

A PHARMACOGNOSTICAL STUDY OF RAUVOLFIA
CAFFRA SOND. 'WHOLE ROOT:

BY:-

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A PROJECT CARRIED OUT AND SUBMITTED IN
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A C K N O W L E D G E M E N T S

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ABSTRACT

A microscopical examination of the powdered root was carried out and the observed diagnostic features recorded.

The powdered root was extracted using suitable solvents and the extract showed the presence of alkaloids when it was tested using Dragendorff's reagent. T.L.C. was used qualitatively to show the presence of reserpine in the root extract using pure reserpine as the reference standard.

Total alkaloidal content was determined by non-aqueous titration and was found to be 0.3048% W/W calculated as reserpine.

Reserpine content was determined by u.v. spectrophotometric assay and was found to be 0.09% W/W, thus accounting for 29.5% W/W of the total alkaloids.

INTRODUCTION

According to Tyler, V.E., (1) it must be emphasised that the name of the drug and that of the genus of the plants from which it derives must be spelled differently. For technical reasons, the genus must be spelled with a V instead of a W. The plant Rauwolfia serpentina is thus the correct botanical origin of the drug Rauwolfia serpentina.

The genus Rauwolfia (Family = Apocynaceae) contains approximately 50 species which grow in tropical and subtropical regions(2). The genus name honours a German physician and botanist Dr. Leonhard Rauwolf who made a study of the medicinal plants of Asia and Africa. The most extensively studied species at the present time are R. serpentina Benth, R. canescens Linn, R. vomitoria Afzel, and R. heterophylla Roem.

For centuries, Rauwolfia serpentina was used by medicinemen in India to treat a variety of maladies ranging from snake-bite to insanity. In 1563, Garcia de Orta mentioned the plant and its uses in his book on drugs of India, but European physicians were skeptical of its properties(3).

Despite this long history, very few pharmacological and chemical studies were undertaken on Rauwolfia until the Indian investigators Bose and Sen reported successful clinical trials with the drug (1941); the Indian

chemists Siddiqui and Siddiqui had isolated the first crystalline alkaloid from the plant in 1931(2).

In addition to the British National Formulary, the drug is official in the British Pharmaceutical Codex, the Pharmacopoea of India and the Pharmacopoea of Japan.

In this project, an attempt is made to carry out a pharmacognostical study of the whole root of Rauvolfia caffra Sond, growing in Kenya, the main aims being; to determine the diagnostic microscopic features of the powdered crude drug; to determine the total alkaloidal content and to quantify the amount of reserpine present in the powdered crude drug.

According to Dale, I.R., (4) Rauvolfia caffra Sond (synonyms Rauvolfia natalensis Sond, and Rauvolfia inebriens K. Schum.) has the following taxonomic characteristics:- it is a tree that grows up to 80 ft. in wet forests. The bark is smooth and grey. The crown is spreading. Leaves appear in whorls of 3 or 4, oblanceolate to 1 ft. long and 3 inches wide, apex acute or acuminate, base long attenuated and more or less decurrent on to the petiole. Flowers are white, subsessile, borne at the ends of secondary branchings of large umbels 4 inches long. The calyx segments are very short, subacute and overlapping at base. The corolla tube is 1/5 inches long and densely villous at the mouth. Carpels are connate at the base. The fruits are obovoid or subglabrous; the berries 1/2 inches diameter. The tree is found in Nakuru, Taveta and Meru Districts.

Wherever it is found, the Rauvolfia caffra plant is put

to some interesting medicinal uses by the local people. A bark decoction of Rauwolfia caffra Sond [Mutondwet (sebei)]; [mutu (meru)] is drunk as a medicine for general body swellings, rheumatism and pneumonia by the sebei and Meru people. The leaves are used by circumcised boys to sleep on when the wounds are still fresh(5).

Rauwolfia caffra Sond is stated to be poisonous in large doses. The Wachagga put the bark in beer to make it more potent. It has the convenient property of putting the drinker right out after only a moderate amount has been drunk and there are no unpleasant after-effects. There are a number of native medicinal uses for the bark of Rauwolfia species and one report has it that the roots of R. rosea K. Schum are very poisonous and are used as an ingredient in arrow poison in the Usambaras.(6)

Watt, J.M. (7) states that Rauwolfia natalensis Sond and Rauwolfia welwitschii Stapf have been sunk into Rauwolfia caffra Sond. He further states that the Mpondo use the bark of R. natalensis Sond for abdominal troubles. The Venda use it to kill maggots in wounds. The Zulu use it as an ingredient in a decoction for scrofula. They also use it as an application to the skin in measles, urticaria and other rashes. They use the milky juice as an emetic for abdominal problems. The bark is bitter and is said to produce severe purgation and abdominal pain, The fruit is sometimes eaten by the dog and results in a type of acute mania ending in convulsions and froathing at the mouth.

The aerial bark of R. caffra Sond has yielded a low percentage of alkaloids which is intractable. The root bark has yielded reserpine in small quantities but the main alkaloid is ajmaline.(7) This to some extent agrees with the work done by McAleer et. al.(8) Who investigated various species of Rauwolfia for their reserpine content (see table 1):

TABLE 1:

Species of Rauwolfia together with their place of origin and reserpine content

SPECIES	ORIGIN	%RESERPINE
R. mombasiana Stapf	Kenya, East Africa	0.116
R. vomitoria Afz.	Belgian Congo	0.09
R. obscura K. Schum	Belgian Congo	trace
R. caffra Sond	Northern Rhodesia & Kenya	0.01
R. natalensis Sond. (=R. caffra Sond)	Union of South Africa	trace
R. communisii Stapf.	Gold Coast, West Africa	0.015
R. canescens L. (=R. tetraphylla L.)	India	0.05
R. hirsuta Jacq. (=R. tetraphylla L.)	Guatemala & Costa Rica	0.06 - 0.08
R. indecora Woods	Guatemala	0.017
R. sarapiquensis Woods	Costa Rica	trace
R. sellowii Muell Arg.	Brazil	trace

W.E. Court et. al. (9) however showed that the reserpine content of R. caffra Sond. root bark compares favourably with other Rauvolfia species. The highest proportion of reserpine is in the inner phloem (0.185%) with 0.157% and 0.153% in the middle and outer phloem respectively. The ajmaline group of alkaloids except for their absence in the cork are more evenly distributed. The alkaloidal content of thin bark exceeds that of thick bark and is very low in the wood of stem and root.

Los, C.W.; and Court W.E. (10) isolated five alkaloids from R. caffra root collected from Northern Transvaal. (see table 2).

TABLE 2:

Alkaloid content of R. caffra root bark

Alkaloid	Corrected Percentage
Ajmalicine	0.1
Ajmaline	1.214
Rescinnamine	0.011
Reserpine	0.065
Serpentine	0.904

Later work by M.S. Habib and W.E. Court(11) however failed to demonstrate the presence of Serpentine in roots collected from Ndola, Zambia and the percentage of the other alkaloids was rather low as shown in table 3.

TABLE 3:

Alkaloids of *Rauwolfia caffra* roots from Ndola, Zambia

Alkaloid	mean percentage (assayed by direct spectroscopy)
Ajmalicine	0.0038
Ajmaline	0.0818
Rescinnamine	0.0499
Reserpine	0.0440

The pharmacological actions of reserpine have been extensively studied as evidenced by reports in various textbooks of pharmacology.

Reserpine depletes the stores of catecholamines and 5 - HT in many organs including the brain and adrenal medulla and most of its Pharmacological effects have been attributed to this action.(12)

The actions and uses of Reserpine have been lucidly put down in B.P.C. 1973(13). Reserpine has central depressant action and produces sedation and a decrease in blood pressure accompanied by bradycardia. Its antihypertensive effect is due to depletion of stores of catecholamines.

When given orally, its effects are slow in onset, seldom appearing within 3 - 6 days of administration and continues for sometime after its withdrawal; it has a cumulative effect. Reserpine is of most value in younger patients with mild labile hypertension associated with

tachycardia. In long established hypertension it is best used in conjunction with more potent antihypertensives.

Patients vary in their response to reserpine and dosage must be adjusted to suit individual requirements. To control mild to moderate hypertension, the dosage for the adult is in the range of 100 - 500 micrograms; usually about 250 micrograms daily by mouth. A thiazide diuretic may be given concurrently to potentiate the antihypertensive effect.

Reserpine is used in mild anxiety states and chronic psychoses due to its sedative action. It has a tranquilising rather than hypnotic action and produces less somnolence compared to the barbiturates. Patients with chronic mental illness treated with reserpine often become relaxed, sociable and co-operative. In mild anxiety, doses of 0.5 to 2 mg. orally are adequate. In severe cases, daily dose of 2 - 3 mg. orally in conjunction with 5 - 10 mg daily by i.m. injection may be given initially, the dosage being subsequently reduced according to the patients response. The optimum dosage may vary widely between patients. Treatment may have to be continued for a long period and withdrawal should not be abrupt.

Toxicity, side-effects and precautions to be taken in the use of reserpine are well explained by Goodman and Gilman (12). Untoward responses to reserpine are predominantly referable to CNS and the g.i.t., and have resulted in a progressive decrease in doses employed in the treatment of hypertension. The mild sedative effects

of small doses may be desirable in some apprehensive patients. However, even doses as small as 0.25 mg per day can produce considerable incidences of nightmares, psychic depression, sometimes severe enough to require hospitalisation or end in suicide. Reserpine should therefore not be administered to patients with a history of depressive episodes and it should be discontinued if suggestive signs or symptoms develop.

Reserpine commonly increases g.i.t. motility with abdominal cramps and diarrhoea. Single doses of 0.25 mg or more quite consistently increase gastric acid secretion. It should not be given to patients with a history of peptic ulcers. A few patients show increased appetite and weight gain .

Hypotensive effects are rare with low doses. Other vascular side effects include flushing and nasal congestion.

Very little pharmacological information is available on ajmaline. Martindale(14) states that it is official in Japanese Pharmacopoea. It has actions similar to quinine and has been used in the treatment of cardiac arrhythmias. Overdosage with ajmaline leads to cardiac arrhythmias, hypotension, shock, anuria and coma.

CHEMICALS AND REAGENTS

Chloroform	BDH Chemicals (Analar)
Ethanol absolute	Riedel-De Haen Ag (Proanalysis)
Methanol	M & B (analytical reagent)
Acetone	BDH Chemicals (Analar)
Cyclohexane	M & B (lab chemical)
Acetic Acid	Merck Damstadt (Pro-analysis)
Acetic Anhydride	Merck Damstadt (Proanalysis)
Perchloric acid	Merck Damstadt (Proanalysis)
Diethylamine	BDH (lab reagent)
Hydrochloric acid	M & B (analytical reagent)
Ammonia	BDH Chemicals (analar)
Sodium sulphate	M & B (lab chemical)
Bismuth subnitrate	Howse & McGeorge (lab chemical)
Potassium iodide	Howse & McGeorge (lab chemical)
Potassium hydrogen phthalate	M & B (lab chemical)
Reserpine (pure crystalline)	Sigma chemical company
Silica Gel G/uv ₂₅₄	Macherey Nagel

Preparation of Dragendorff's Reagent

This was prepared according to the method outlined by E.G.C. Clarke. (15)

(a) 2 g of bismuth subnitrate was mixed together with 25 ml. of acetic acid and 100 ml. of water.

(b) 40 g of Potassium iodide was dissolved in 100 ml. of water.

10 ml. of solution (a), 10 ml. of solution (b), 20 ml. of acetic acid and 100 ml. of water were then mixed together to give the final solution.

COLLECTION AND PREPARATION OF THE PLANT MATERIAL

The roots used in this study were collected from Taveta in December 1978. They were quite dry by the time the experimental work was started.

The roots varied in diameter from 2 - 4 cm and appeared as short pieces of 15 - 20 cm length. The bark of the roots is brownish to grey in colour while the woody part is yellowish to cream coloured.

The roots were cut into very small pieces using a knife and a pair of shears, care being taken not to lose any bark which had the tendency to flake off when the roots were subjected to great forces.

The small pieces were subjected to coarse grinding using a Lee Household mill. The resulting material was then ground into a fine powder using a small laboratory mill (Krupps Type 208, made in Germany).

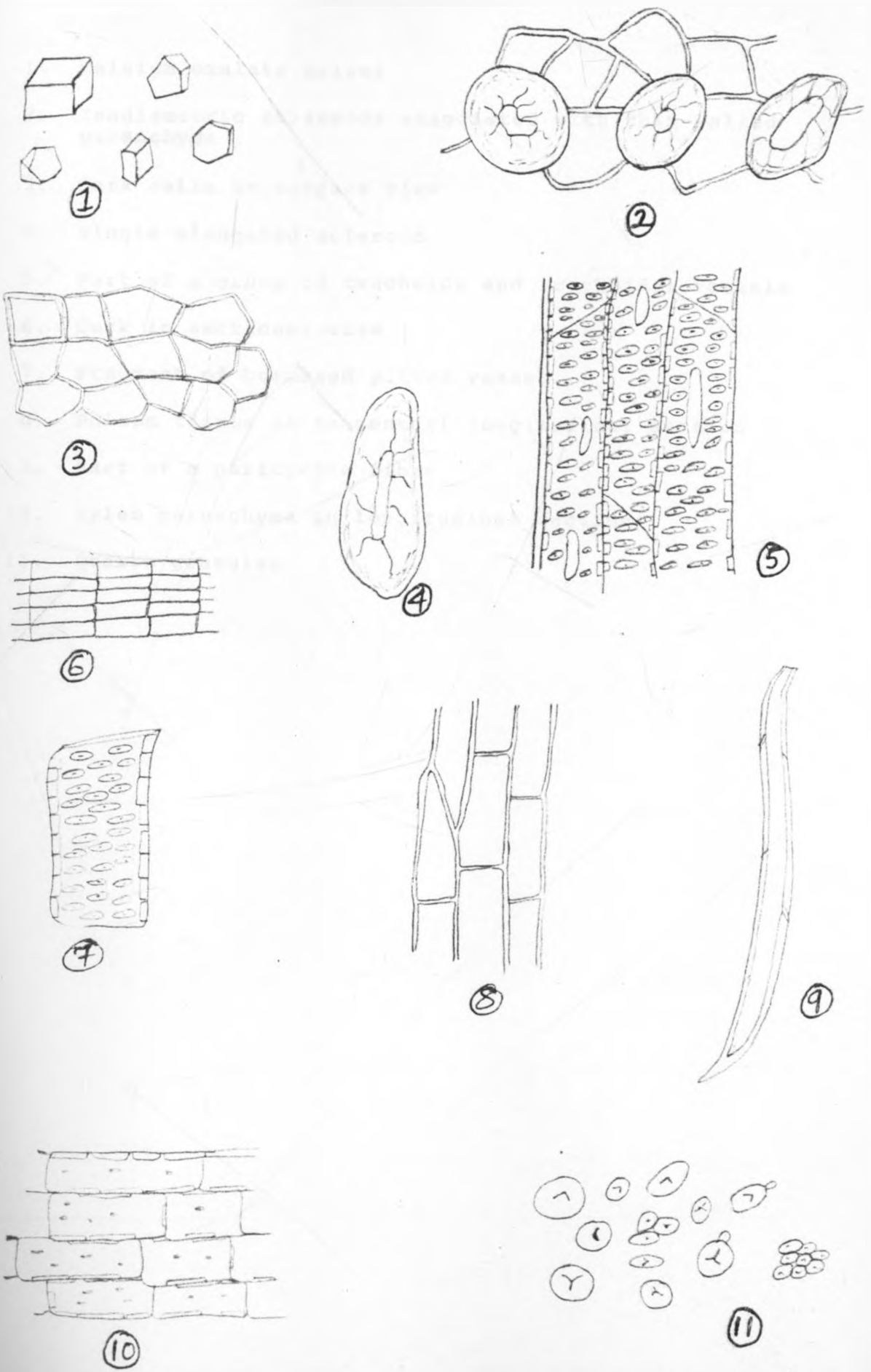
The powder (brownish-grey coloured, odourless with a characteristic slightly bitter taste) was then stored in an air tight amber coloured bottle.

MICROSCOPIC EXAMINATION OF THE POWDERED ROOT

The characteristic features observed under the microscope are enumerated below and digramatically represented in figure 1.

- (a) Fairly abundant starch granules which are mostly simple, spherical to ovoid in shape with a slit shaped or stellate hilum. A number of compound granules also occur with two, three, or more granules.
- (b) Numerous sclereids occuring singly or in groups associated with thin-walled parenchyma. The shape varies from iso-diametric to elongated. The walls are thickened and lignified.
- (c) Fibres which are frequently fragmented and are either scattered or associated with xylem tissue.
- (d) Abundant lignified fragments of xylem composed of vessels, tracheids and xylem parenchyma. The tracheids usually occur associated with smaller vessels and they also have numerous bordered pits.
- (e) Calcium oxalate crystals are abundant and usually scattered. They appear as prisms of irregular shapes.
- (f) Pericyclic fibres are occasionally seen. These are large, non-lignified and are usually found fragmented.
- (g) Reddish-brown cork cells which are polygonal or elongated in surface view. They are strongly lignified.

CHARACTERISTIC FEATURES SEEN UNDER THE MICROSCOPE



(x 400)

KEY TO FIGURE 1

1. Calcium oxalate prisms
2. Isodiametric sclereids associated with thin-walled parenchyma
3. Cork cells in surface view
4. Single elongated sclereid
5. Part of a group of tracheids and tracheidal vessels
6. Cork in sectional view
7. Fragment of bordered pitted vessel
8. Phloem tissue in tangential longitudinal section
9. Part of a pericyclic fibre
10. Xylem parenchyma in longitudinal section
11. Starch granules

DETERMINATION OF MOISTURE CONTENT

According to Brain & Turner(16), excess moisture in a drug suggests not only that the purchaser is paying a high price for unwanted water, but also that the drug has been incorrectly prepared, or subsequent to preparation, has been incorrectly stored. Excess moisture can result in the breakdown of important constituents by enzymatic activity and may also encourage the growth of yeasts and fungi during storage.

Moisture content limits are usually stated in pharmacopoeial monographs, but where no limit statement is expressed, the limit by inference is that the drug should be air dried. This only requires that the drug has reached equilibrium with the surrounding humidity, which will vary according to the location, in place and time, of the material.

Experimental

The method of heating to constant weight was adopted^P since R. caffra powder is not known to contain volatile material at the temperature that was employed.

2.0011 g of the powder was weighed accurately onto a crucible that had previously been dried to constant weight at 105°C. This was then placed in an oven at 105°C and dried to constant weight, care being taken to use a desiccator when transferring the material from the oven to the weighing room and back. See table 4 for summary of the weighings.

TABLE 4

Heating of *R. caffra* powder to constant weight

Weight of crucible after 30 minutes drying (g)	33.3945
Weight of crucible after 15 minutes drying (g)	33.3915
Weight of crucible after 15 minutes drying (g)	33.3903
Weight of crucible after 15 minutes drying (g)	33.3900

$$\begin{aligned} \text{Weight of dry crucible + the powder sample} \\ &= 33.3900 \text{ g} + 2.0011 \text{ g} \\ &= \underline{\underline{35.3911 \text{ g}}} \end{aligned}$$

Weight of crucible + powder after 60 mins drying (g)	35.3563
Weight of crucible + powder after 30 mins drying (g)	35.3122
Weight of crucible + powder after 30 mins drying (g)	35.3120

$$\begin{aligned} \text{Loss on drying} &= 35.3911 \text{ g} \\ &\quad \underline{35.3120 \text{ g}} \\ &= 0.0791 \text{ g} \\ \\ \text{Moisture content} &= \frac{0.0791 \text{ g}}{2.0011 \text{ g}} \times 100 \\ &= \underline{\underline{3.95\% \text{ W/W}}} \end{aligned}$$

EXTRACTION OF THE ALKALOIDS

The general method for extraction of alkaloids as outlined by K.R. Brain and T.D. Turner(17) was adopted. The principle of the method is that alkaloidal salts are soluble in aqueous solvents and insoluble in organic solvents, while as the free bases, they are insoluble in water and soluble in organic solvents.

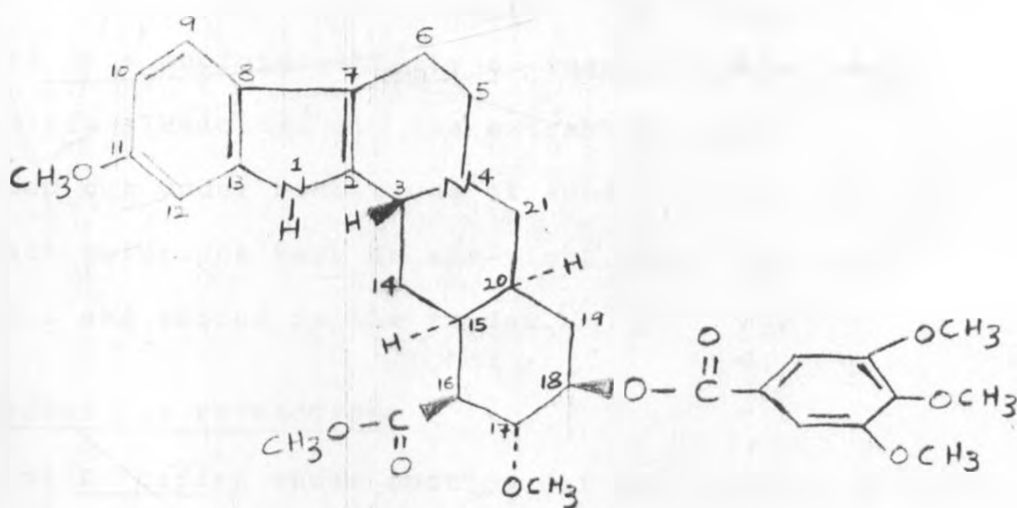
Reserpine and its preparations discolour rapidly when exposed to light but loss in potency is usually small. Solutions in benzene and chloroform are very sensitive to light, rapidly losing potency on exposure(18).

Wilson, C.O.(19) also reiterates this fact and explains the instability from the point of view of the chemistry of reserpine. Reserpine has the following structure

(see figure 2)

FIGURE 2:

Structure of reserpine



In common with other compounds with an indole nucleus, reserpine is susceptible to decomposition by light and oxidation especially when in solution. The decomposition occurs without appreciable colour change, thus colour change can

not be used as an index of the amount of decomposition.

There are several possible points of breakdown on the reserpine molecule. Hydrolysis may occur at C - 16 and C - 18 (See figure 2). Reserpine is stable to hydrolysis in acid media, but in alkline media, the ester group at C - 18 may be hydrolysed to give methylreserpate and trimethoxybenzoic acid (after acidification). If in addition, the ester group at C - 16 is also hydrolysed, reserpic acid and methyl alcohol are formed. Citric acid helps maintain reserpine in solution and in addition stabilises the alkaloid against hydrolysis.

Storage of reserpine solutions in daylight causes epimerisation at C - 3 to form 3 - isoreserpine. Oxidation also takes place to form 3 - dehydroreserpine. Oxidation will also take place in the dark in the presence of large amounts of oxygen.

Due to this photosensitivity of reserpine and related Rauwolfia alkaloids, all the extractive procedures were carried out under conditions of subdued light and the extract solutions kept in air-tight amber coloured bottles and stored in the fridge.

PROCEDURE FOR EXTRACTION

10 g of R. caffra whole root powder was weighed out into a beaker and moistened with 2.0 M ammonia solution, this being necessary to convert the alkaloidal salts in the plant material into free bases. The moistened material was placed in a thimble, placed in soxhlet apparatus and

continuously extracted for three hours using 250 ml. of chloroform.

This extract was purified as follows: It was extracted in a 500 ml. separatory funnel with 5 successive aliquotes each of 50 ml. of 1.0 M HCl. Complete extraction was tested for using the Dragendorff's spot test. (A drop of the chloroform phase is placed on a filter paper impregnated with Dragendorff's reagent after each extraction till no orange-red colouration is produced on the filter paper). The chloroform layer containing non-alkaloidal impurities and pigment material was discarded and the combined aqueous extract containing the alkaloids as their hydrochloride salts was made alkaline to litmus using 2.0 M ammonia solution. This was then extracted with 5 successive aliquotes each of 50 ml. of chloroform, the Dragendorff's spot test also being carried out at this stage on the aqueous phase to ensure complete extraction. The combined chloroform extract was dried using a little anhydrous sodium sulphate, filtered and concentrated to a volume of about 30 ml. in vacuo. It was then packed in an air-tight, amber coloured bottle and stored in a refrigerator ready for use in Thin-layer chromatographic studies.

THIN-LAYER CHROMATOGRAPHIC STUDY OF THE EXTRACTED ALKALOIDS

T.L.C. ON MICROSCOPE SLIDES

Some of the mobile phases suggested by Stahl (1959) were tried on microscope slides to determine which of them would give the best separation.

Technique: One-way ascending

Adsorbent: Silica Gel G/UV₂₅₄ for T.L.C. (Macherey Nagel)

- Mobile phases:
- I chloroform: diethylamine (90:10)
 - II cyclohexane: chloroform: diethylamine (50:40:10)
 - III chloroform: acetone: diethylamine (50:40:10)
 - IV methanol

Temperature: Room temperature (25°C)

Sample: Chloroformic extract of R. caffra alkaloids

Standard: 0.025% W/V solution of reserpine

Visualisation: Examination in screened u.v. light (short wavelength) followed by spraying with Dragendorff's reagent

Procedure:

A slurry was prepared by mixing 35 g of silica gel G/UV₂₅₄ with 100 ml. of chloroform/methanol solution (2:1) and shaken well before use. Two clean microscope slides were held back to back and dipped into the slurry. They were then withdrawn slowly and placed face-up on the bench to dry for about 10 minutes before spotting. Two spots, one of the standard and the other of the alkaloidal extract were spotted on the baseline and development done in small tanks. The results are summarised in table 5.

TABLE 5:

Summary of the results of trial T.L.C.

Mobile phase	Observation
I Chloroform: diethylamine (90:10)	Test mixture separated into three spots
II Cyclohexane: chloroform: Diethylamine (50:40:10)	No separation, the test mixture moved as a broad band
III Chloroform: acetone: Diethylamine (50:40:10)	No separation observed, the test mixture is seen as a broad tail.
IV Methanol	Test mixture and standard are both retained on the base line.

Mobile phase I (Chloroform: diethylamine (90:10) showed the best separation and was therefore chosen for use on larger plates.

T.L.C. ON 10 x 20 CM PLATES

Technique: One way ascending

Adsorbent: Silica Gel G/uv₂₅₄ for T.L.C. (Macherey Nagel)

Mobile Phase: Chloroform : diethylamine (90:10)

Temperature: Room temperature (25°C)

Sample: Chloroform extract of R. caffra alkaloids

Standard: 0.025% W/V solution of reserpine.

Visualisation: Observation in screened UV. light (short wavelength) followed by spraying with Dragendorff's reagent.

Procedure:

The slurry was prepared by thoroughly shaking 30 g of silica gel G/uv₂₅₄ with 60 ml. of distilled water. It was then cast on 10 x 20 cm plates using a Desaga spreader to produce a layer of 250 Microns thick. Excess water was allowed to evaporate and the plates then activated at 110°C for 30 minutes.

50 ml. of the mobile phase was poured into a development chamber into which saturation paper (Whatman No. I) was placed, the chamber covered and allowed to saturate for one hour before development was carried out.

The standard and test material were spotted on the activated layers and development carried out under conditions of subdued light since Rauwolfia alkaloids are photosensitive(18).

Visualisation was done by observing the plates under screened u.v. light and subsequently spraying with Dragendorff's reagent.

A copy of the document of a representative chromatogram is shown in figure 3. The hRf and Rx values of the separated alkaloids were calculated and the results summarised in table 6.

TABLE 6

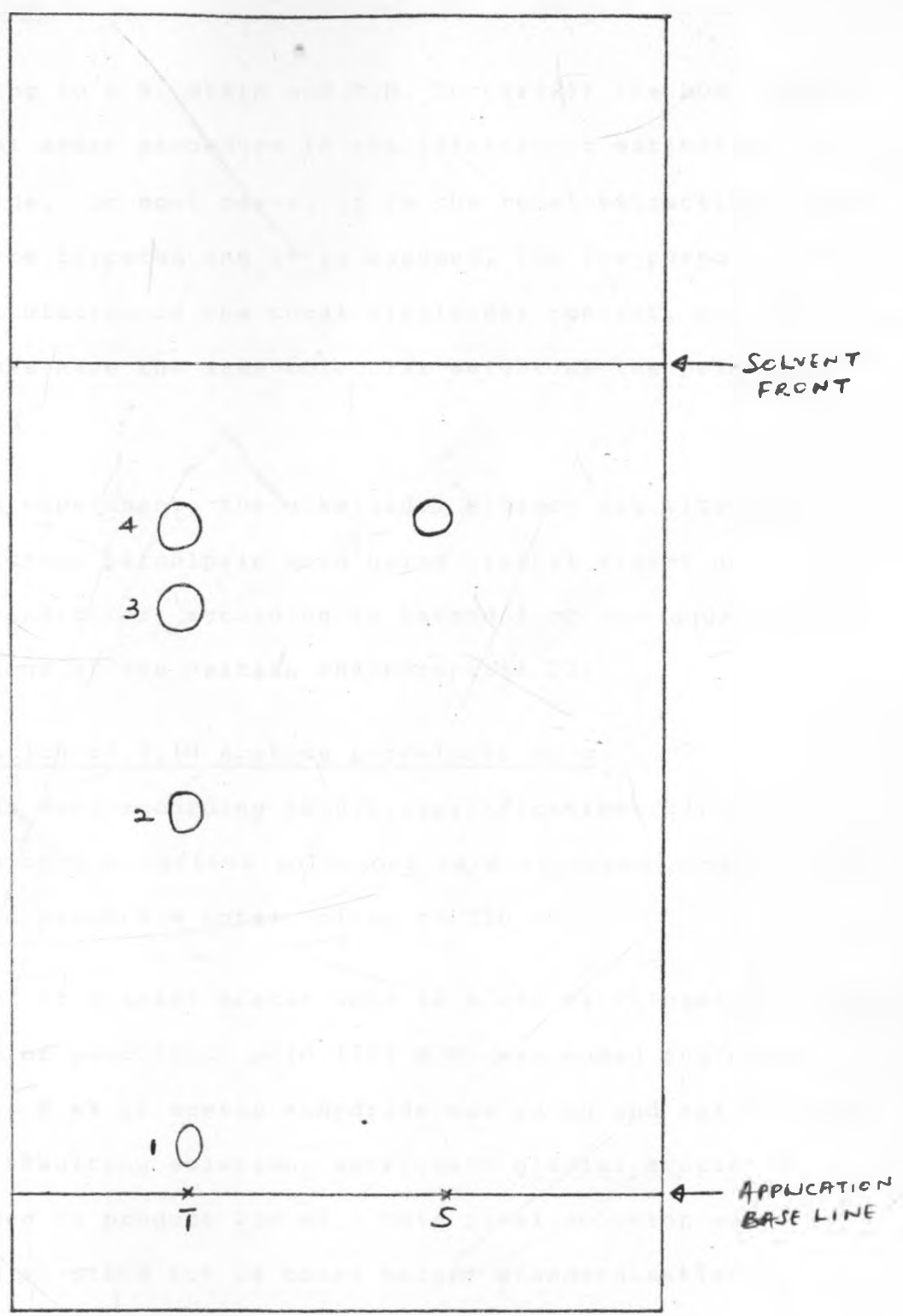
hRf and Rx values of the separated alkaloids
(Distance moved by solvent front = 12.8 cm)

Substance	Distance moved	hRf	Rx	Identity
Spot 1	0.7 cm	5.47	-	unknown
Spot 2	5.9 cm	46.09	-	unknown
Spot 3	9.1 cm	71.09	-	unknown
Spot 4	10.3 cm	80.47	1	Reserpine
Standard	10.3 cm	80.47	-	Standard Reserpine

The results show that under the conditions applied, the extract separated into four alkaloids out of which one can be positively identified as reserpine since it has Rx value of 1 with reference to reserpine. The other alkaloids could not be identified due to lack of reference standards.

FIGURE 3

Chromatogram of Rauwolfia coffee alkaloids



T = Alkaloid extract
S = Standard Reserpine

DETERMINATION OF TOTAL ALKALOIDAL CONTENT BY NON-AQUEOUS TITRATION

According to K.R. Brain and T.D. Turner(21) the most common chemical assay procedure is the titrimetric estimation of alkaloids. In most cases, it is the total extractible bases which are titrated and it is assumed, for the purposes of the calculation of the total alkaloidal content, that all the bases have the same molecular weight as the principle alkaloid.

In this experiment, the alkaloidal extract was titrated with acetous perchloric acid using crystal violet as a visual indicator, according to method 1 of non-aqueous titrations of the British Pharmacopoeia(22).

Preparation of 0.1N Acetous perchloric acid

This was done according to B.P. specifications(23). The volumes of the various solutions were adjusted proportionately so as to prepare a total volume of 250 ml.

To 225ml of glacial acetic acid in a 250 ml volumetric flask, 2.05 ml of perchloric acid (72% W/W) was added and mixed. To this, 8 ml of acetic anhydride was added and again mixed. To the resulting solution, sufficient glacial acetic acid was added to produce 250 ml. This final solution was allowed to stand for 24 hours before standardisation.

Standardisation of the acetous perchloric acid

The exact strength of the acetous perchloric acid was ascertained by titrating it with approximately 0.5 g (see actual figures table 7) of potassium hydrogen phthalate

(previously dried at 120°C for 2 hours) using method I of non-aqueous titration(22).

TABLE 7

WEIGHINGS

	Sample I	Sample 2
Weight of potassium hydrogen phthalate plus weighing bottle (g)	1.3609	1.3615
Weight of weighing bottle 'empty' (g)	0.8604	0.8619
Weight of potassium hydrogen phthalate (g)	0.5005	0.4996

TABLE 8

TITRATIONS

	Sample I	Sample 2
Final burette reading (ml)	23.30	46.50
Initial burette reading (ml)	0.00	23.30
Titre (ml)	23.30	23.20

PROCEDURE FOR STANDARDISATION

Each sample of potassium hydrogen phthalate was dissolved in 20 ml. glacial acetic acid previously dried by addition of acetic anhydride. The dissolution process was aided by the use of gentle heat. The solution was then titrated against the acetous perchloric acid to a blue-green end point using crystal violet indicator.

The results are summarised in table 8.

CALCULATION OF THE NORMALISING FACTOR

Sample 1

Titre = 23.30 ml.

1 ml. of 0.1N perchloric acid is equivalent to

0.020414 g $C_8H_5O_4K$ (23)

Hence 0.5005 g of $C_8H_5O_4K$ weighed out (table 7)

$$= \left(\frac{0.5005 \text{ g}}{0.020414 \text{ g}} \times 1 \right) \text{ ml. of } 0.1N \text{ HClO}_4 \text{ assuming } F=1$$

= 24.5175 ml.

$$V_1 F_1 N_1 = V_2 F_2 N_2$$

Where V_1 is the volume of 0.1N (N_1) perchloric acid of known factor ($F_1 = 1$), and V_2 is the volume of 0.1N (N_2) perchloric acid of unknown Factor (F_2)

$$\text{Hence } F_2 = \frac{24.5175}{23.3} = 1.052253$$

Sample 2

A similar calculation as for sample 1 gives a value of

$$F_2 = 1.0548879$$

Hence average normalising factor

$$= \frac{1.052253 + 1.0548879}{2} = 1.05357$$

TITRATION OF THE ALKALOIDAL EXTRACT WITH THE STANDARD ACETOUS PERCHLORIC ACID

10 g of Rauvolfia caffra whole root powder was weighed out accurately and extracted as described previously by continuous soxhlet extraction.

The final purified -

chloroform extract was evaporated to dryness in-vacuo and the residue dissolved in 40 ml. of glacial acetic acid previously dried using acetic anhydride(24). The resulting solution was then titrated with the standard 0.1N acetous perchloric acid using one drop of crystal violet indicator to a blue-green end-point. The same process was repeated on another 10g sample of R. caffra whole root powder. The results are summarised in table 9.

Titration Results (TABLE 9)

	Sample 1	Sample 2
Final burette reading (ml)	0.45	0.50
Initial burette reading (ml)	0.00	0.00
Titre (ml)	0.45	0.50

CALCULATION OF TOTAL ALKALOIDAL CONTENT

Sample 1

Titre = 0.45 ml.

Hence volume of 0.1N (F= 1.05357) acetous perchloric acid equivalent to total alkaloids = 0.45 ml.

But 1 ml of 0.1N acetous perchloric acid is equivalent to 0.06090 g total alkaloids calculated as reserpine(24)

Hence total alkaloids

$$= (0.45 \times 1.05357 \times 0.06090) \text{ g}$$

$$= 0.028873 \text{ g}$$

But this amount is present in 10 g of R. caffra whole root powder.

Hence percentage content of alkaloids in the powder

$$= \frac{0.028873}{10} \times 100 = 0.28873\% \text{ W/W}$$

Sample 2

A similar calculation as for sample 1 gives a total alkaloidal content of 0.3208% W/W

Hence average total alkaloidal content

$$= \frac{0.28873 + 0.3208}{2}$$

$$= \underline{0.3048\% \text{ W/W calculated as reserpine}}$$

DETERMINATION OF RESERPINE CONTENT BY U.V. SPECTROPHOTOMETRIC ASSAY

The reserpine content of the alkaloidal extract was estimated by determining the absorbance of an alcoholic solution of the extracted alkaloids and reading off the reserpine concentration from a calibration graph prepared using pure crystalline reserpine. Reserpine in absolute ethanol has a characteristic u.v. absorption with lambda max. at 268 nm. (10) The method described here is adopted in view of the fact that other Rauvolfia alkaloids likely to be present in the extract absorb at different wavelengths and do not therefore interfere with u.v. absorption of reserpine at 268 nm as shown in table 10(10).

TABLE 10

Absorbance of some Rauvolfia alkaloids in alcoholic solutions

Alkaloid	lambda max (nm)
Ajmaline	252, 291
Ajmalicine	225, 275, 282, 290
Rescinamine	228, 303
Reserpine	235, 268, 299
Serpentine	253, 308, 360

Preparation of the calibration graph

0.04 g of pure crystalline reserpine (sigma chemical company) was weighed out accurately into a 100 ml volumetric flask. It was then dissolved in absolute ethanol and the volume made up to the mark using the same solvent. A series of solutions of increasing concentration were then prepared by pipetting

specific volumes of the stock solution into 10 ml volumetric flasks and making up to the mark using absolute ethanol. The absorbances of the final solutions were read off at 268 nm using spectronic 21 (Bausch & Lomb). The results are summarised in table 11 and graphically represented on the attached graph paper. (figure 4)

TABLE 11

Absorbance of standard reserpine solutions in absolute ethanol

Volume of stock Solution pipetted (ml)	Concentration after dilution to 10 ml (mg/ml)	Absorbance at 268 nm
0.5	0.02	0.51
1.0	0.04	0.98
1.5	0.06	1.44

Preparation of the ethanol extract of the alkaloids

10 g of R. caffra whole root powder was accurately weighed out and extracted as previously described. The final purified chloroformic extract was evaporated to dryness in-vacuo and the residue dissolved in 200ml of absolute ethanol. The absorbance of the resulting solution was read off at 268 nm using spectronic 21 (Bausch and Lomb).

The corresponding concentration of reserpine was read off from the calibration graph.

Calculation of Reserpine Content

The absorbance of the alcoholic extract = 1.1.

From the calibration graph, this corresponds to a concentration of 0.045 mg/ml.

Hence total reserpine present in 200 ml of the

absolute ethanol solution

$$= 0.045 \text{ (mg/ml)} \times 200 \text{ ml}$$

$$= 9 \text{ mg}$$

$$= 0.009 \text{ g}$$

This amount of reserpine is present in 10 g of R. caffra whole root powder (weighed out).

Hence % reserpine content

$$= \frac{0.009 \text{ g}}{10 \text{ g}} \times 100 = \underline{0.09\% \text{ W/W}}$$

From the previous calculation (Page 29) the total alkaloid content calculated as reserpine was found to be 0.3048% W/W

Hence in a 10 g sample of R. Caffra powder, the weight of total alkaloids will be

$$\frac{0.3048}{100} \times 10 = 0.03048 \text{ g}$$

The content of reserpine = 0.09% W/W (see above)

Hence in a 10 g sample, the weight of reserpine

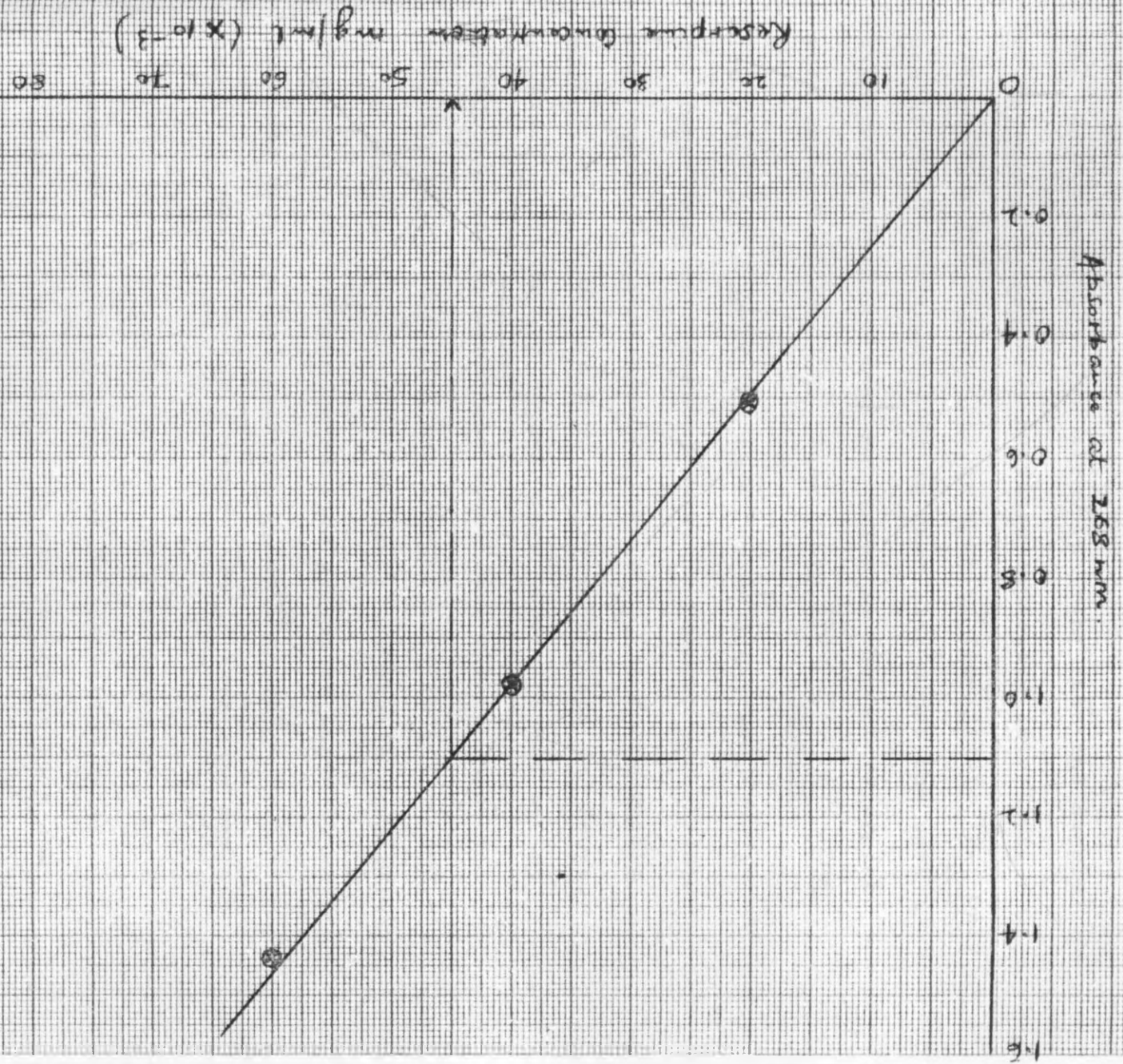
$$= \frac{0.09}{100} \times 10 = 0.009 \text{ g.}$$

Hence the amount of reserpine as a percentage of the total alkaloids

$$= \frac{0.009 \text{ g}}{0.03048 \text{ g}} \times 100$$

$$= \underline{29.5\% \text{ W/W}}$$

FIGURE 4 : CALIBRATION GRAPH FOR DETERMINATION OF RESERPINE CONTENT



DISCUSSION OF RESULTS AND CONCLUSION

The results of the T.L.C. study indicate that the sample of Rauwolfia caffra root examined separated into four alkaloids under the conditions applied (see figure 3). Only one of these was identified as reserpine since the spot numbered (4) has the same R_f value as that of reserpine pure reference standard. (see table 6)

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The reserpine content was found to be 0.09% w/w. This is higher than the value reported by McAleer *et. al.* (8). They reported a reserpine content of 0.01% in Rauwolfia caffra roots collected from Northern Rhodesia and Kenya. (Table 1). It is also higher than that reported by LOS and Court, and by Habib and Court (see tables 2 and 3 respectively). The last two sets of researchers worked on material collected from Transvaal and Zambia respectively. The alkaloidal content in various plants is known to vary from one geographical location to another and even from season to season, hence a comparison in this case may not be justified.

Sources of error in this kind of determination exist. Reserpine and related alkaloids are photosensitive and it is not possible to strictly protect all solutions from light, right from the extraction procedures upto the assay. The error is even more magnified when the reserpine content is to be determined using solutions eluted from preparative T.L.C. plates. Not only are the samples susceptible to degradation during development, but efficient and reproducible separation is necessary for

accuracy.

If spectrophotometric methods of assay are employed to determine the reserpine content in such eluted solutions, it is essential to prepare blanks to account for the absorption of the material that may be extracted from the adsorbent. Due to this proneness to degradation, such procedures as extraction, elution and spectrophotometric assays have to be standardised and carried out fairly fast and this calls for expertise.

The method employed in this project, on the other hand, avoids elution from preparative T.L.C. plates but the possibility of interference by other absorbing material at the chosen wavelength, however small, can not be ruled out.

The total alkaloidal content was found to be 0.3048% W/W. The B.P.C. 1973 sets the limit of the content of reserpine-like alkaloids in R. vomitoria at not less than 0.2% and in R. serpentina at not less than 0.15%. These two unfortunately are the only species of *Rauvolfia* recognised by the B.P.C. as official plant sources of reserpine.

Rauvolfia caffra Sond growing in Kenya qualifies, on the strength of this piece of project work, to be included in the official compendia as a commercial plant source of reserpine-like alkaloids.

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