

THE PHARMACOGNOSTICAL STUDY OF THE
BARK OF CINCHONA LEDGERIANA MOENS
CULTIVATED IN KENYA, //

BY

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

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D E D I C A T I O N

TO

MY BROTHER REV. FATHER GABRIEL N. MUVERETHI,
WHO HAS BEEN AN UNFAILING SOURCE OF HELP AND
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A B S T R A C T

The aim of this study was to evaluate the cinchona bark obtained from plants cultivated in Kenya. The bark was examined microscopically and by chemical methods. The bark was found to contain 8.5 percent of total alkaloids calculated as quinidine sulphate (on a moisture free basis). The TLC study revealed the presence of quinine and quinidine in the bark extract in the ratio approximately 1:1.

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

According to Trease, (1), cinchona bark consists of various species, races and hybrids of cinchona, family rubiaceae. Large trees which are indigenous to Colombia, Ecuador, Peru and Bolivia are grown in tropical regions for commercial purposes. The European pharmacopoea recognises *Cinchona succirubra* pavon and its varieties and hybrids containing not less than 6.5 percent of total alkaloids, 30 - 60 percent of which consists of quinine - type alkaloids. The former importance of cinchona bark and its alkaloids in the treatment of malaria has been lessened by the introduction of synthetic drugs, however it still has some importance and quinine and quinidine are included. In most world's pharmacopoeas e.g. ~~the~~ ~~British Pharmacopoea~~ and the British Pharmacopoea, ~~the~~ former collection of the bark from wild trees was replaced by cultivation and much research was undertaken by the Dutch in Java and the British in India to obtain hybrids which are rich in alkaloids. Whilst Indonesia and India remain important producers of cinchona, a high percentage of the total crop is now grown on plantations in the Congo, Guatemala and Bolivia.

The bark was originally obtained by felling wild trees, and as none were replanted the trees were exterminated in many districts. Ruiz (1792) and Royle (1839) suggested the cultivation of Cinchonas in other parts of the world. Weddell germinated seeds in Paris in 1848 and the plants were introduced in Algiers in 1849 but without much success. A further attempt by the Dutch was made in 1854, seeds and plants being obtained from Peru by Hasskarl and introduced into Java. An English expedition under Markham in 1860 led to the introduction of cinchona calisaya Weddell into India. Seeds of cinchona ledgeriana moens were obtained from Bolivia by Charles Ledger in 1865 and were bought by the Dutch for their Javanese plantations. World War II and subsequent fighting in Malaya and Vietnam increased the demand for cinchona and stimulated cultivation in Africa and Central and South America.

According to Tyler 1976 (2) and Claus 1965 (3), cultivation of cinchona trees gives an opportunity to select seeds from plants yielding high-quality bark, and also to hybridize one choice strain with another. For example hybrids of cinchona ledgeriana Moens and Cinchona calisaya pavon produce a higher yield of alkaloids than either of the parent species. Selected seeds planted in seed beds develop into young plants suitable for transplanting within two years. The stems tend to die and drop off, and the tree crowns are very close, thus shading the trunks.

Shade is favourable to the production of quinine. Trees 6 to 9 years old possess maximum amount of alkaloids in the bark. They can easily be uprooted with tractors, following which the fresh bark of both trunk and roots can be removed by hand. The dried bark of the young tree may have an alkaloidal content three times as great as bark from an old tree. A considerable amount of cinchona bark enters into the manufacture of vermouth and certain bitter liqueurs. Distribution of quinine and quinidine in the stem is a function of the bark thickness, they decrease from the base upwards (4).

The commercial bark occurs in squills which are upto 30 cm in length and usually 2-6 mm thick. The bark for manufacturing purposes occurs often in form of small curved pieces. The outer surface of the bark frequently bears lichens or mosses. The cork may or may not be longitudinally wrinkled and usually bears longitudinal and transverse cracks, which vary in frequency and distinctness in the different varieties. The inner surface is striated and varies in colour from yellowish to deep-brown. The fracture is short in the outer part but somewhat fibrous in the inner part. It has slight odour and bitter astringent taste.

In view of the number of hybrids which are cultivated, the distinction of various commercial cinchona barks is difficult.

According to Trease (1) all cinchona barks have a similar microscopical structure. The cork is composed of several layers of thin-walled cork cells, arranged in regular radial rows and appearing polygonal in surface view. Their cell contents are dark reddish in colour. Within the cork cambium is a phelloderm of several layers of regular cells with dark walls. The cortex is composed of tangentially elongated thin walled cells containing amorphous reddish-brown matter or small starch grains 6-10 mm in diameter. Scattered in the cortex are the idioblasts containing microcrystals of calcium oxalate and secretion cells. The phloem consists of narrow-sieve tubes showing transverse sieve plates, phloem parenchyma resembling that of the cortex and large characteristic spindle shaped phloem fibres with thick conspicuously striated walls traversed by funnel-shaped pits. The phloem fibres occur isolated or in irregular radial rows. The distribution and size of the phloem fibres differ in the various species (those of *C. succitubna* are 350-1400mm in length and 30-100mm in diameter. The medullary ray cells being thin walled and somewhat radially elongated.

According to Tyler, 1976(2), the alkaloids chiefly occur in the parenchymatous cells of the middle layers of the bark. Cinchona contains some 25 closely related alkaloids of which the most important are quinine, quinidine, cinchonidine and cinchonine. The average yield being 6-7 percent of which from 50-75 percent quinine. Another consistment of cinchona bark is cinchotannic acid from 2-4%, which decomposes into the nearly insoluble cinchona red, occuring in red barks to the

extent of 10%. In commerce, cinchona bark is priced either on the basis of its total alkaloid content or more often on its quinine content.

According to Goodman (5), quinine effects such a large variety of biological systems that it has been called "general protoplasmic poison". With some reservations, this appraisal is probably correct because it is toxic to many bacteria and other unicellular organisms such as trypanosomes infusoria, yeast, plasmodia and spermatozoa.

Until the 3rd decade of the present century, the cinchona alkaloids represented the sole chemotherapeutic agents for the specific treatment of malaria. Since the advent of synthetic antimalarials, quinine has been relegated to a secondary role.

Quinine is effective both as a suppressive drug and in the control of overt clinical attacks. Its primary action is schizontocidal and it is also gametocidal.

Quinine resembles salicylates and related drug in its analgesic property. Adequate doses obtund moderate pain, especially muscle and joint pain. Site of action is central.

Toxic amounts of quinine may cause abortion, but it is questionable whether this is always due to a direct uterine action of the drug. Quinine passes the placental barrier and can produce poisoning in the fetus.

Quinine has curare-like effects on skeletal muscles and can antagonise the actions of physostigmine on skeletal muscles as effectively as does curare.

Soluble salts of quinine are extremely bitter and very small amounts of cinchona preparation are used as bitter-tonics. Larger doses of alkaloids may inhibit vagat-mediated gastric secretion. Irritant properties of cinchona alkaloids cause considerable gastric distress, nausea, vomiting and diarrhoea are prominent when large doses are taken orally.

Chronic poisoning by quinine is referred to as cinchonism; it is characterised by ringing in ears, headache, nausea and disturbed vision; this include disturbed colour perception, photophobia, diplopia, night blindness and mydriasis.

EXPERIMENTAL

1. Collection of Plant Material

The samples of dried bark of cinchona ledgeriana moens were obtained from Kericho Plantation in 1979 and January, 1980. The plantation is run by the Brook-Bond company for many years. It is situated near the tea plantation about 3 kilometres from Kericho.

2. General Test for presence of Quinine

The presence of quinine in the bark was confirmed by the Thalleiognin test (6). To the bark extract, bromine water was added and shaken for 10 minutes with 25% ammonia solution. A green colour indicated the presence of quinine.

3. The study of Cinchona bark extract

Preparation of the slurry and covering of the plates were performed according to technique described by STAHL(11) and Randerath (8).

A slurry of silica gel g (30g) in water 60ml. was applied to five plates (20x20cm) by means of the Desaga applicator to give a layer 250 μ thick prior to use, the plates were dried at 110^o for 30 minutes and stored in the dessicator over anhydrous silica gel.

In preliminary examination, the best mobile solvent was found to be

Benzene: ether: diethylamin (55:35:10) as recommended by STAHL(7).

4. Preparation of Reference substances

Quinine and quinidine sulphate tablets were used. 0.1g of powdered quinine and quinidine sulphate tablets by addition of 5ml of 25% percent ammonia solution; the mixture was extracted with 10ml chloroform. The extract was separated and used in TLC examination. Reference substances according to STAHL(7) were as follows:

- a) 35mg quinine dissolved in 10 ml methanol.
- b) 1 mg quinidine dissolved in 10ml methanol.

5. Preparation of Plant Extract

Extraction of alkaloids from the bark was performed according to STAHL(7).

To 1.0g of powdered cinchona bark, 2 drops of 25 percent concentrated ammonia solution, 5 ml of chloroform were added. The content was shaken for 5 minutes and then filtered.

6. Quantitative determination of alkaloids

Determination of alkaloids content was carried out by potentiometric method described in British Pharmacopoea (9).

0.1N perchloric acid was standardised by dissolving 0.2g of potassium phthalate in 50ml glacial acetic acid. The mixture was titrated against perchloric acid using a potentiometer noting the EMF after every 1 ml addition of the acid.

Non-aqueous titration followed by the titrating the extract residue in 50ml glacial acetic acid, with 0.1N perchloric acid previously standardised by the PH meter. EMR was recorded after 1 ml addition of perchloric acid.

7. Determination of moisture content

Determination of moisture content was performed by gravimetric method, 1.0g of the bark was thoroughly weighed and heated to a constant weight at 105°C and the average result of three determinations was calculated.

8. Microscopical examination of the bark

The transverse and longitudinal sections of the bark were studied microscopically and the results compared with those given by other authors.

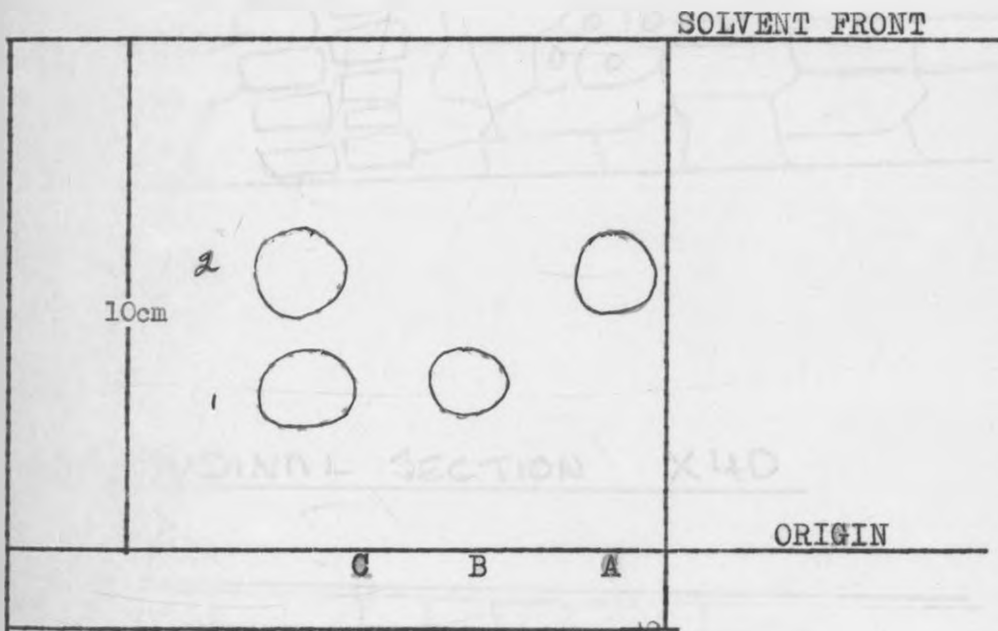
Level	mic
1	20
2	30
3	40
4	50

RESULTS + DISCUSSION

Figure 1

Thin Layer chromatogram of the Cinchona bark extract

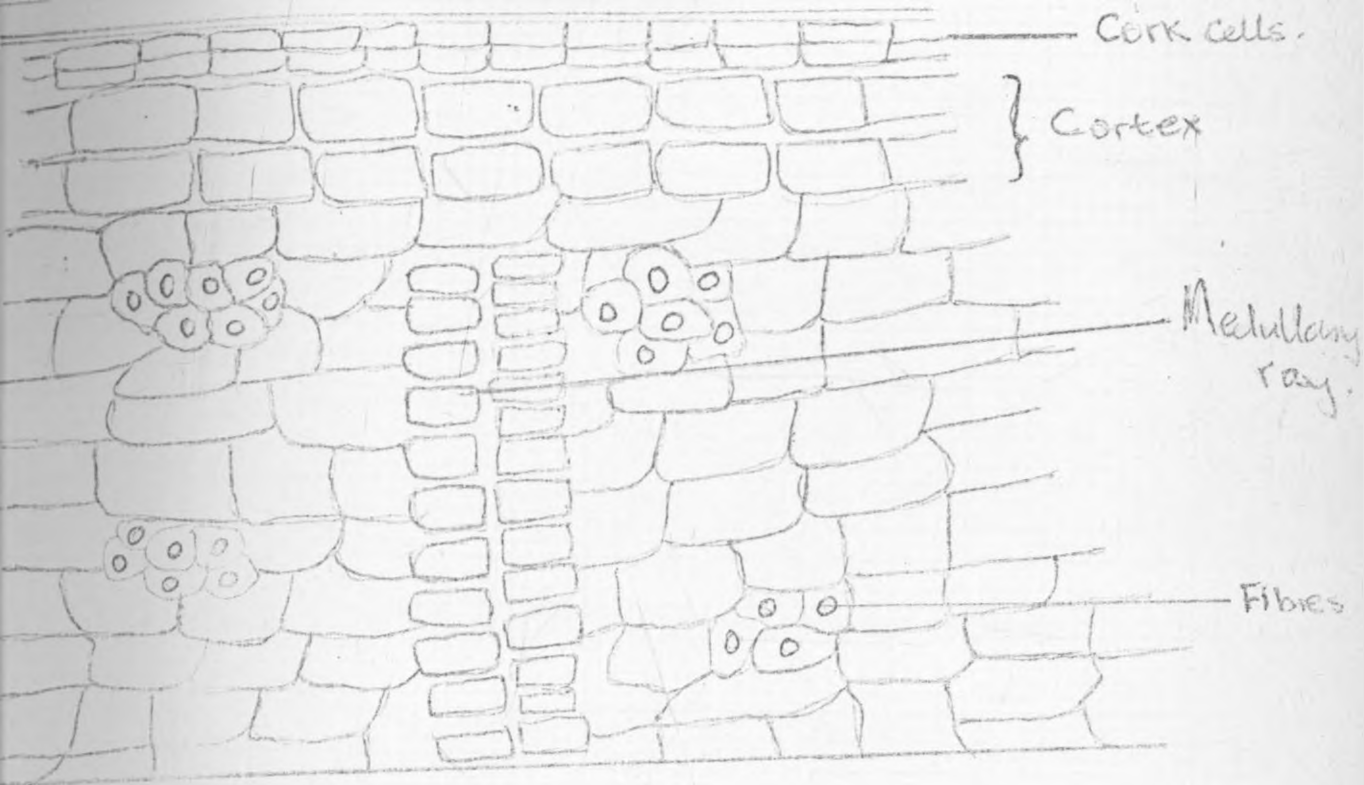
- A - Quinidine
- B - Quinine
- C - Cinchona bark extract (sample)



SPOTS	hRf
A	35
B	20
C Spot 1	20
" 2	35

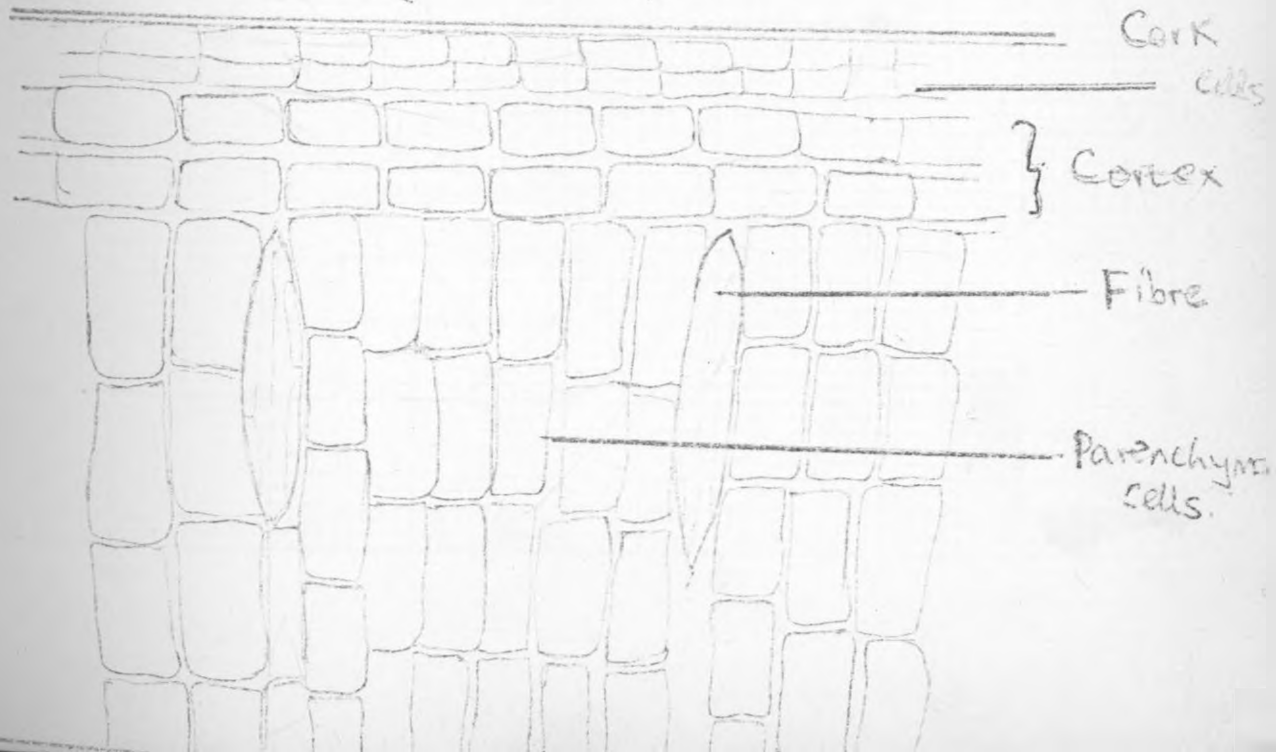
MICROSCOPICAL STUDY OF THE BARK.

TRANSVERSE SECTION X40.

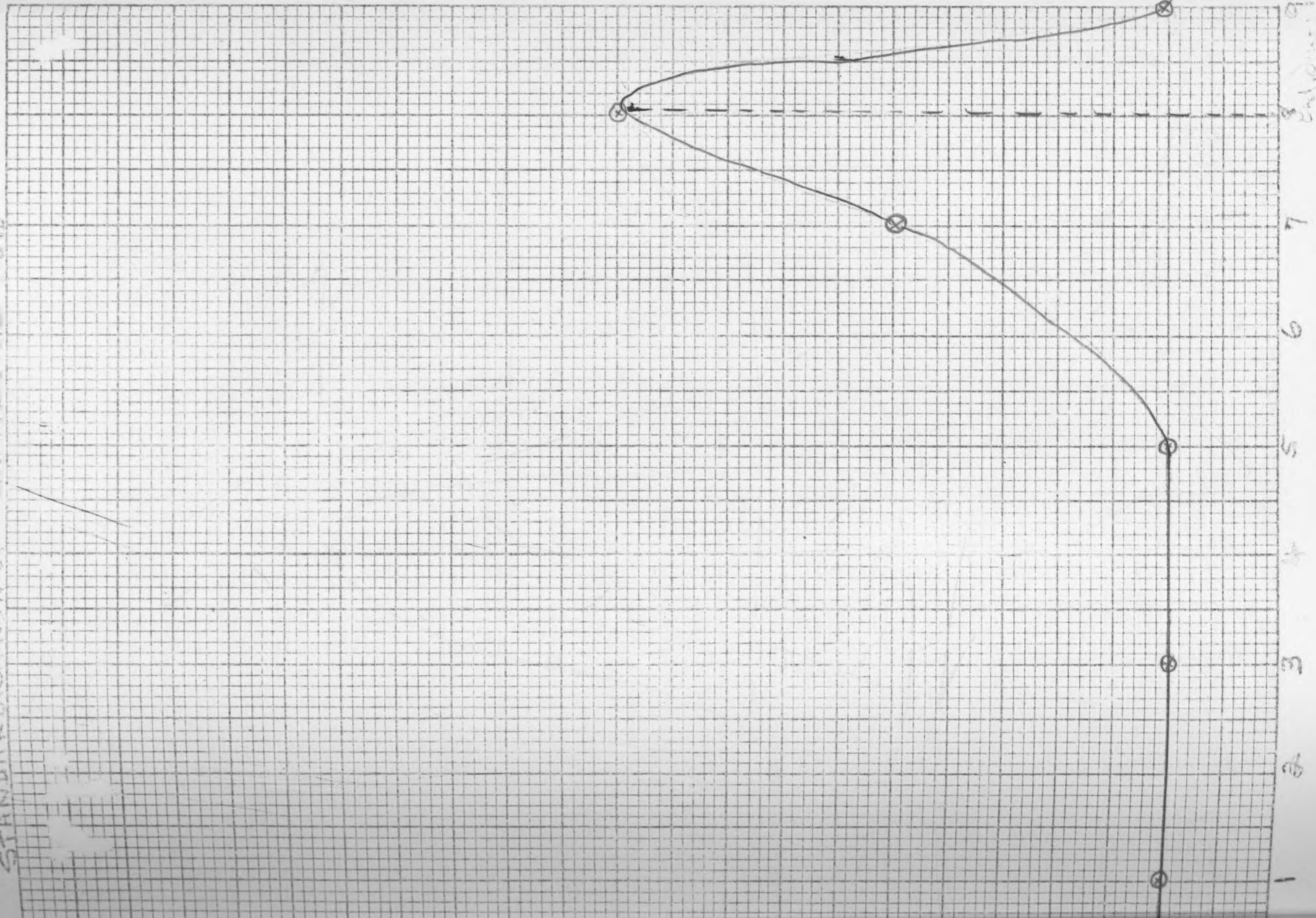


LONGITUDINAL SECTION X40

3

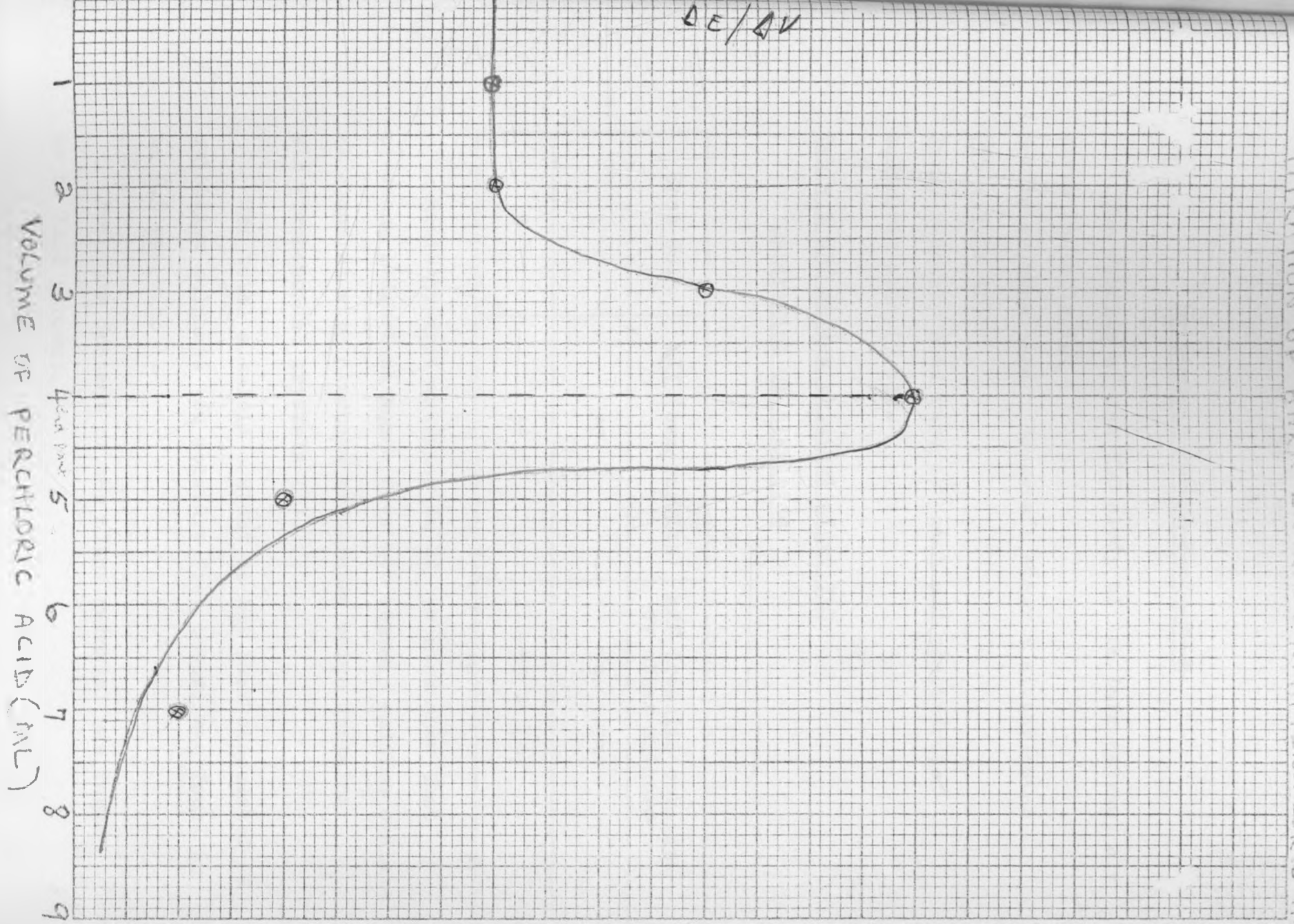


STANDARDIZATION OF PEROXYLACETIC ACID



VOLUME OF PEROXYLACETIC ACID IN ML.

TITRATION OF BARK EXTRACT WITH PERCHLORIC ACID



D I S C U S S I O N

The results of the TLC study of the bark extract are given in table 1.

Mobile solvent used : Benzene : ether : diethylamine
(55 : 35 : 10)

Spray reagents used were methanol and concentrated sulphuric acid (9 :1).

Table 1 hRf values obtained after TLC

Separation of alkaloids

Spot No.	hRf (own results)	hRf (results obtained by other authors)
1	20	19 (9)
2	35	33 (9)

Microscopical examination of the bark show that cinchona ledgeriana moens does not contain calcium oxalate as compared with cinchona succirubra which contains microspenoidal crystals (1).

The total alkaloid content in the bark calculated as quinidine sulphate (on moisture free basis) was found to be 8.5 percent.

Moisture content of the plant material was found to be 11.2 percent.

As may be seen from table 1, TLC examination of the bark extract revealed two spots of approximately same area and therefore the ratio of alkaloids is approximately 1:1. There was spot enlargement when the reference substances were added to the sample. This indicated that the sample contained the same substances as the reference substances used.

The R_f value of spot, No.1 corresponds to that given by quinine. Both spots were yellowish-white after spraying. Combining this observation with the result of the general chemical test, it may be concluded that the crude drug contains quinine. Spot No.2 has the same R_f as that given by quinidine and enhancement of the spot by adding pure quinidine to the sample leads to increased regularly shaped spot. Thus the crude drug contains quinidine. Only two alkaloids were detected in TLC examination of the bark extract. Other alkaloids occur only in very small amounts (traces).

Microscopical study of the crude drug revealed presence of characteristic large lignified fibres which are similar to those found in other cinchona species. However cinchona ledgeriana moens cultivated in Kenya does not contain microsphenoidal crystals of calcium oxalate as found in other related species. Total alkaloids content of the bark of cinchona ledgeriana was found to be 8.5 percent (calculated on moisture free basis). This result corresponds well with those obtained in other countries(2).

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