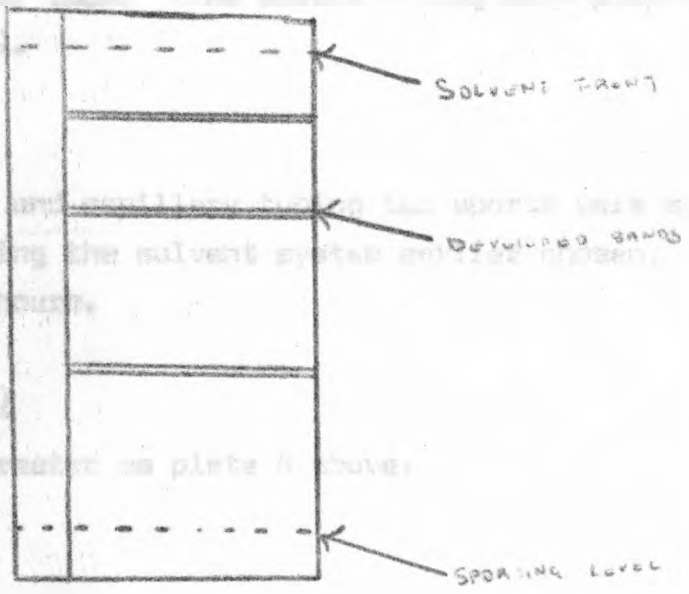
Of the three combination of developing solvents, Benzene, Ethyl formate and ferric acid gave the best sports.

After choosing the solvent, TLC was done to get the Rf values of the sports on both secundiflora and cape aloe.

A preparation T.L.C. was also done and the sports isolated and Anthraqehe extracted, using non destructive method. One side of the plate was sprayed with Alcoholic Hydroxide. The bonds spoted were scraped from the plate, into a beaker, 10 ml of chloroform was added to extract the Anthraquinone derivatives present.

This was then filtered and the resulting solution analysed in the infra-red light. Two sports of cape aloe and two sports of secundiflora aloe were taken.

Figure 2



The results are over-leaf.

It is documented the Aloes have different derivatives of Anthraquinones as their Aglycone portion. The purpose of the I.R. analysis was to find if the Aloe-secundiflora being studied had any structurally different Aglycone from the already studied Cape Aloe.

The different isolated sports from T.L.C. were marked numerically on the I.R. chart. Due to failling and perhaps some combination of the products, the I.R. charts were overlapping somehow.

The plates were prepared according to the method given by EGON STAHL (6).

The plates used were 20 x 20. Three plates were used i.e. one for cape aloes, another for Aloe-secundiflora species and a third one for both of them. (The plates having been prepared for adsorption T.L.C.).

PLATE A (CAPE ALOE)

Using a template and capillary tubing two sports were applied and then developed using the solvent system earlier chosen. The development took two hours.

PLATE B (SECUNDIFLORA)

This plate was treated as plate A above.

PLATE C

The two Aloe species were studied together by sporting a sport of cape aloe and a sport of Aloe secundiflora. Thetwo sports were then developed under the same conditions and their Rf values measured. This ensured that variations in temperature conditions were eliminate.

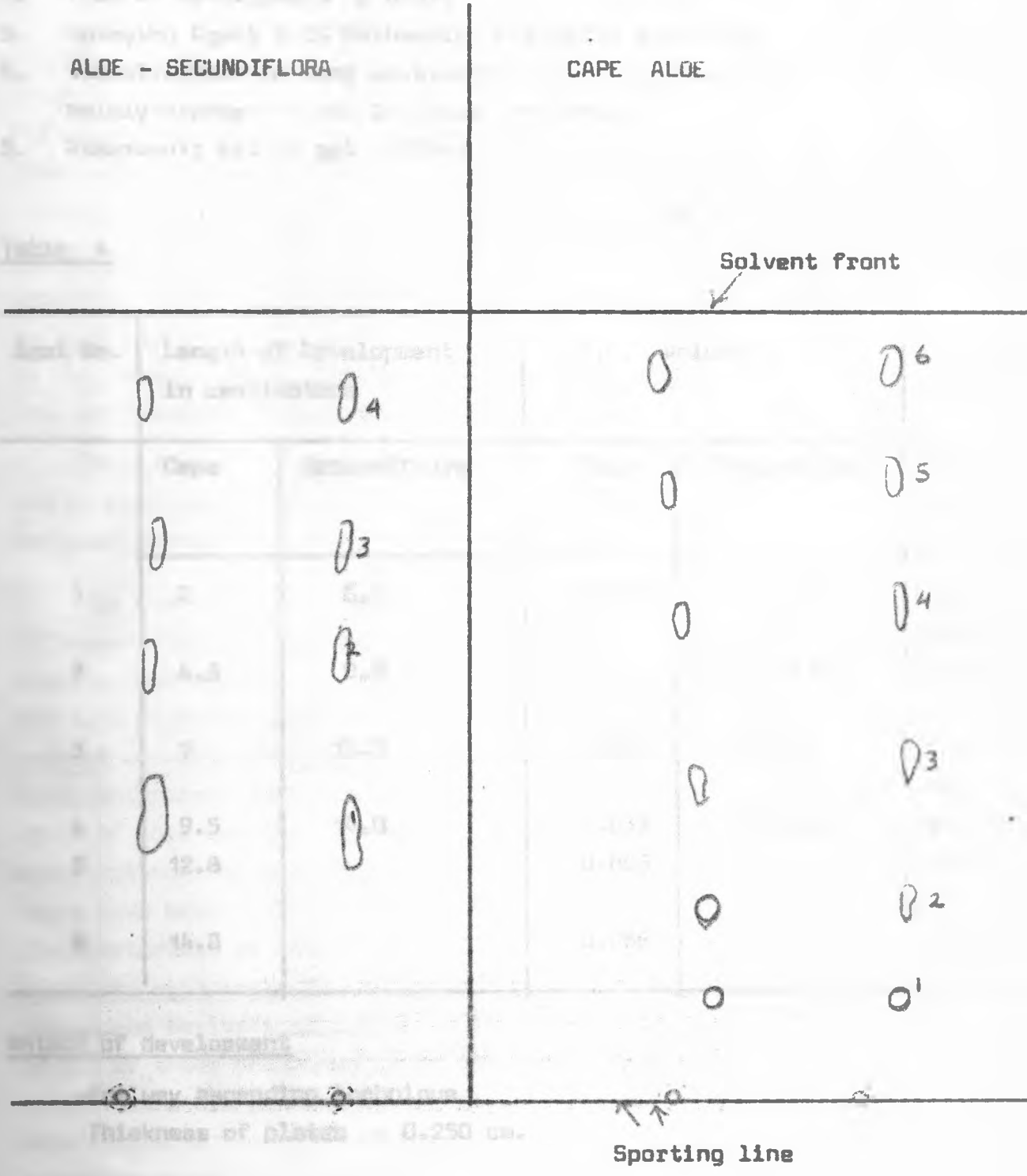
PREPARATIVE T.L.C.

After comparison of the Rf values of the two species from the T.L.C., the constituents of the two species were further compared by developing on preparative T.L.C. and then isolating the sports by scrapping out then extracting the aloe contents to find out if Infra-red Analysis could give any major chemical change that could be noticed.

Thick plates of .75 cm were prepared as before, they were activated and sported. Two plates were used. After developing for 2 hours then sporting was done.

Figure 1

THIN LAYER CHROMATOGRAM OF BOTH SPECIES



Rfs of Both Cape Aloe and Aloe Secundiflora

1. Solvent Front 15 cm
2. Time of Development 2 hours
3. Spraying Agent 2.5% Methanolic Potassium Hydroxide
4. Visualisation in long wavelength U.V., they fluorescence mainly orange and red in these wavelengths
5. Adsorbent; silica gel (G254)

Table 4

Spot No.	Length of Development in centimeters		R.F. Values		
	Cape	Secundiflora	Cape	Secundiflora	Colour of Spot
1	2	6.5	0.133	0.433	Light Orange
2	4.5	9.5	0.3	0.633	Orange-Violet
3	7	13.0	0.466	0.866	Orange-red
4	9.5	14.0	0.633	0.986	Yellow
5	12.8		0.855		Orange-red
6	14.8		0.986		Orange

Method of development

-One way ascending technique

Thickness of plates = 0.250 cm.

D I S C U S S I O N

From the presults over-leaf, it shows that Aloes, contain a number of anthraquinone glycosides, the principal one of which is barbaloin (Aloe-emodin)anthraquinone C-10-glycoside). O-glycosides of barbaloin with additional sugar have also been isolated from certain samples of cape Aloe.

The active constituents of Aloe vary quantatively and qualitatively according to the species from which the drug is obtained. Analysis have revealed that curacao Aloe is superior to Cape Aloe, containing two and a half times as much aloe-emodin as the latter. Curacao aloe also contain an appreciable amount of free and combined chryophanic Acid not present in the other types.

The secundiflora Aloe on the Basis of the chemical tests done has on comparative terms lower amount of the active principles analysed chemically by the simple tests done.

The anthracene derivatives occur in these plant materials in different forms at different oxidation levels. That is they may occur as derivatives of anthraquinone, or anthrane, or anthranol. The term Aloin is sometimes used in the literature to mean an extract of Aloes containing a mixture of such anthracene glycosides These anthracene derivatives give a red colour with alkali. On the Basis of this reaction and under standard conditions, colorimetric assay methods for estimating the anthracene constituents of the plant drugs have been devised. Contents of anthracene compounds in these plantmaterials as estimated by such colorimetric methods do not correlate with their cathartic action, as the different forms of anthracene derivatives differ in their cathartic activity. The action of these compounds is on the large intestine. When these constituents are present in the plant material as glycosides, the sugar helps to transport the anthracene aglycone intact to the large intestine where the aglycone is liberated by enzymes.

These anthracene derivatives, without the sugar are mostly broken down and only a small proportion is able to reach the large intestine to exert the cathartic action (15).

There appears to be also some evidence (16) indicating that those with two phenolic hydroxyl groups are active while those with one phenolic hydroxyl groups are not active and removal of acetylation of the phenolic hydroxyl group in these anthracene derivatives leads to loss of cathartic action.

Aloes are widely distributed in different parts of the world They have all been knownto contain different derivatives of anthraquinones. This depends on species of plant. The secundiflora-Aloe was compared using chemical tests giving colour change results. Colour tests showed that one, the concentration of anthraquinones in secundiflora-Aloe was rather low. It is documented that the glycoside content of the plant varies from species to species, and at time of year of collecting the plant. Normally for maximum yield collection, the glycosides must be collected at a season when there is muximum concentration.

The quantities of specific anthraquinone derivatives also varied from Cape Aloe to Secundiflora Aloe e.g. the Borntrager Test gave more intense colour on Cape Aloe than on Secundiflora Aloe. The secundiflora species did not give colour change with Nitrous Acid Test for isobabaloin. The cape Aloe contained more anthranols, than the secudiflora Aloe as evidenced by Borax Test.

There is however close approximation between the values of Rf, on both species except Cape Aloe had more spots than Secundiflora. Thus both species had anthraquinone derivatives which were rather similar i.e. only slight difference in Rf values.

It was tried if a major difference in the anthraquinone structures could be detected by use of I.R.

The structures as determined by the Infra-red did not reveal major difference in the structures. Although using I.R. data alone it is impossible to structurally determine a compound. It does confirm the presence of O-H groups which have stretching vibration of between 3200-3600.

It also confirms the presence of Ketonic group which absorb at 1675 - 1725.

The Cape Aloe and Secundiflora charts have varying peak differences. Slight though they might be the cause of separation into the various sports.

Aloe-Secundiflora can thus be used for laxative purposes having ~~purposes having~~ the anthraquinone derivatives present in Cape Aloe.

The Aloe-secundiflora studied has some free Anthraquinone derivatives i.e. those not glycosidically linked to the pentose sugar in the plant. This could be due to degradation of the glycosides after combination. The Anthraquinone derivative may be found before combination with the sugar moety.

There is greater awareness in the cosmetic industry that Aloe Latex is a good humectant and this might be exploited to a greater degree in future.

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CONCENTRATION

THICKNESS

SOLVENT CCl_4

REMARKS

SCAN MODE

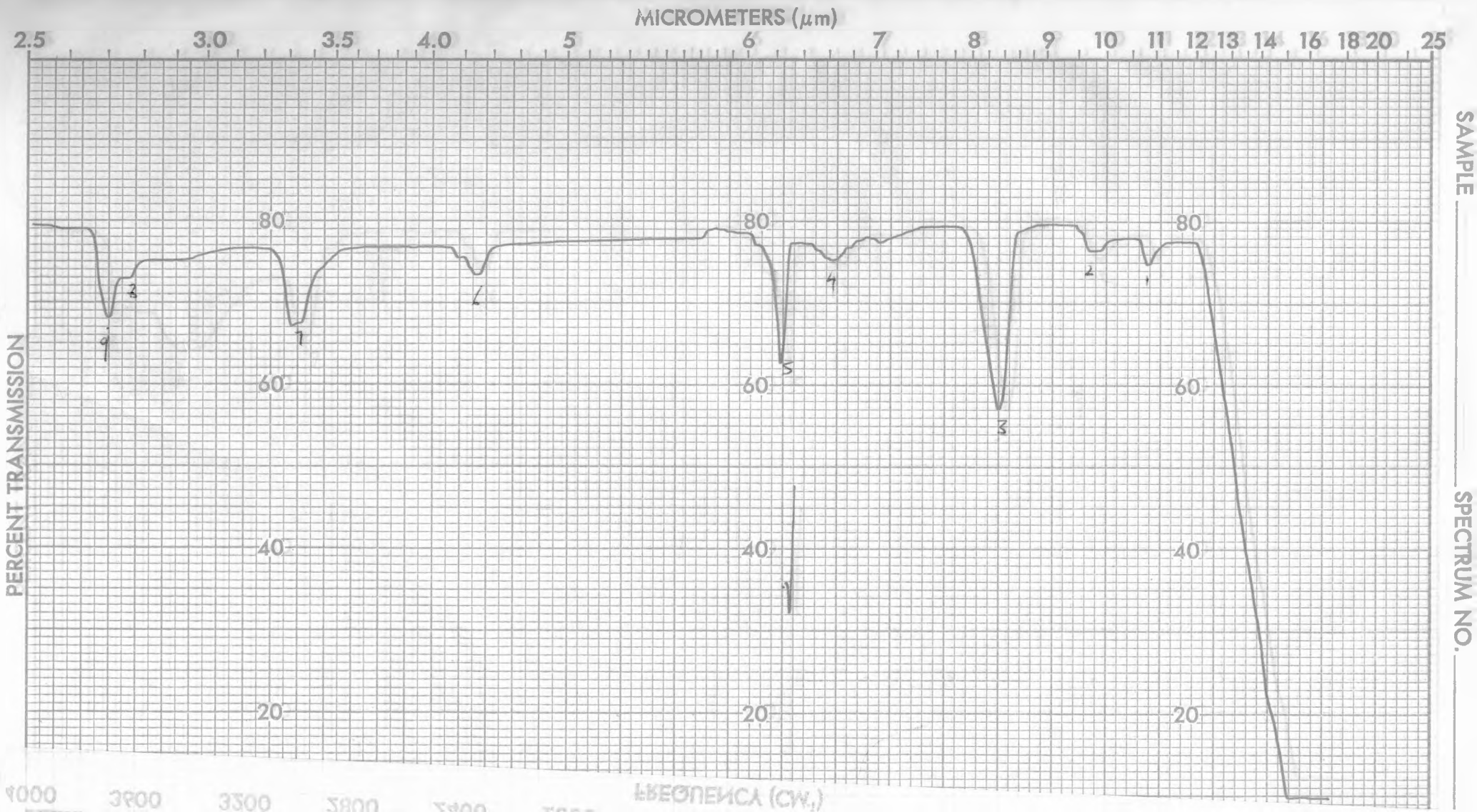
HI ENERGY ☐ CAL. ☐

RESOLUTION ☐

OPERATOR KAMMU S.N DATE 23-2-84

SAMPLE S_2 (SECOND SAMPLE) 3

ORIGIN



CONCENTRATION

THICKNESS

PHASE CHCl3

REMARKS

SCAN MODE

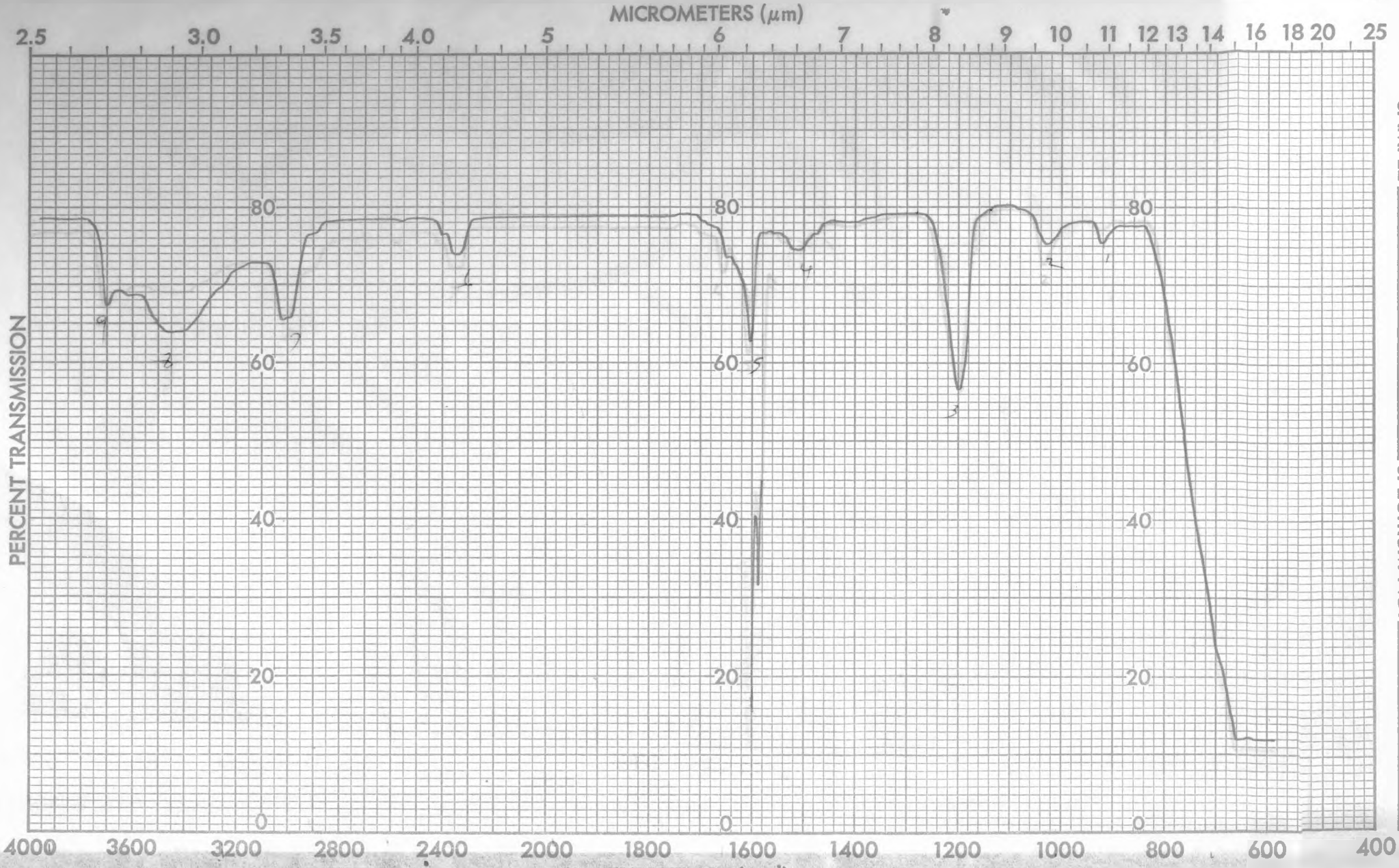
HI ENERGY ☐ CAL. ☐

RESOLUTION ☐

OPERATOR KAMAL S.N DATE 23-2-84

SAMPLE 2 CHCl3 FILM (2)

ORIGIN



CONCENTRATION

THICKNESS

PHASE

REMARKS

SCAN MODE

ACCY. ☐ SURVEY ☐

HI ENERGY ☐ CAL. ☐

RESOLUTION ☐

OPERATOR DATE

SPECTRUM NO.

SAMPLE SECUNDIFLORA (1)

ORIGIN