A COMPARATIVE PHARMACOGNOSTICAL STUDY OF CINCHONA LEDGERIANA MOENS AND CINCHONA SUCCIRUBRA

BY

PETER JAMES OWINO

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FACULTY OF MEDICINE,
DEPARTMENT OF PHARMACY,
UNIVERSITY OF NAIROBI.

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C O N T E N T S

A B S T R A C T

The aim of this project was to compare two pecies of Cinchona, namely Cinchona Ledgeriana and Cinchona Succirubra.

This entailed examing the drugs microscopically as well as evaluating the content of the principle alkaloid Quinine.

Microscopically there was very little difference observed between the two species.

The bark of Cinchona Ledgeriana had a total Alkaloid content of 8.02% o which 5.14% was found to be Quinine. For Cinchona Succirubra the total alkaloid content was 6.51% of which 1.68% was found to be Quinine.

It was found that whereas the amount of Quinine in Cinchona Ledgeriana represented more than 60% of the total alkaloids the amount of Quinine in Cinchona Succirubra represented only 25% of the total alkaloids.

" QUININE REMAINS.... THE KEYSTONE IN THE TREATMENT OF MALARIA"

- TYAGI N.

I N T R O D U C T I O N

For over 300 years the word 'Cinchona' has been synonymous with Malaria, which for centuries had been a major scourge and still continues to remain so in most parts of the world today.

Malaria was first mentioned in the ancient Egyptian papyri, the Elbus Papyrus of 1550 B.C. The cause and cure of malaria had eluded people for more than three thousand years and it was not until the 19th century that the actiology of malaria was understood.

The movement of the then powerful Spanish Armada's to what was to become known as the 'New World' completely revolutionised medicine in that the vast virgin forests of the Andes had within them a rich collection of medicinal flora. It has been argued that of the discoveries the greatest of them all was the 'discovery' of Cinchona and according to Ramazzini (1633 - 1717), 'Cinchona produced a revolution in medical treatment comparable to the introduction of gunpowder in warfare' (5) No choice of words could best describe this discovery as malaria was by then the most dreaded killer. It has been credited with killing more people than all wars and plagues and threatened successive civilisations with collapse. Malaria still retains this distinction to some extent today as according to the world Health Organisation statistics it is resopnsible for about three million deaths annually.

The first comprehensive description of Cinchona is credited to Jesuit priests among them Antonio de la Calancha and his companion Bernabe Cabo who introduced the bark by then known as 'Jesuits Bark' to Europe before 1633.

The introduction of the bark in Europe initially met with opposition from many European physicians in the continent who resisted the new remedy partly because it was not sponsored by their profession and also because of hostility towards the Jesuits as a whole particularly in Britain where Sir Robert Talbor the self-styled 'Feverologist' treated many famous cases of malaria only to be revealed later on that his secret potion was indeed none other than the Jesuits bark causing extreme embarassment to the Royal College of Physicians which had vigorously campaigned against the bark as a remedy for malaria (8).

One man who single handedly fought for the acceptance of Cinchona bark was Cardinal Joseph Lugo and after the Jesuit congress of 1649 initiated by him, the Jesuits started using the bark in their mission hospitals all over the world.

The importance of Cinchona bark thus grew rapidly and by 1677 it was intoduced in the London Pharmacopoeia as 'Cortex Peruanus'.

The botanical genus was first established by Linnaeus in 1742 who also name the three Cinchona apparently in honour of the Countess of Chinchon, the wife of the Spanish viceroy of Peru who had reportedly been cured of Malaria by the bark, a belief that was later disputed as a mere legend(5,8).

The Cinchona bark consists of various species, races, hybrids of Cinchona, family Rubiacae. The bark of various species of Cinchona yield a number of closely related alkaloids belonging to the Quinoline group (3).

Joseph Pelletier and Joseph Caventous in September 1820 discovered Quinine, the alkaloid in Cinchona bark reponsible for the cure of malaria. This discovery revolutioned the treatment of malaria as it was now possible to give a measured dose of the pure alkaloid instead of the intensly bitter decoction.

The Cinchona trees are large evergreen trees sometimes reaching a height of 100ft with a trunk diameter of upto 0.6 metres at the base. They are indigenous to Columbia, Peru, Bolivia and Equador where they grow at an altitude of 1000-3000 metres. There are over 36 known species and hybrids of Cinchona and they are widely cultivated in large plantations in the far-east and some parts of Africa.

The admission of Cinchona in the London Pharmacopoeia in 1677 and its importance as the only antimalarial increased its demand astronomically leading to its extensive cultivation by the Dutch and British in their vast colonies in India, Ceylon, Java, Indonesia, Malaya, Singapore and later in Tanganyika.

The introduction of Cinchona seedlings in the Dutch East Indies in 1865 by Charles Ledger was an important landmark in the cultivation of these trees as the natural forests of South America were rapidly loosing their large reserves of the trees since no care was taken to ensure the replacement of the felled trees forcing the Bolivian government to ban the collection of

Cinchona seeds for export. However, the cultivation of these trees was so rapid that by 1882, India alone had 4,731,608 trees according to Dymcock et al (3). The distribution of these trees were as follows:-

Cinchona Succirubra	-	3,873,285
Cinchona Calisaya	-	566,695
Hybrids	-	291,628

This trend continued and by 1941 the Dutch East Indies were producing 1017 tons which was about 90% of the worlds supply of Quinine thus giving the Dutch a virtual monopoly in the trade. (5)

The chief species yielding commercially important barks are C. Officiralis L. (pale or crown bark); C. Calisaya WEDD (yellow bark); C. Ledgeriana MOENS; C. Succirubra PAVON; C. Lancifolia MUTIS; C. Nitida RUIZ et PAVON; C. Micaranth RUIZ et PAVON; and C. Peruviana HOWARD (2).

Cultivation and Collection:-

The most widely cultivated species of Cinchona are C. Succirubra and C. Ledgeriana. These two do not increase much in alkaloidal value after six years of age and are therefore not barked when young. Ledgers are known to thrive best at altitudes between 2000 - 3000 feet and require quite an amount of sunshine.

According to Cromwell (2) the alkaloids are present in the cortical tissues of the stems and the roots of plants of all ages and have also been isolated in small quantity from the seeds of some species.

The alkaloids first appearing in young plants and in leaves and twigs have been found to be in an amorphous state but as growth continues they become crystalline. Hence it is possible that the latter are produced from the former.

There are several methods or systems of collecting or harvesting the bark. These are:-

a) The coppicing system - This involves leaving the tree to grow for seven to eight years after which it is cut down and the bark removed. After this, adventitious shoots are allowed to grow from the stool and a bushy growth encouraged. Fine quills of bark are removed from the shoots and eventually the tree is dug out and the root bark which is rich in alkaloids removed.

b) The Uprooting system - This is the most economical and by far the most widely used. After a period of six or seven years of growth the trees are thinned out and the bark collected from roots and stems. This process of thinning out is continued each subsequent year until all the plants have been removed after which the ground is then replanted.

c) The Mossing system - This system was introduced in 1863 to obviate the necessity of felling old trees. It involves the removal of narrow strips of bark followed by covering of the wounds with moss or other protective material. Cambial activeity then leads to the production of a new bark rich in alkaloids. However, this system proved to be very expensive and is rarely used nowadays.

The trees are preferably barked during the rainy season when the bark 'lifts' or is more easily removed.

According to the British Pharmaceutical Codex ('73) commercially available stem barks are quilled or curved pieces of length of about 30 cm or more and having a thickness of about 2 - 6 mm. The outer surface are usually grey or brownish-grey frequently bearing lichen or mosses.

Depending on the species the outer surface is usually rough with transvers fissures or may be longitudinally wrinkled. The inner surface is usually striated and varying in colour. It has a characteristic odour and an intensly bitter and astringent taste.

Microscopical Characteristics

Though there are many species and hybrids of Cinchona they share similar microscopical structures. (9)

These consist of the outer cork which contain several layers of thin walled cork cells arranged in regular radial rows and appearing polygonal in surface view. However, it is worthy to note that in some of the commercially available quills the cork layer may be missing.

The phelloderm within the cork combium is made up of several layers of regular cells with dark walls. Next to this region is the cortex which is composed of tangentially elongated thin walled cells containing reddish-brown

amorphous matter or small starch grains. Also scattered within the cortex are idioblasts containing sphenoidal microcrystals of calcium oxalate.

The medullary rays are radially elongated. The Phloem consists of narrow sieve tubes showing transverse sieve plates with Phloem Pareuchyma resembling that of the cortex.

However, the distribution and size of the Phloem fibres differ in various species. The Cortical parenchyma of the stem and roots contain an abundance of alkaloids (2).

Constituents:-

Cinchona barks vary in amount of total alkaloids of which more than twenty alkaloids have been isolated and characterized. (2) However, majority of these alkaloids are amorphous and non-crystallizable. These are collectively known as 'Quinoidine', a term that is strictly applicable only to the residue left after the removal of all the major alkaloids.

The major alkaloids of Cinchona species are four, namely Quinine, Quinidine, Cinchonine and Cinchonidine. These four alkaloids are infact two pairs of Diastereoisomers (Fig. 2).

Quinine is perhaps the most important and widely known. The proportion of Quinine remains fairly constant for each species averaging 70 - 80% in Ledgers, 60 - 70% in Officinalis and 20% in Succirubras (3). The total alkaloidal content usually ranges from 5 - 10%.

The other minor alkaloids that have been isolated from various species are Quinicine, epi- Quinine, epi-Quinidine, Hydroquinine, Hydroquinidine, Cincotine, Cinchamidine, Cinchonamine, Cupreine, Quinamine, Conquinamine, Paricine, Aricine, Cusconine, Concusconine, Chairamine, Conchairamine, Chairmidine, Conchairamidine (2).

The alkaloids exist in combination with quinic acid $C_7H_{12}O_6$ and to a lesser extent with Cinchotannic acid $C_{14}H_{16}O_9$. The content of quinic acid varies from 5 - 8% and Cinchotannic acid from 3 - 4%.

Cinchotannic acid has been identified as the astringent principle of the Cinchonas. It is soluble in water and easily recipitated by acids and gives a green colour with Ferric Chloride. In addition to these, Cinchonas contain a glycoside quinovin, cinchona red, quinovic and oxalic acids, starch,

colouring matters, waxes and fats. (3)

Pharmacology:-

Of all the Cinchona alkaloids only Quinine and Quinidine have found useful medical applications.

Quinine is widely referred to as a 'general protoplasmic poison' and apart from its anti-malarial and anti-febrile properties it also acts on various body organs.

As an antimalarial, Quinine is schizonticidal i.e. it destroys the asexual erythrocytic forms of the parasite. Thus, it is effective in treating acute clinical attacks of malaria. It can also give supressive cure but it is not recommended for proylaxis of malaria unless the synthetic anti-malarial e.g. Proguanil, Pyrimethamine etc. are unavailable.

With the advent of the synthetic anti-malarials just before world war II, the role of quinine has greatly been diminished. This is mainly because of its toxicity and necessity of frequent administration. However, the emergence of strains of malarial parasites resistant to Proguanil, Mepacrine, Pyrimethamine and recently Chloroquine will most likely result in quinine being restored to its former position of prominence. Another factor that will most likely contribute to its resurgence is its cost. It is much cheaper and this is a distinct advantage particularly in the developing countries.

On the uterus, quinines causes contractions and in high doses it is an abortifacient. It has curare-like effects on skeletal muscles and antagonises the actions of Physostigomine on skeletal muscles as effectively as Curare. It has thus been used successfully in the treatment of Night Cramps(14).

Quinine also has analgesic properties and relieves muscle and joint pains. Due to its intense bitter taste it is sometimes used as a bitter.

Quinidine on the other hand is used in the treatment of Paroxysmal Ventricular tachycardia, and of atrial fibrillation of recent origin in the absence of congestive cardiac failure. Quinidine acts by prolonging the refractory period of cardiac muscle therefore reducing the rate at which succesive contractions can take place.

It is usually formulated as the sulphate or bisulphate. Severe poisoning by Quinine or any of the Cinchona alkaloids gives rise to a wide

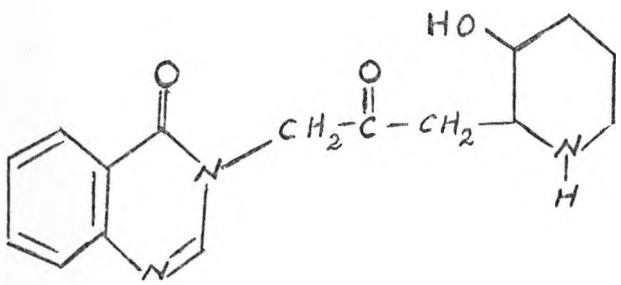
variety of toxic effects known as 'Cinchonism'. It is characterised by headache, fever, vomiting, excitement, confusion, blindness, deafness and loss of consciousness. Death usually occurs as a result of Respiratory failure.

The only other alkaloid that has been found to be effective against malaria is Febrifugine (below) obtained from the ancient Chinese drug Chan'g Shan.

Chan'Shan consists of powdered roots of plants of the Saxifragaceae family in particular Dichroa febrifuga but the bilogically active principle has also been isolated from species of Hydrangea (5).

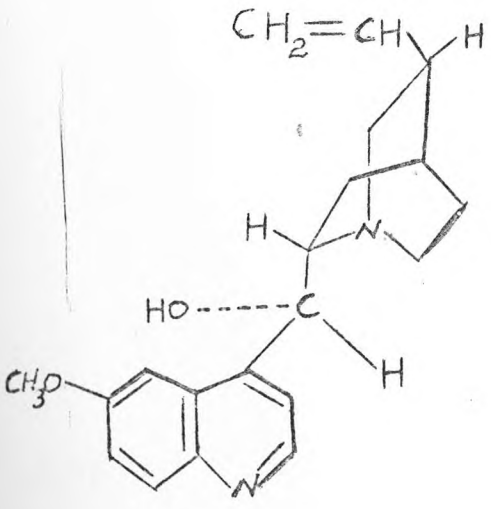
However, Febrifugine was found to be effective only against P.Vivax and P. Falciparum. (Tsu '47, Coatney '50) and the theapeutic indices were also found to be low.

Fig I

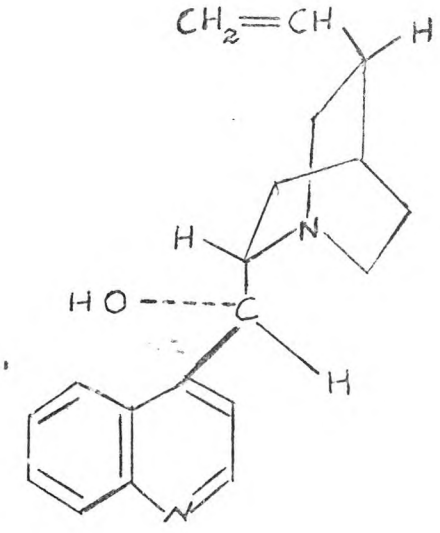


FEBRIFUGINE

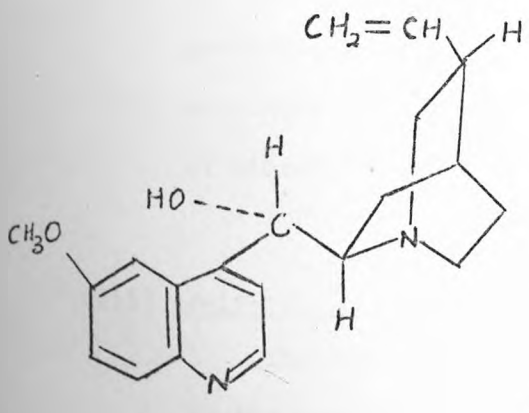
FIG. 2: STRUCTURAL RELATIONSHIPS OF THE FOUR MAJOR CINCONA ALKALOIDS



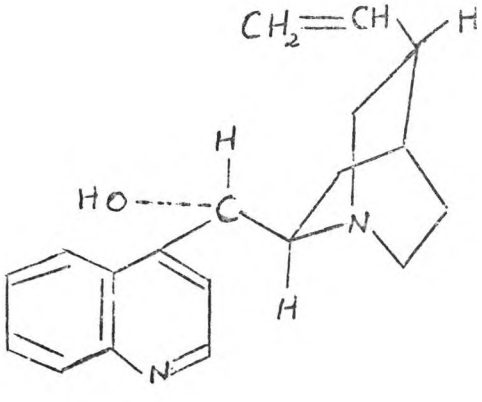
(a) QUININE



(b) CINCHONINE



(c) QUINIDINE



(d) CINCHONIDINE

EXPERIMENTAL WORK AND RESULTS

i) Collection of Plant Material

The samples of Cinchona Ledgeriana bark used originated from the Brocke-Bond plantations situated at Kericho, Kenya. The bark was aquired by the Department of Pharmacy in 1980.

The samples of Cinchona Succirubra bark was also aquired by the school of Pharmacy in 1980 from South America but the exact locality is unknown.

(ii) Macroscopical and Microscopical Examination of the Barks

a) Cinchona Succirubra - Gives a red-brown powder with a characteristic smell. It has a bitter and somewhat astringent taste.

b) Cinchona Ledgeriana - Gives a light-brown powder having a similar taste and smell to that of Cinchona Succirubra.

A transverse section of each bark was examined under both low and high magnification and the tissue organisation and structure recorded.

The powdered bark was also examined microscopically under high power (x400) and various structures identified. Microchemical tests were also done using Iodine and Phloroglucinol to detect the presence of starch and lignified fibres respectively.

(iii) Moisture Content Determination

The determination of the moisture content of each bark was done by the Gravimetric method. One gram of the finely powdered bark was accurately weighed in a petri dish and then dried in an incubator to a constant weight at 105°C. The percentage moisture content of Cinchona Ledgeriana and Cinchona Succirubra were found to be 14.5% and 12.3% respectively.

(iv) Preparation of the Alkaloidal Bark Extract

The General procedure for the extraction of alkaloids (1) was adopted for extraction of the alkaloids as free bases.

About 10 grams of the ground drug was moistened with water and Ammonium hydroxide (20ml) mixed and left to stand for about 20 minutes with occassional stirring. This was then filtered using glass wool. 25ml of choloroform was then added to the filtrate and after agitation the aqeous phase was discarded.

The Chloroform phase was then extracted with 20ml of 1% dilute Sulphuric acid in a separator. The Chloroform layer containing Resins and Fats was discarded and the aqeous acidic phase containing the alkaloidal salts made alkaline with dilute Ammonium Hydroxide.

The Alkaloids were then extracted with successive 10ml protions of chloroform. The Chloroform layers were then combined and then reduced to a volume of about 20ml by slight heating.

Qualitative reactions of the Extracts

2ml of the extract was placed in an evaporating dish and evaporated to dryness. This residue was then dissolved in 1ml of 1% Sulphuric acid and the solution tested for the presence of alkaloids.

(i) Mayers Reagent: About 0.5ml of the above solution was taken and 2 - 3 drops of Mayers reagent addes. A whittish-brown precipitate was obtained indicating the presence of alkaloids.

(ii) Dragendorff's Reagent: 0.5ml of the the solution was taken and 2 - 3 drops of Dragendorff's reagent added. An Orange-red precipitate was obtained thus indicating the presence of alkaloids.

(iii) Thalleioquin Test: This is the specific test for the determination of the presence of Quinine.

2ml of the extract was evaporated to dryness and the residue taken up in about 1ml of dilute acetic acid. One ml of water was added. Followed by 2-3 drops of Bromine water and the mixture shaken. A sharp green colouration was produced on addition of a few drops of concentrated ammonia. When the solution was neutralised slowly with dilute Sulphuric

acid a bluish colour was observed with slowly turned red as more acid was added.

This test confirmed the presence of Quinine in both Cinchona Ledgeriana and Cinchona Succirubra bark extracts.

(v) Separation of Cinchona Alkaloids by Thin Layer Chromatography

In these studies bark extracts from both C. Ledgeriana and C. Succirubra (prepared according to method outlined above) were compared under similar conditions using various solvent systems. The slurry was prepared by the method outlined in Appendix I. In all cases Silica Gel (MN - Kreselgel G/UV 254) was used and the spotting reagent was methanol: concentrated sulphuric acid (9:1) which gave intense blue-violet spots under U.V. light. This reagent was found to be much more superior to Dragendorff's reagent during preliminary investigation work.

Reference standards of Quinine and Quinidine were prepared from Quinine Sulphate and Quinidine Sulphate tablets respectively (see app.II) The asceding technique was used on the 10 x 10 chamber after allowing for a chamber saturation time of about 15 minuts. (see Tables 1 - 4).

(vi) Determination of the Total Alkaloid Content

The estimation of the total alkaloid content of each bark was carrie out by the titrimetric method adopted from Loustalot and Pagan by B.T. Cromwell. (2).

2 grams of the finely powdered bark was weighed into a 100ml beaker and about 0.5g calcium hydroxide added. A smooth homogenous paste was made using about 7 - 10ml of water and allowed to stand for 10 minutes. The paste was then transferred to a 200ml volumetric flask with 100 - 150ml ethanol, shaken vigorously and then allowed to stand for one hour with occassional shaking. The flask was then made to volume with ethanol and then filtered. 100ml of the ethanolic bark extracted (representing one gram of the bark) was then pipetted into a 400ml beaker.

10ml of 0.1N hydrochloric acid and 100ml of distrilled water were then added and the excess acid was then titrated to $_{p}H$ 6.2 with 0.05N Sodium hydroxide using a $_{p}H$ meter. Two determinations were carried out. A blank determination was then done by titrating the ethanol and acid with the Sodium hydroxide.

The total alkaloid content was calculated by using the equivalence given below (2).

1ml 0.05N NaOH = 0.0155g of total alkaloid

Results:

C. Succirubra:- Titre difference found were 4.1ml and 4.3ml.

∴ Average = $\frac{4.1 \quad 4.3}{2}$

= 4.2ml

∴ Amount of Alkaloids = 4.2 x 0.0155g

= 0.0651g

% Alkaloid content = 6.5%

C. Ledgeriana:- Titre difference obtained were 5.15 ml and 5.20ml

∴ Average = $\frac{5.15 \quad 5.2}{2}$

= 5.175

∴ Amount of alkaloids = 5.175 x 0.0.55g

= 0.08021g

% Alkaloid content = 8.02%

(vii) The Isolation and Determination of Quinine

The extraction of this major alkaloid was done according to the general principles for the isolation of the crystallirable alkaloids of Cinchona. The amount of Quinine was determined gravimetrically both as the free base expressed as a percentage ratio of the total alkaloid, content determined previously.

12g of the finely powdered bark was throughly mixed with about 4g of calcium hydroxide and the mixture made into a stiff paste with a sufficient quantity of 5% solution of sodium hydroxide. The paste was

then transferred to a soxhlet extractor and continously extracted with Benzene for about 10 hours.

The alkaloids were then extracted from the benzene by agitating the organic solvent with successive quantities of dilute sulphuric acid at 60°C until extraction was complete. The combined acid extracts were adjusted to $_{p}H$ 6.5 while still warm with dilute sodium hydroxide and the solution cooled. Crystals of the neutral Quinine sulphate were then freed from Cinchonidine and Cinchonine by repeated recrystallization using hot water and the crystals dried and weighed.

To prepare the base i.e. Quinine, the crystals of Quinine Sulphate were dissolved in dilute sulphuric acid with slight heating to effect dissolution and the solution made slightly alkaline by adding dilute ammonia with continous stirring. An amorphous precipitate was readily formed which gradually became crystalline in nature. The crystals were then washed with water and dried at 40°C. The weight of the crystals was then determined.

Results:

a) (i) C. Succirubra

Weight of Quinine Sulphate - 02331g

Amount of Quinine Sulphate obtained from 1g of bark

$$= \frac{0.2331}{12}$$

$$= 0.0194g$$

Amount of Quinine obtained from 12g of bark - 02019g

This is equivalent to 0.01682g/g of bark.

% Quinine content of total Alkaloids

$$= \frac{1.68}{6.51} \times 100$$

$$= 25.81\%$$

% w/w Quinine content in Succirubra Bark = 1.68%

(ii) C. Ledgeriana

Weight of Quinine Sulphate obtained - 07379g

1g of bark should yeild - $\frac{0.7379}{12}$ = 0.06149g

This is equivalent to 6.149%

Amount of Quinine obtained from 12g of bark - 0.6167g

This is equivalent to 0.05139g/g of bark

% Quinine content of total Alkaloids

$$= \frac{5.139}{8.02} \times 100$$

$$= 64.1\%$$

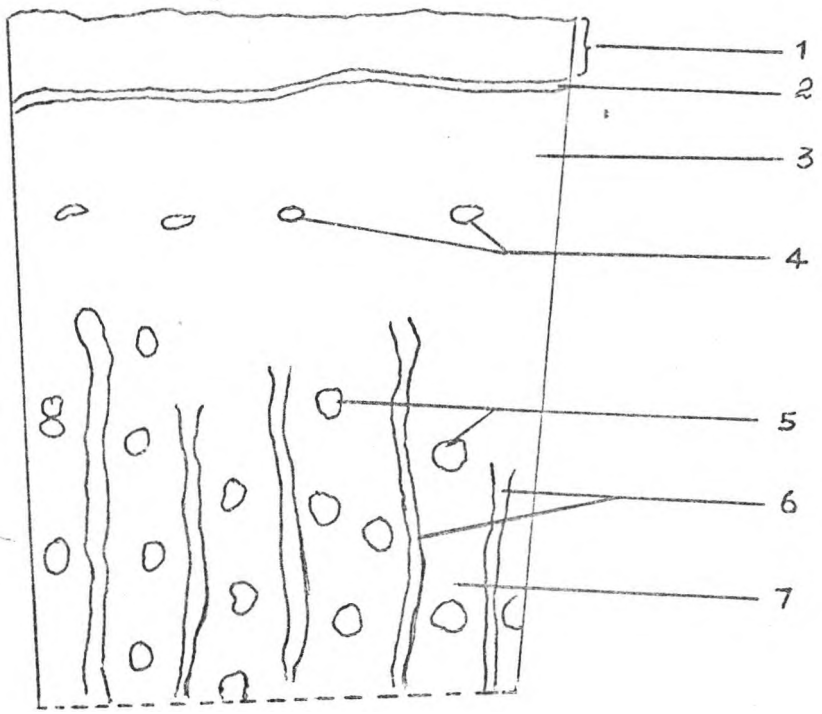
% w/w Quinine content in Ledgeriana bark = 5.14%

b) Melting point range of the Quinine Sulphate crystals obtained were 209 - 213°C.

c) Optical rotation - Quinine was found to be levorotatory

1. MICROSCOPICAL STUDY OF CINCHONA BARK

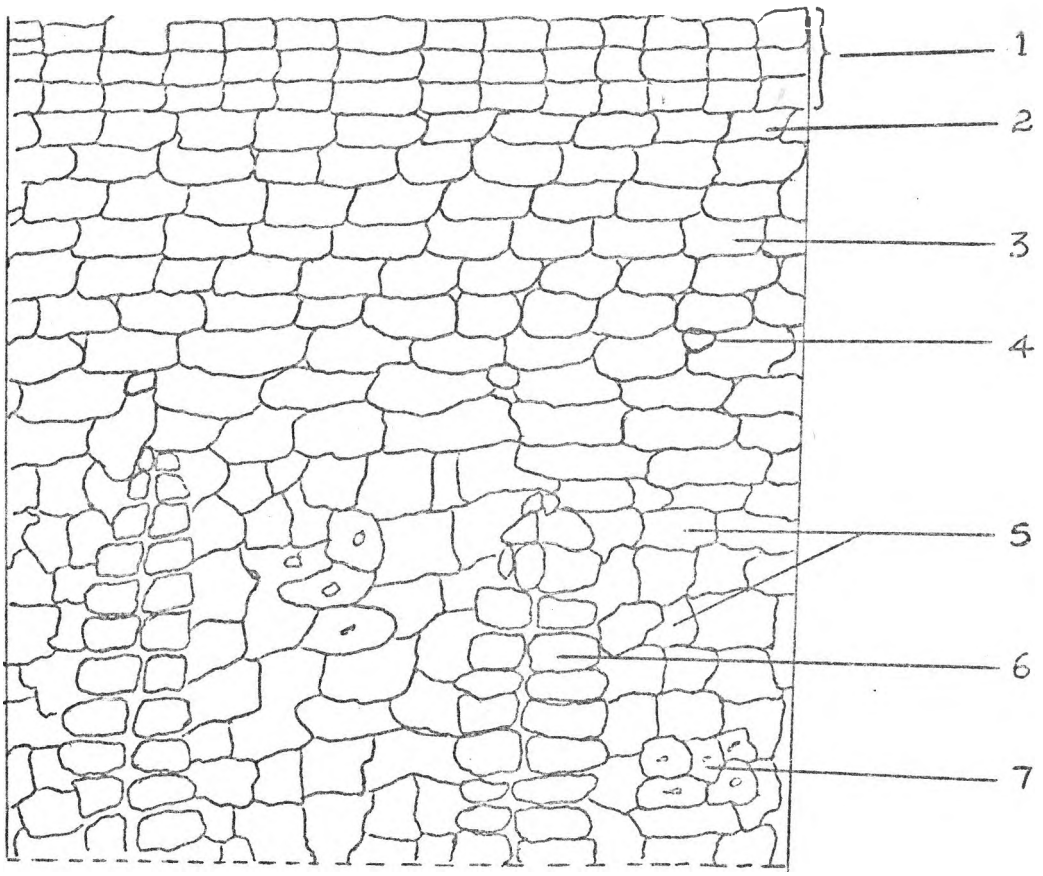
(a) Transverse section (low power) - regions of Differentiation



KEY:

1. Cork
2. Phellogen
3. Cortex
4. Secretion ducts
5. Bundle of Phloem fibres
6. Medullary ray
7. Phloem parenchyma

(b) T.S. C. Ledgeriana and C. Succirubra (high power)

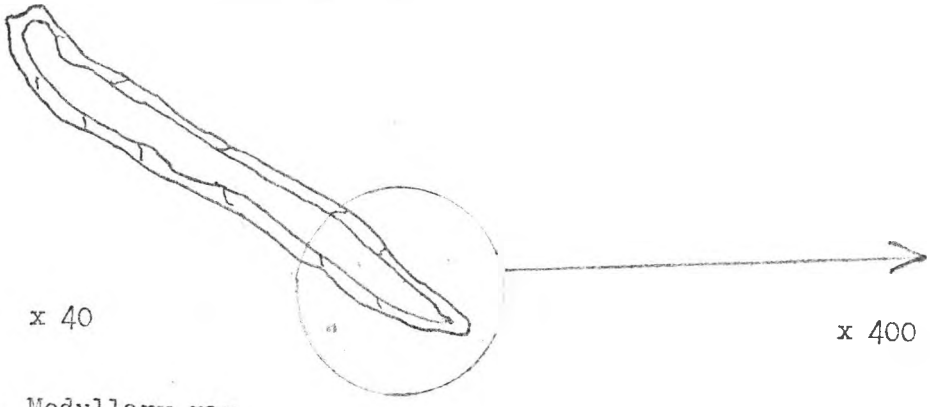


KEY:

1. Cork
2. Phellogon
3. Cortex
4. Secretory duct
5. Phloem parenchyma
6. Medullary ray
7. Phloem fibre

II. CHARACTERISTIC FEATURES OF POWDERED CINCHONA BARK

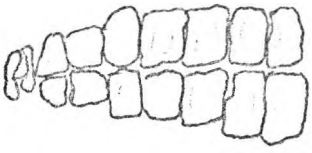
a) Bast Fibres (+ + +)



x 40

x 400

b) Medullary ray



Frequency = + +

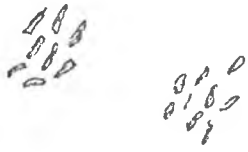
c) Cork (Sectional view)

Frequency = +

d) Starch granules

Frequency = +

d) Calcium Oxalate (C. Succirubra only).



Frequency = + +

KEY

+ + + - ABUNDANT

+ + - MODERATE

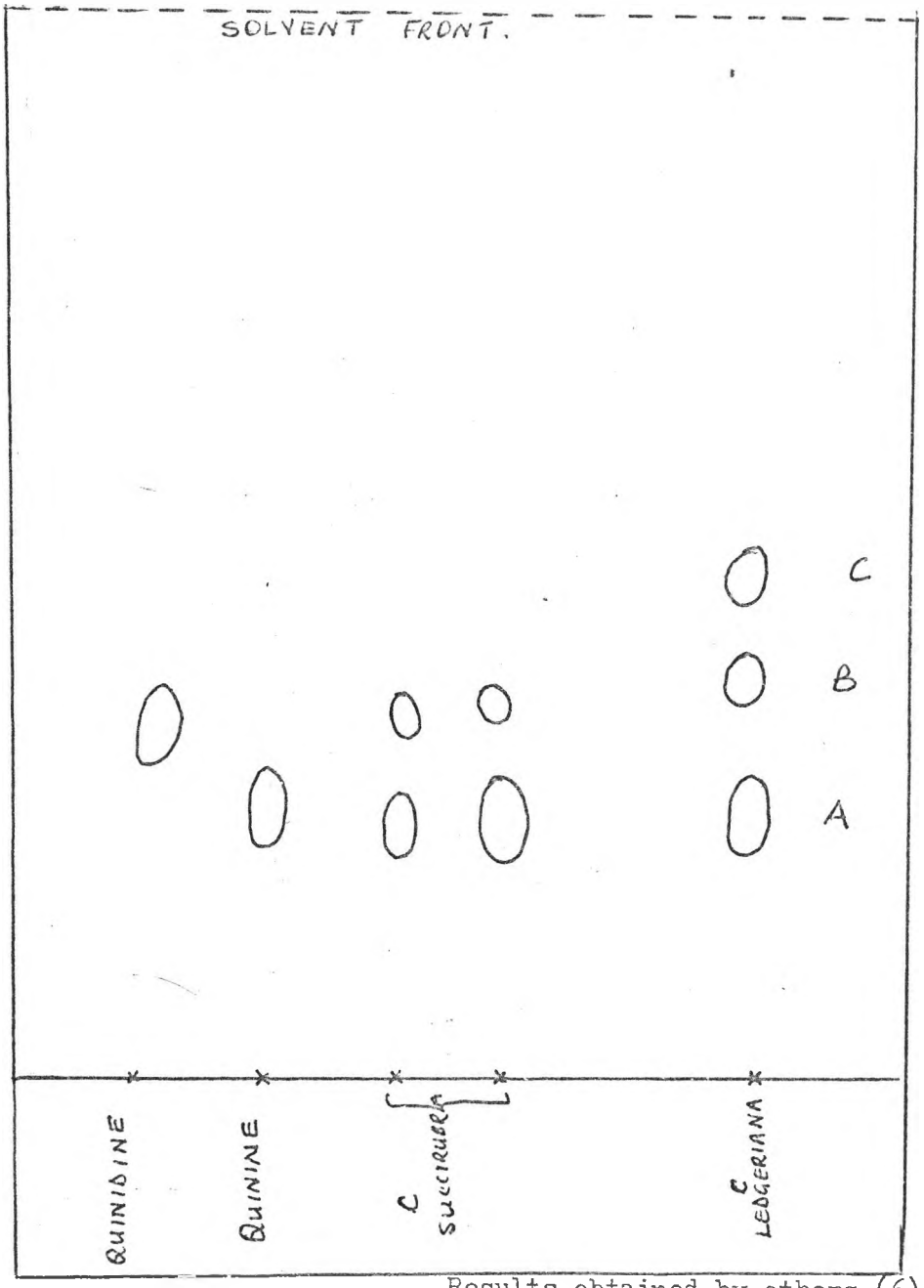
+ - SCARCE

T.L.C RESULTS

a) Table 1

Solvent Systems: Chloroform: Diethylamine (90:10)

	hRf Values			References	
	SPOT A	SPOT B	SPOT C	QUININE	QUINIDINE
C. Succirubra	23.9	35.2	-	25.4	33.1
C. Ledgeriana	24.7	37.3	46.5	-	-



Results obtained by others (6)

Quinine - 26

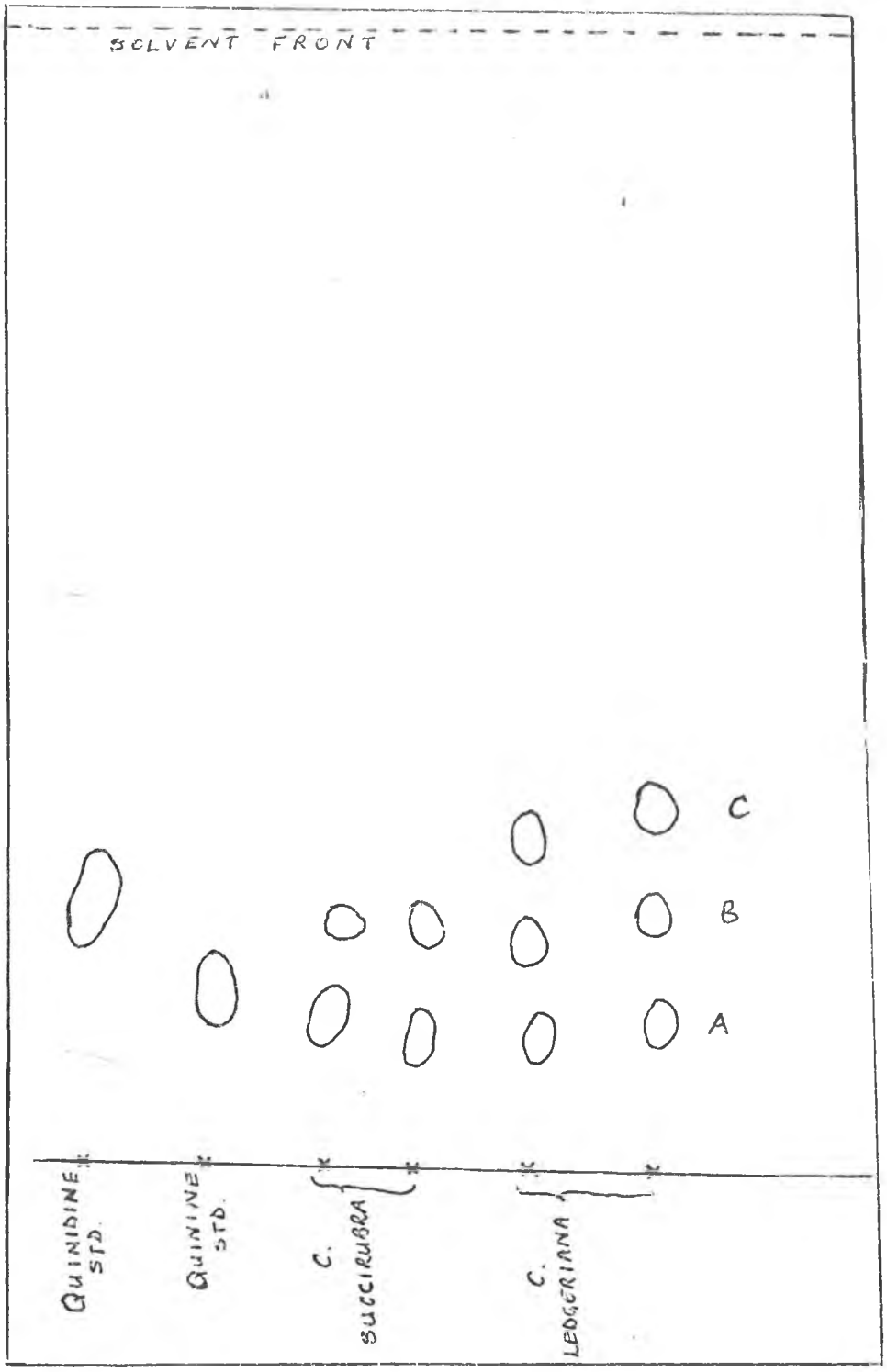
Quinidine - 40

Cinchonine - 44

b) Table 2

Solvent System: Beneze: Ether: Diethylamine (55:35:10)

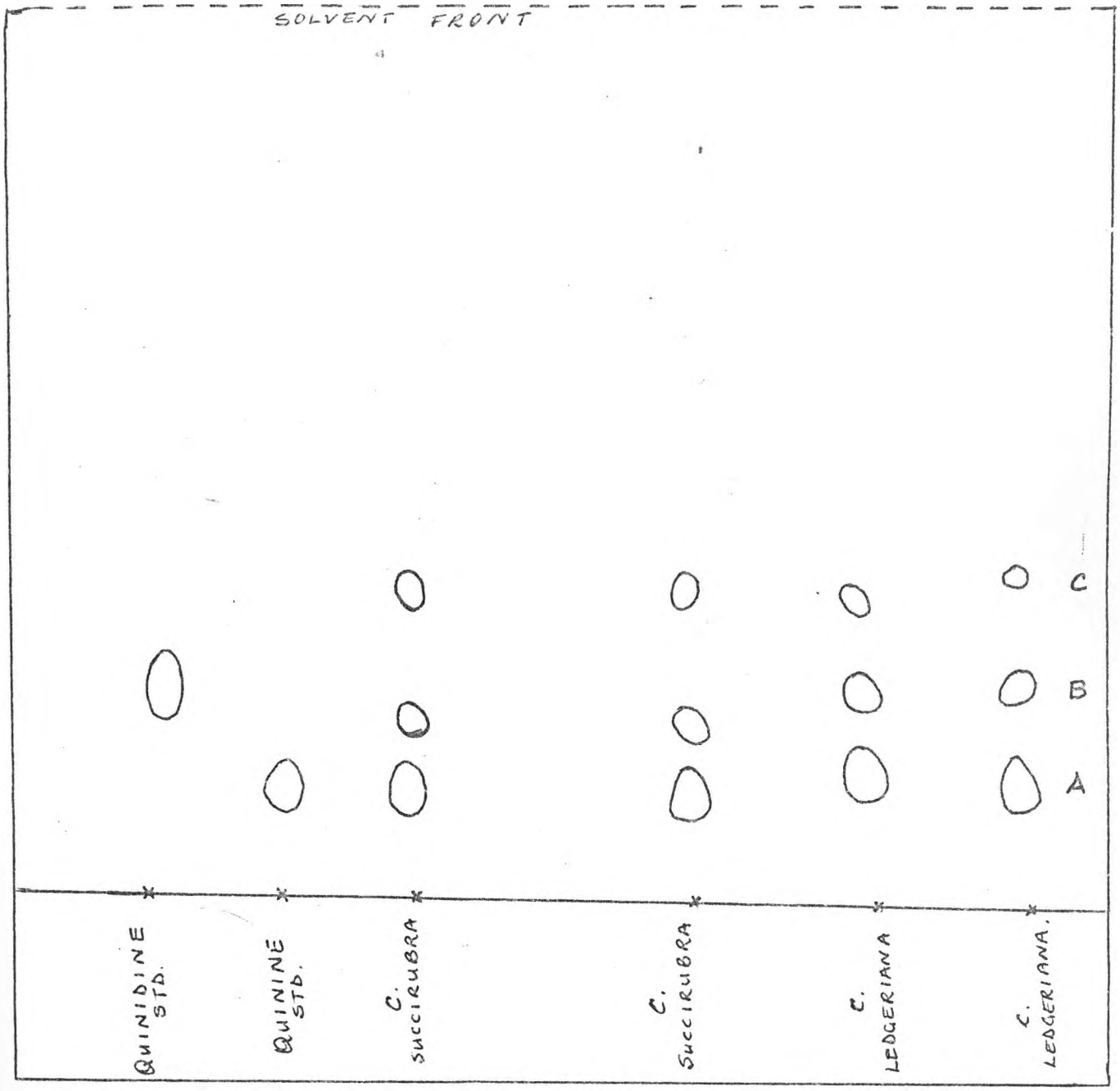
	hRf Values			References	
	SPOT A	SPOT B	SPOT C	QUININE	QUINIDINE
C. Succirubra	13.1	21.3	-	15.6	23.1
C. Ledgeriana	13.8	22.5	31.9		



c) Table 3

Solvent System: Benzene: Ethylacetate: Diethylamine (70:20:10)

	hRf Values			Reference	
	SPOT A	SPOT B	SPOT C	QUININE	QUINIDINE
C. Siccirubra	11.3	19.9	34.8	12.1	22.7
C. Ledgeriana	14.2	24.1	34.0		



Value obtained by others (6)

Quinine - 17

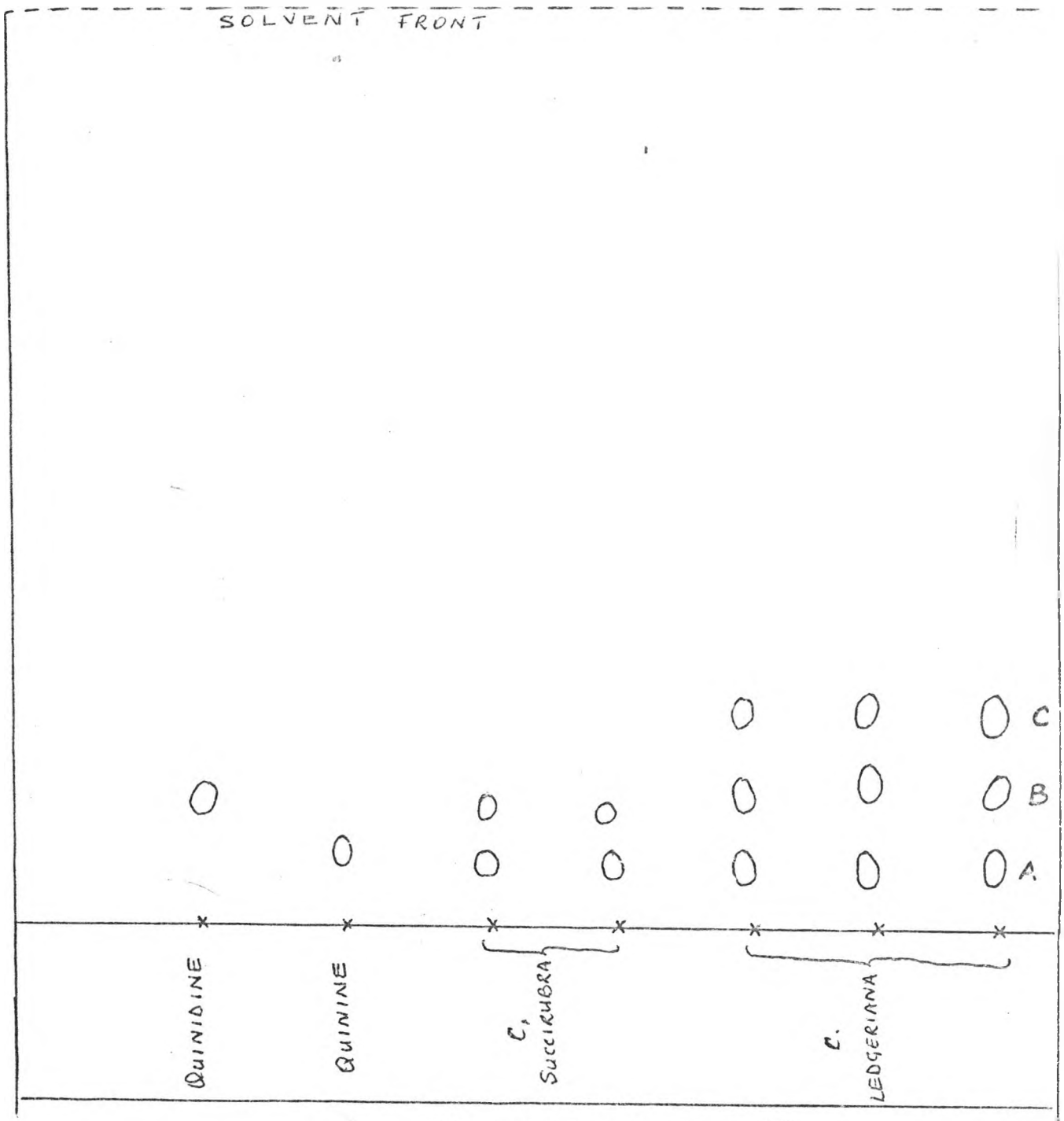
Quinidine - 25

Cinchonine - 27

d) Table 4

Solvent System: Cyclohexane: Chloroform: Diethylamine (50:40:10:)

	hRf Values			References	
	SPOT A	SPOT B	SPOT C	QUININE	QUINIDINE
C. Succirubra	5.96	11.9	-	7.3	13.2
C. Ledgeriana	5.96	14.7	23.2		



Values obtained by others (6)

Quinine - 7.0

Quinidine - 15.0

Cinchonine - 17.0

D I S C U S S I O N

Macroscopically, the only major difference between the two species is that Cinchona Succirubra gives a reddish-brown powder whereas C. Ledgeriana bark gives a yellowish-brown powder. They however, showed a similarity in the taste and aroma.

Similarly, the microscopical examination also showed no fundamental differences. The similarity between the two barks conforms with what has been observed by other workers (9). Among the prominent features observed on examination of the powdered drugs were the presence of large spindle shaped bast fibres which are clearly diagnostic of Cinchona species, starch granules, bits of Phloem Parenchyma and medullary ray.

However, a significant difference between the two species was the abundance of microsphenoidal crystals of calcium oxalate in C. Succirubra. These crystals were however conspicously absent in C. Ledgeriana.

In the TLC examination of the bark extracts, the alkaloids that were detected were three. These being Quinine, Quinidine (both confirmed) and the third one suspected to be Cinchonine. This was however done by using hRf values obtained by other workers elsewhere (see table 1 - 4) since no standard was available. It was found that whereas C. Ledgeriana showed all three spots i.e. Quinine, Quinidine and Cinchonine, the extracts from C. Succirubra gave only two spots corresponding to Quinine and Quinidine. However, the Benzene: Ethylacetate: Diethylamine system gave three spots. Thus, this was the only system that readily gave three spots with the C. Succirubra extract.

The total alkaloid content of C.Succirubra and C. Ledgeriana were found to be 6.51% and 8.02% respectively. These values are within the common range of 6 - 9% since variations are expected due to environmental differences, age and method of collection of bark.

In comparison, the value obtained with C. Succirubra is however, higher than the variety grown in some parts of Asia which vary from 6.04 - 6.25% (3).

The value obtained with the locally grown C.Ledgeriana is however, lower than those grown elsewhere which in most cases is about 8.5%.

C. Ledgeriana was found to have higher content of Quinine than C. Succirubra per unit weight of bark. Relating this to the total alkaloid content of which C. Ledgeriana was found to have 64.1% and C. Succirubra 25.8%. It is observed that Quinine accounts for more than half of the alkaloids present in C. Ledgeriana whereas in C. Succirubra it accounts for a quarter of the alkaloids. The values more or less agree with what has been observed in most cases as ledgers have a relatively high content of Quinine.

Thus the locally grown C. Ledgeriana has both a higher alkaloid and Quinine content than the C. Succirubra variety obtained from South America.

A P P E N D I X

1) Preparation of Plates for TLC Examination

The slurry was prepared by mixing 30g of Silca Gel (type MN Kiesolgel G/VV254) with 60m of water and shaking throughly for about 2 minutes. This was then applied to five plates (10 x 10) by means a Desaga spreader using a 250μ applicator at a constant speed to give the desired slurry layer of 250μ.

After allowing the slurry to dry, the plates were then dried in an oven at 110^{o}C for 30 minutes, then stored in a dessicator prior to use.

2) Preparation of Reference Substance

Quinidine and Quinine Sulphate tablets B.P. were used.

About 0.3g of the powdered tablets was mixed with 20ml of dilute ammonia solution. This mixture was then extracted with 30ml of chloroform using a separator. The Chloroform extracts were then stored in a tightly capped amber coloured bottles and later used in the TLC examinations.

3) Reagents and Instruments

All reagents used were analytical grade reagents except in cases where these were absent then other grades were used e.g. General laboratory reagents.

Instruments:

a) Pye Unicam $_{p}$H meter (model 290 MK 2)

b) Gallenkamp melting point determination apparatus

c) Atago Polarimeter

d) U.V. spot locating chamber.

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