

EXPERIMENT NO-1

THE MORPHOLOGICAL, HISTOLOGICAL, POWDER CHARACTERISTICS, EXTRACTION AND DETECTION OF CINCHONA BARK

AIM OF THE EXPERIMENT: To study the morphological, histological, powder characteristics, extraction and detection of Cinchona bark.

REFERENCES:

REQUIREMENTS :

1. Apparatus: Compound Microscope, Petri plate, cover slip, Glass-Slide, beaker dropper, filter paper, forceps, tripod stand, wire gauze, dissecting needle, sharp razor, Soxhlet apparatus, etc.
2. Chemicals: Conc. HCL, Fluroguinol, Glycerine, alcoholic KOH, NaOH, conc. H₂SO₄, toluene, ethyl acetate, diethyl-amine, ammonia, etc.

THEORY:

Synonyms: Jesuits bark, Peruvian bark, Cinchona bark.

Biological Source: It consists of dried bark of cultivated trees of Cinchona Calisaya, C. ledgeriana, C. officinalis.

Family: Rubiaceae

Chemical Constituents: The primary alkaloids in cinchona are quinine, quinidine, Chinchonine, Cinchonidine. Other constituents are quinic acid, cinchotannic acid.

Uses: Cinchona used to treat a variety of diseases such as anti-malarial, anti-pyretic and analgesic. The drug is also useful in cases related to cardiac problems like arrhythmia. It is also useful in dyspepsia and gastric pain.

A. MORPHOLOGICAL CHARACTERS:

Colour: Externally greyish brown, internally yellowish brown in colour.

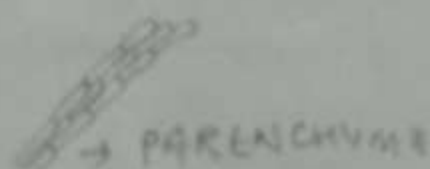
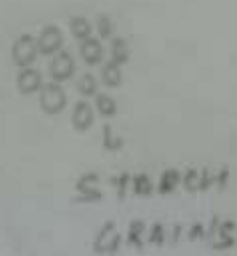
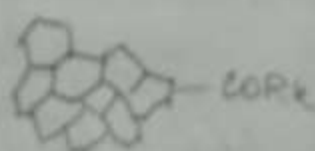
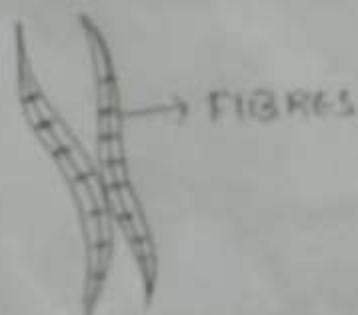
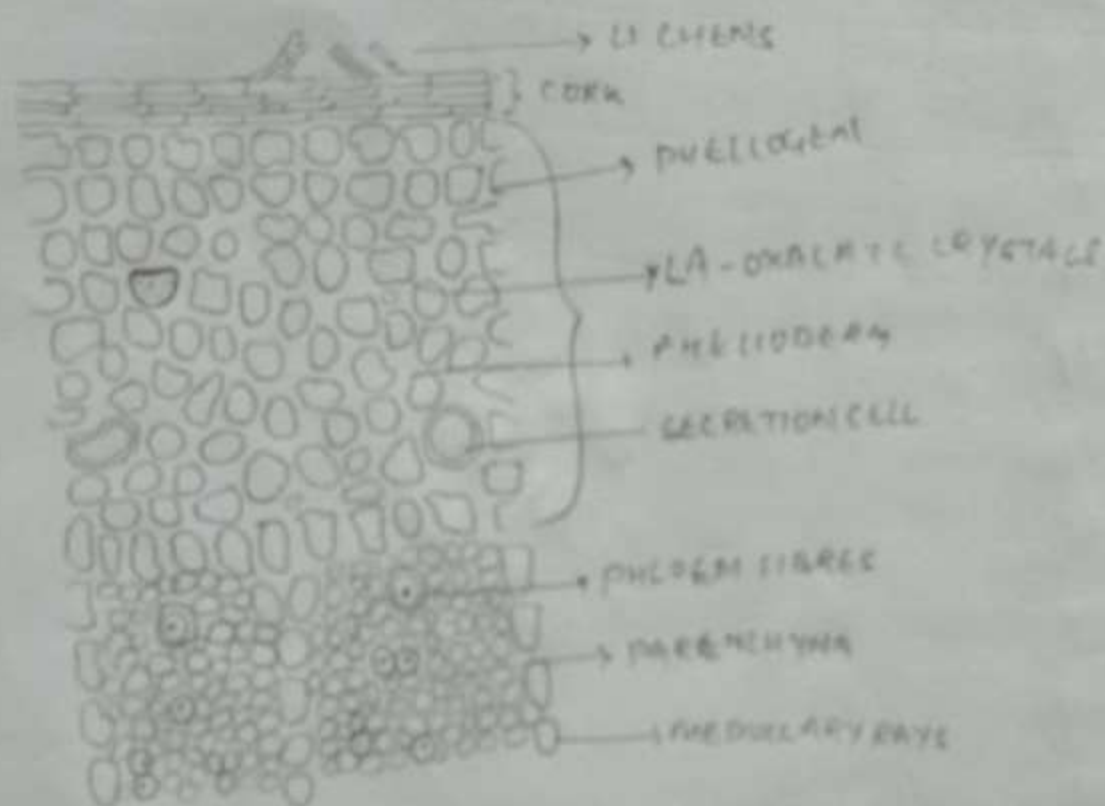
Odour: Slight and characteristics

Taste: Intensely bitter and slightly Astringent.

Size: The quills are about 15-25 mm long and 2-6 mm thick

Fracture: Short in outer bark and fibrous in inner part.

TS of CINCHONA



Extra features: Cinchona trees are evergreen with simple, oppositely arranged leaves. The flowers are small and usually creamy white or rose in colour. The flowers are borne in terminal clusters and the fruit is a small capsule.

B. MICROSCOPICAL CHARACTERISTICS :

Periderm: cork, several layers of thin walled flat, polygonal cells with reddish brown content, impregnated with suberin. (2)

Phellogen: 2-3 layers of thin walled cells without any cellular content.

Phellogen: 6-8 layers of thin walled rectangular cells without any cellular contents.

Cortex: Several layers of thin walled tangentially elongated cells containing reddish brown matter. Calcium oxalate crystals 2-6 μm long microsphenoidal crystals. Starch Grain: Rounded 6-10 μm in diameter.

Secondary Phloem:

Sieve tubes: The compact cells being about 200 μm long and 15-20 μm wide and having narrow companion cells, most of the sieve tubes are compressed and collapsed.

Fibres: Numerous large, fusiform, lignified phloem fibres, having striated walls mostly isolated, sometimes in groups of 2 to 3 fibres.

Phloem parenchyma: Thin dark reddish brown walls, some with micro-prism of calcium oxalate.

Medullary rays: 1-3 seriate extended up to cortex cells, radially elongated and contain starch grain

C. POWDER CHARACTERISTICS:

CORK: Thin walled flat, polygonal cells with reddish brown matter

Phloem fibres: Numerous, large, fusiform, lignified phloem fibres, striated walls having pores 30 to 60 μ in width and 500 to 800 μ in length.

Calcium oxalate crystals: micro-prism of calcium oxalate crystals, about 2 to 6 μ long, in dark coloured parenchyma.

Starch Grain: Minute, simple and Compound grains about 6-10 μm in diameter

D. EXTRACTION OF ALKALOIDS FROM CINCHONA:

The dried coarse powder of cinchona bark is moistened with alcoholic KOH (20%) and NaOH (5%) solution that will help to convert cinchona alkaloid to free bases. The wet powder then charged into Soxhlet apparatus for extraction using benzene as solvent. After

Micro-chemical Test.

Sl. No.	Reagent	Observation	Characteristics
1	Phloroglucinol conc. HCl (1:1)	Pink	Wagnier's phloem fibres
2	Dil. Iodine sol ⁿ	Blue	Starch
3	Dil. Acetic acid	Insoluble	Calcium oxalate crystals
4	Dil. HCl	Soluble	Calcium oxalate crystal
5	Sulphuric acid (6V)	Calcium sulphate crystals formation	Calcium oxalate crystal

(2)

Chemical Test

Sl. No.	Test	Observation
1	Heat the powder with glacial acetic acid	Reddish brown
2	Treat the brick with conc. sulphuric acid & observe under UV light	Blue fluorescence
3	Thallogen test - heat powder with few drops of bromine water shake & strong ammonia.	emerald green bloom developed changed to blue / neutralization

Calculations

$$\% \text{ yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100 = \frac{0.726}{100} \times 100 = 0.726$$

$$R_f \text{ value} = \frac{\text{Distance travelled by solvent}}{\text{Distance travelled by solute}} = \frac{3.5}{5.0} = 0.7$$

complete extraction, add 5% sulphuric acid to the extract. Take this mixture in separating funnel and separate the acidic layer from benzene layer. To the acidic layer add sodium hydroxide to adjust the pH to 6.5. Precipitates of quinine sulphate are formed, filter and recrystallise with hot water. To isolate the other cinchona alkaloids treat this quinine sulphate with dil. sulphuric acid, to it add ammonia solution for separation of quinine crystals from the mixture. After addition of ammonia to the remaining mixture precipitates as quinidine, cinchonine and cinchonidin.

E. DETECTION BY TLC:

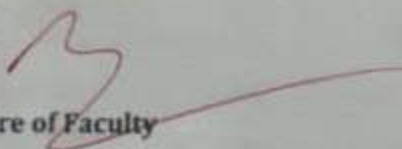
Quinine and quinidine was identified by thin layer chromatography by using silica gel as stationary phase and Toluene: ethyl acetate: diethylamine (70:20:10) as mobile phase. After drying the TLC plate, Quinine and quinidine is detected at 366 nm under UV light, at 0.16 Rf value by spraying dragendroff reagent (orange brown colour).

RESULT:

From the above experiment the morphological, histological & powder characteristics of cinchona bark was studied.

⑤ The percentage of yield of cinchona alkaloids from cinchona bark was found to be 0.726%.

The extracted cinchona alkaloids were identified by TLC method & Rf value is 0.7%.


Signature of Faculty

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