



## Research Article – Phytochemistry

# Pharmacognostic and Physico chemical standardization of *Cinchona officinalis* Linn.f

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## Abstract

*Cinchona officinalis* Linn.f. is commonly known as Crown bark in English belongs to family Rubiaceae. Stem bark of *C. officinalis* have profound application in homeopathy. Aim of the present study is to standardizing *C. Officinalis* mother tinctures by taking from three different sources [Dr. D.P. Rastogi, CRI (H) Noida (A), two from market (B, C)] for pharmacognostic, physio chemical studies and comparative analysis for authentication of drug. Pharmacognostic studies of *C. officinalis* Linn.f on bark have been carried out; physico chemical parameters of raw drug viz., ash values, formulation, besides weight per ml, total solids, alcohol content along with High Performance Thin Layer Chromatography (HPTLC) studies have been worked out for all the three mother tinctures at Dr. D.P. Rastogi, CRI (H) Noida and Homeopathic pharmacopeia laboratory Gaziabad . Bark, quelled or curved pieces up to 30 cm or more long with a thickness occurs between 2 to 6 mm; outer surface dull brown- grey, bearing lichens and mosses; usually rough with numerous small transverse cracks with re-curved edges; inner surface pale in colour; fracture short in external layers, fibrous in inner layers; odour slight and characteristic; taste intensely bitter and astringent. Physicochemical properties and HPTLC profile of mother tinctures of the drug are standardized and described. The taxonomy and fingerprinting profile are salient features to establish the standards for the drug and comparative finger printing of mother tinctures genuinity of drug.

**Keywords:** Pharmacognosy, *Cinchona officinalis*, High performance thin layer chromatography, Standardization

## Introduction

*Cinchona officinalis* Linn.f is an evergreen cylindrical tree 10-15m in height, rough, brown, yellow with black and whitish marking on bark; leaves are small, opposite, elliptical, ovate- lanceolate, entire, glabrous along with reddish petioles; flowers are reddish-brown in short cymbiform, compound cymes, terminal, axillary; calyx tubular, 5-toothed, obconical, sub-tomentose, sub-companulate, acute, triangular, dentate, hairy; corolla tube 5 lobed, densely silky with white depressed hairs, slightly pentagonal; stamens 5; style round, stigma submersed; fruit is capsule ovoid-oblong; seeds are elliptic with winged margin ocraceous, crinulate-dentate (Sangeetha *et al.*, 2011; Saxena *et al.*, 2013; Chikezie and Ojiako, 2015).

*C. officinalis* is used as tonic, cholagogue, aperitive, digestive, astringent, febrifuge, antiprotozoal, antiseptic and wound healing agent. It is suitable for loss of appetite, liver dysfunction, flu, asthenia, convalescence, prophylaxis and the treatment of cardiac difficulties and arrhythmias, cramps and myalgia. Externally it is applied as astringent, purifying and disinfectant agent and also in cases of hair loss. It is equally used in some dental products due to its astringent properties (The Wealth of India, 1949; Rao *et al.*, 1978; Verma and Vaid, 1981; Yarnell, 2007; El-Naggar, 2010).

## Materials and Methods

The plant material *C. officinalis* was supplied by the Survey of Medicinal Plants and Collection Unit, Nilgiris, Tamil Nadu. Transverse sections were taken with the help of

microtome. In house mother tincture was prepared from authentic material and other two mother tinctures were purchased. All the chemical used in the experiment were of analytical grade are chloroform, Di ethyl amine (DEA), ammonia were procured from Merck Specialties Pvt. Ltd, Mumbai, India.

Apparatus: Instruments Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttentz, Switzerland); Syringe: 10µL Hamilton (Bonadug, Switzerland); TLC chamber: Glass twin trough chamber (20× 10); Densitometer: TLC scanner 3 with CATS software; CAMAG; HPTLC Plate: 20×10cm, precoated silica gel aluminium 60F254; Merck.

## Physicochemical

Dried stems were coarsely powdered and subjected to determination of loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, physicochemical constants, and ultraviolet (UV) aspects of mother tincture following official methods. Mother tincture was prepared as per Homoeopathic Pharmacopoeia of India [HPI] by percolation method at Dr.D.P. Rastogi CRI (H) Noida (Anonymous, 1971).

## High-Performance Thin Layer Chromatography

Analysis 20 ml (3x20ml) mother tincture of each (Dr D.P. Rastogi CRI sample A, sample B, sample C) was evaporated on a water bath to remove the alcohol. The mother tinctures were basified with ammonia and residue was extracted thrice with 20 ml chloroform. The

concentrated chloroform extract was used for the High Performance Thin Layer Chromatography (HPTLC) study. The concentrated chloroform extract was spotted in the form of band of width 8.0 mm with a Camag microliter syringe on precoated Silica gel aluminum plate 60F<sub>252</sub>, (20 cm × 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttens, Switzerland). A constant application rate of 3 and 5 µL/s was employed. The slit dimension was kept at 6.00 mm × 0.30 mm and 20 mm/s scanning speed were employed. The mobile phase consisted of CHCl<sub>3</sub>:DEA (9:1 v/v) and 10 ml of mobile phase was used for Chromatography. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttens, Switzerland) saturated with the mobile phase at room temperature for 25 minutes. The length of the chromatogram run was 8 cm and subsequent to the development, the TLC plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III- Camag Switzerland) at 254 nm and 366 nm by reflectance scanning and operated by Wincats software (Camag) resident in the system (Stahl, 1961; Wagner *et al.*, 1984; Sethi, 1996).

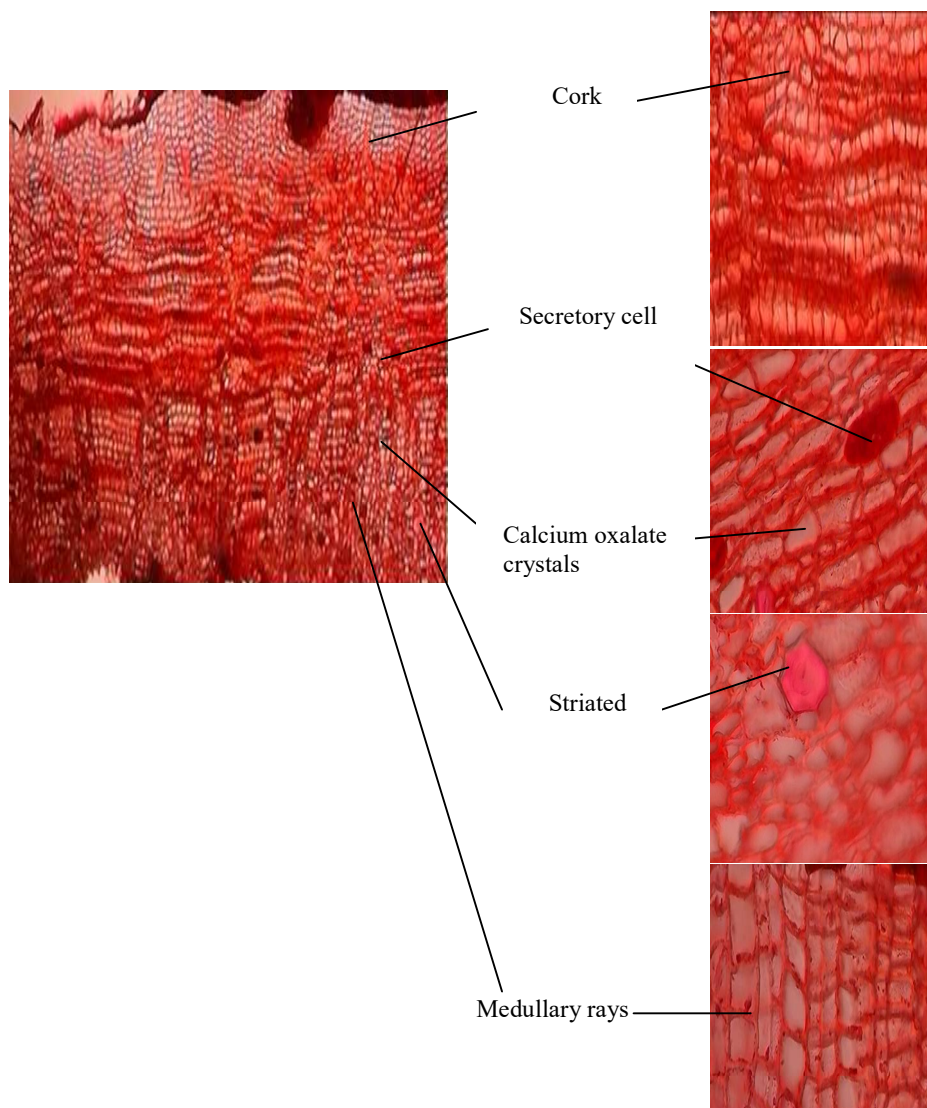
## Observations and Results

### Morphology

*Cinchona officinalis* is a 10 to 20 m tall tree with a dense and irregular globular crown and a straight trunk about 30 cm in diameter. Its leaves are oval, single, dark green in colour, opposed, petiolate, with a thick central nerve and a full margin. The fragrant white or pinkish flowers are arranged in terminal inflorescences (panicles) with white hairs. Its fruit is a dark brown glabrous oblong capsule, 2 to 4 cm long, containing 3 to 4 seeds.

### Microscopy

Transverse section of stem bark composed of several layers of flat, thin walled cork cells. There are several layers of phelloderm in cork cambium. Cortex composed of thin walled parenchyma cells contain in small starch grain 2-6 having 10-15 µm diameters. In cortex few idioblasts containing calcium oxalate crystal .Phloem having narrow sieve tubes having transverse plates and the most important feature of the phloem, is fusiform, lignified phloem fibre. These fibres are about 90 µm wide 1230 µm long. Medullary rays are 2-3 cells wide, sclereids are absent Fig.1.



**Fig.1** Transverse section of bark (10X).

### Physico chemical Studies

The determined data under the physico-chemical study for the raw drug is summarized in Table 1 and that of mother tincture preparation and its standardization in Tables 2 and 3 respectively. Qualitative Phytochemical Tests Loss on drying reveals the presence of water in the plant and also some volatile organic matter. Results of physico-chemical studies are summarized in Tables 1-3.

**Table 1.** Standardization of raw drug

Parameters	Quantitative values
Loss on drying at 105°C	Not more than 18.9% w/w
Total ash value	Not more than 2.66% w/w
Acid insoluble ash	Not more than 0.12% w/w
Water soluble ash	Not more than 0.95% w/w

**Table 2.** Formulation of mother tincture (percolation technique used)

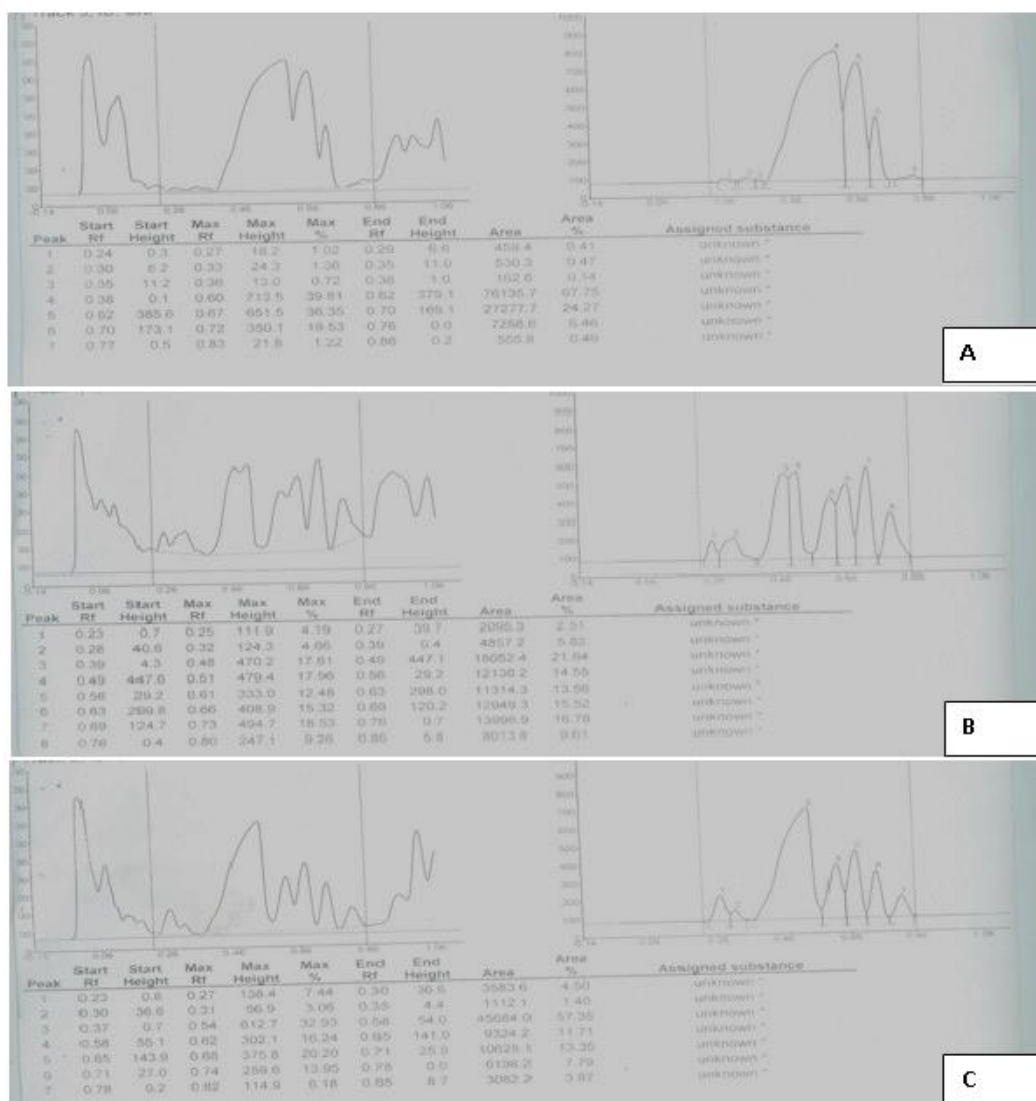
Drug strength	1/10
Preparation	
<i>Cinchona officinalis</i> coarse powder	100gm
Strong alcohol	824ml
Purified water	200ml

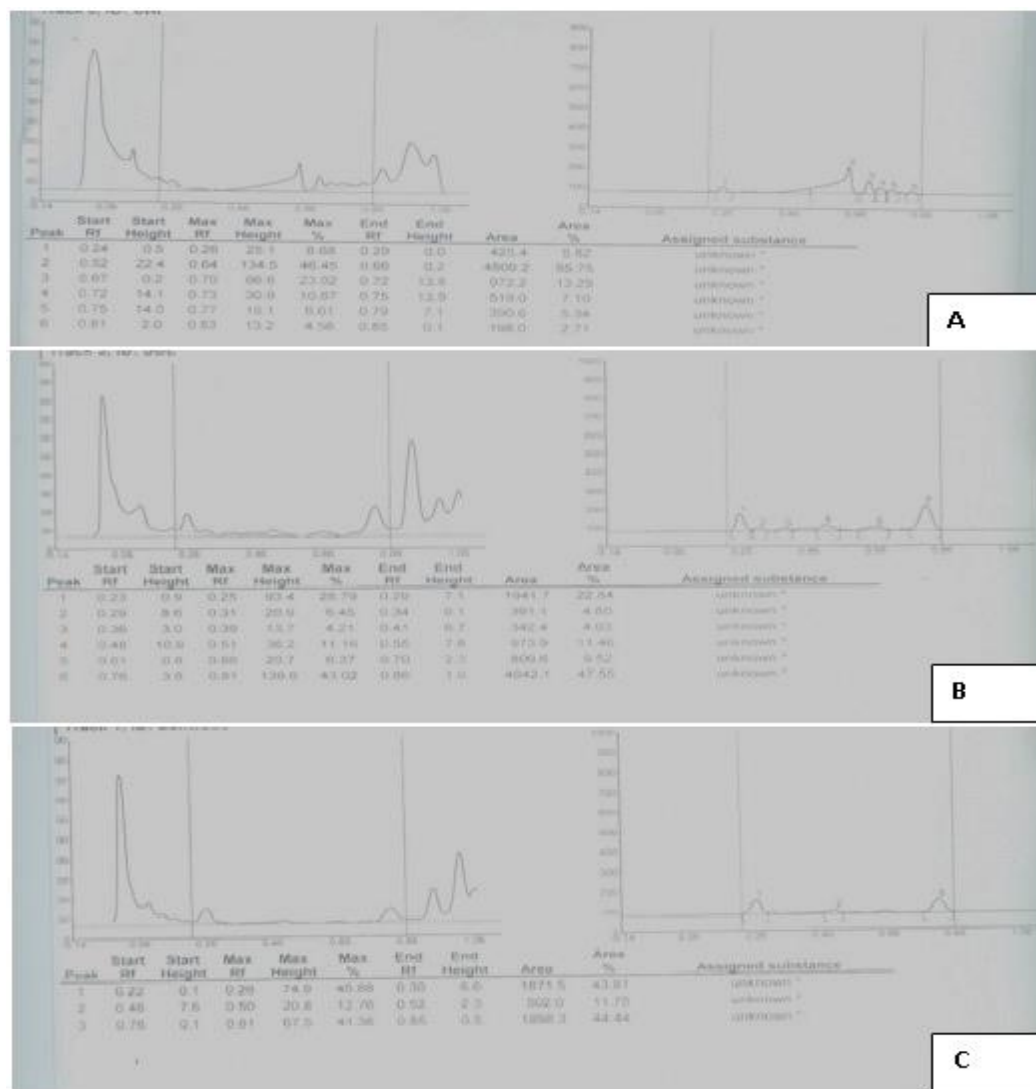
**Table 3.** Standardization of mother tincture

Parameters	Observations
Organoleptic properties	
Appearance	Clear liquid
Colour	Amber
Odour	Characteristic
Physicochemical tests	
Sediments	Nil
Wt. per ml	0.8729
Total solid	1.24 % w/w
Alcohol content	78.6% v/v
pH	5.40

### High Performance Thin Layer Chromatography Fingerprinting

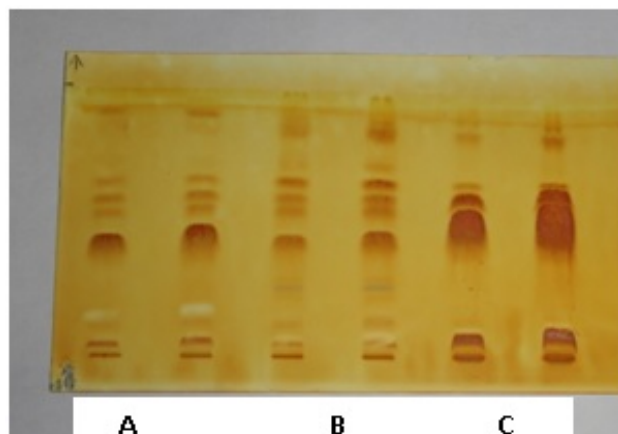
The profile of chromatographic separation scanned at 254 nm of all the three mother tincture samples (Noida sample A, sample B, sample C), reveals similar peaks Fig (2). While, chromatogram scanned at 366 nm, also similar peaks Fig (3), not only peaks but the spots are also similar Fig (4). It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the mother tincture.





**Fig 3:** High Performance Thin Layer Chromatography (CHCl<sub>3</sub>:DEA 9:1v/v) of *Cinchona officinalis* scanned at 366nm.

The TLC profile of both market sample (B, C) was similar to the in-house prepared mother tincture (A). These are considered valuable standards in Pharmacopoeia. These are a vital finger print parameters to ensure the reliability and reproducibility of the drug.



**Fig. 4.** High Performance Thin Layer Chromatography finger printing of *Cinchona officinalis*

## Discussion

### Pharmacognosy

*Cinchona officinalis* is a slender, evergreen tree up to 8m tall. Transverse section shows an outer layer of cork consists of 10 to 12 layers of thin walled, flat cells, reddish brown in colour; cork cambium 1 to 2 layered, radially arranged and tangentially flattened; phelloderm parenchymatous, contains simple starch grains and few cells with sandy crystals of calcium oxalate, inner phelloderm with large secretory canals; secondary phloem consists large, fusiform, lignified phloem fibre with concentric striation, conspicuous tubular funnel shaped pits; fibres long, mostly solitary, occasionally in short 2 to 4 radial rows; some cells of phloem parenchyma filled with dark reddish-brown content; medullary rays 1 to 3 seriate. These are the characters on the basis of which we can identify the plant.

### Physicochemical

The phytochemical analysis using various reagents showed the presence of secondary metabolites like tannins and phenolic compounds, alkaloids, and volatile oils. Physicochemical constants viz., ash and other parameters can be used as a reliable aid to check the identity, purity and strength.



Thin Layer Chromatography is done as an important tool for the authentication of herbal drugs and formulations. The results obtained from the study could be utilized for scientific validation and formulating standards for the quality assurance of the drug. In HPTLC, the developed chromatogram and RF values of bands will be specific for the drug with the selected solvent system. UV spectroscopic study exhibits, prominent peaks, which serve as characteristic standards.

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