

# Pharmacognostical, Pharmaceutical Studies and HPTLC Fingerprint of *Terminalia chebula* Retz. Fruits

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**Abstract:** *Terminalia chebula* Retz. is a valuable medicinal plant used in treatment of various ailments in traditional medicines due to the presence of large number of biological active chemicals and biochemicals. This plant is used by the pharma industry to prepare many medicinal formulations to cure different diseases. Ayurveda has large number of effective single and poly herbal formulations against diseases. In the past decades increasing the demand for traditional medicines, maintaining the quality standards are must necessary. Fruits of *Terminalia chebula* Retz. was subjected to pharmacognostical, physicochemical, phytochemical, microbial limit test (MLT) and HPTLC analysis as per standard protocols. The experimental findings of Pharmacognostical study of the drug showed group of sclereids, mesocarp, epidermal cell, starch grains, simple pitted vessels and fibres. The % yields of physicochemical values such as loss on drying, water soluble extractive, alcohol soluble extractive, total ash, acid insoluble ash and pH are 8.54, 52.5, 47.76, 2.39, 0.21 and 3.80. Phytochemical studies showed the presence of alkaloids, carbohydrates, protein, resin, tannin and saponin. MLT parameters are as per API standard protocol. HPTLC fingerprint of *Terminalia chebula* Retz. fruit were developed.

**Keywords:** *Terminalia chebula*, Pharmacognostical, HPTLC

## 1. Introduction

*Terminalia chebula* is a medium to large size tree found throughout India, mostly in deciduous forest [1], [2]. It belongs to the Family Combretaceae. This is a very valuable medicinal plant has been extensively used in folklore and traditional medicines viz. ayurveda, unani and homoeopathic system of medicines. The Sanskrit name of *T. chebula* is 'Haritaki' which means yellowish dye (harita) that contains the god Siva (Hari, i.e. the Himalayas) and it is known to cure (harayet) all the diseases. Its other synonym Abhaya, refers to the "fearlessness" provides in the face of the disease [3]. *T. chebula* fruit contains chebulin, palmitic, stearic, oleic, linoleic, arochidic and belenic acids, chebulnic acid, tannic acid, gallic acid, resin, glycosides of anthroquinons derivative etc. [4]. These biologically active chemical is associated with large number pharmacological activities due to this reason the plant is used in traditional medicine [5]. Fruits of *T. chebula* are one of the important ingredients of Triphala formulation. *T. chebula* possesses a wide variety of activities like antioxidant, antimicrobial, antiviral, astringent and carcinogenic, hypocholesterolemic, radio-protective, antispasmodic, antipurgative, stomachic, tonic and laxative. Researchers showed significant effect of gallic acid on lipid profile [6].

Previous research reported that the demand of herbal medicines increase from past few decades in all over the worlds for primary healthcare due to wide range of pharmacological activities, safety and lower costs. However, one of the impediments in the acceptance of the traditional medicines is the lack of standardization. Standardization of plant drugs is essential to evaluate the quality of drugs. Pharmacognostical (powder microscopy) studies is the method of authentication, pharmaceutical (evaluation of various physicochemical, phytochemical and microbial limit test (MLT) parameters) parameters evaluate the quality, strength and purity of drugs. Chemo profiling and marker

compound analysis using modern analytical techniques is high performance thin layer chromatography (HPTLC) method has an important tool for the identification and quantification of phytochemicals in herbal drugs and formulations.

## 2. Methods / Approach

### Sample collection, processing and powder preparation

The fruits of *Terminalia chebula* fruit were collected from Chitahara forest of Majhagawan of Satna district (M.P.), India. The plant material were identified and authenticated. The collected fruits were washed with water, shade dried under room temperature; all fruits were powdered and stored in closed container tightly for further studies.

### Organoleptic characters

Colour, odour and taste of fruits of *T. chebula* were recorded [7].

### Morphological studies

Morphological characters such as shape, size, ridges and grooves of fruits were observed [8].

### Microscopical studies

About 1g powdered drug of fruits of Haritaki was treated with Chloral hydrate and 5% nitric acid solutions, boiled and cooled separately. Wash the treated samples with water 4-5 times for Chloral hydrate and 1-2 times for 5% nitric acid. Prepared slides of treated powder sample with iodine water, Sudan III, mounted with the help of glycerine separately and observed under the compound microscope at 40 X 10X magnification of the trinocular research microscope fitted with digital Camera Lucida [9], [10], [11].

### Physico-chemical analysis

The physicochemical analysis such as loss on drying (LOD) at 105°C, water soluble extractive (WSE), ethanol soluble extractive (ESE), total ash (TA), acid insoluble ash (AIA)

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and pH of 10% (w/v) aqueous solution of sample were carried out according to the standard methods [12], [13].

### Phytochemical screening

Phytochemical screening of aqueous and ethanolic extracts of fruits of *Terminalia chebula* were carried out by standard methods [14], [15], [16].

### Test for microbial limits

Carry out the microbial limit test (MLT) for the determination of microbial load of *Staphylococcus aureus*/g, *Salmonella spp*/g, *Pseudomonas aeruginosa*/g, *Escherichia coli*, Total bacterial count (TBC) and Yeast & Mould (Y & M) in the *T. chebula* fruits curna as per standard methods [17], [18]. In this test specified agar and enrichment media were used is Himedia grade and purchase from Privet limited Mumbai.

### High Performance Thin Layer Chromatography (HPTLC)

For HPTLC, 5 g of coarsely powdered drug was taken in 250 ml Stoppard iodine flask and extracted with 100 ml ethanol for 24 hours by maceration technique with occasional shaking. The extract ((25 ml of) thus prepared was further diluted to 100 ml. A portion from this extract (25ml) was concentrated on a water bath and used for HPTLC. Similarly ethanol extract was prepared for the both the samples. HPTLC of the extracts of all the test and reference samples was carried out on Silica Gel 60 F<sub>254</sub> precoated plates (0.2 mm thickness; from Merck India Limited). Camag Linomat 5 applicator was used for band application and Desaga Video documentation Unit 3 was used for documentation of fingerprints profile. (7: 3). The plate was developed over a distance of 8 cm in a saturated development chamber (Twin trough chamber (10×10 cm) with SS lid, and visualized under visible light, UV (254nm and 366nm). The plates were also visualized after spraying with 5% methanolic-sulphuric acid followed by heating at 105°C for 5-7 min [19], [20], [21], [22]. Gallic acid standard was obtained from Himedia laboratories Pvt. Limited, Mumbai. For the experiment Analytical grade toluene, ethyl acetate, glacial acid, formic acid and methanol were used and were procured from E. Merck, Mumbai and solvent system with toluene : ethyl acetate : glacial acid : formic acid (25:45:25:5) in a Camag Twin trough chamber.

## 3. Results & Discussion

### Organoleptic characters

Organoleptic characters of fruits of *T. chebula* were observed (Table: 1).

### Morphological studies

The fruits are ovoid in shape, 2.5 – 4.0 mm in length and 0.8 – 2.5 mm wide. Wrinkles are abundant and 6-ridges and 6-grooves were observed (Figure1). Similar characters were observed in previous study [2].

### Powder microscopy

Powder characteristics of *T. chebula* fruits shows single and groups of sclereids, epidermal cell, mesocarp cell, simple pitted vessels, fibres, with peg-like out growth and simple, round and oval shape starch grains (Figure2).

### pH

The pH of 10% (w/v) aqueous solution of fruit of *T. chebula* is 3.80.

### Physico-chemical analysis

All the physicochemical parameters of fruits of *T. chebula* were analyzed and showed values under within permissible limit<sup>20</sup> such as loss on drying, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive were 8.54%, 2.39%, 0.21%, 47.76% and 52.5% respectively (Graph1).

### Phytochemical screening

Phytochemical screening showed the presence of alkaloids, carbohydrate, proteins, resin, saponin and tannin (Table: 2). Same results were also reported past studies carried out by researchers [2], [4], [5], [23]. These phytochemical are primary and secondary metabolites which play a major role in therapeutic and pharmacological properties of plants in the prevention and cure of various types of diseases.

### Microbial Limit Test (MLT)

The microbial profile of the *T. chebula* fruit sample was found under the permissible limits of both API and WHO. Total bacterial count (average 119 cfu/g), Yeast and Moulds (average 51 cfu/g) counts were reported less than the limit set by WHO (Anonymous, 1998) and pathogenic bacteria, i.e. *Salmonella*, *Pseudomonas*, *Staphylococcus* and *E.coli* were found to be absent (Figure 3 and Table: 3).

### HPTLC Profile

The TLC plates were examined under ultra violet light at 366 nm and at visible light for both before and after derivatization with 5% methanolic-sulphuric acid reagent (Figures.1). The R<sub>f</sub> values and colour of the bands obtained were recorded. The results revealed a major spot under ultraviolet light (366 nm) it showed major band at R<sub>f</sub> at 0.08 (Sky blue), 0.17 (blue), 0.63 (Light yellow) and 0.94 (Red). After spraying the plates with 5% methanolic-sulphuric acid reagent followed by heating at 110°C for about 7 min the plates were observed under ultraviolet light. It showed major bands under ultraviolet light (366 nm) at R<sub>f</sub> 0.08 (Sky blue), 0.16 (blue), 0.79 (Red), 0.84 (Red), 0.90 (Red) and 0.94 (blue). The major spot and colour under visible light at R<sub>f</sub> 0.95 (Brown) (Figure 4).

### Marker analysis of Gallic acid

After developing and drying, the plates were observed under UV light and Visible light for the presence of GA, which was detected by prominent black colour spot at 254nm (BD) and dark brown spot in visible light (BD). HPTLC chromatogram of methanolic extract of *Terminalia chebula* fruit together with the reference standard (GA) has been described (Figure 5); the R<sub>f</sub> of GA in plates were 0.31 (Figure 5).

## 4. Conclusion

The present study was carried out on pharmacognostical, pharmaceutical and HPTLC fingerprints of *Terminalia chebula* Retz. fruits which are helpful in identification and authentication of plant. The result of present study can be considered as reference values for similar research work in

future and also helpful in development of standard manual of plant.

**Table 1:** Organoleptic characters of fruits of *T. chebula*

Colour	Odor	Taste
Yellowish brown	Astringent and bitter	Characteristics

**Table 2:** Phytochemical studies in aqueous and ethanolic extracts of fruits of *T. chebula*

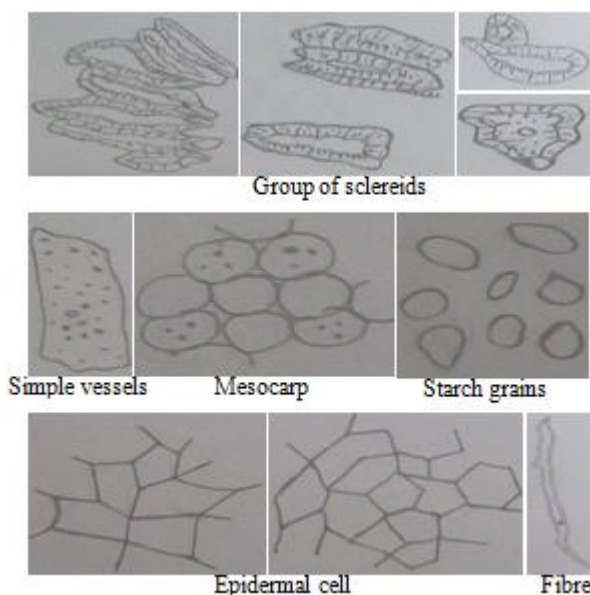
Name of experiments	Aqueous extract	Ethanolic extract
Alkaloids (Wagner's test)	+	+
Carbohydrate (Fehling's test)	+	+
Proteins (Bieuret's test)	+	+
Resins	+	+
Saponin (Foam test)	+	-
Starch test	-	-
Flavonoid (Shinoda's test)	-	-
Tannins (Lead acetate test)	+	+

**Table 3:** Standard limit of microorganism per pathogen in powder sample

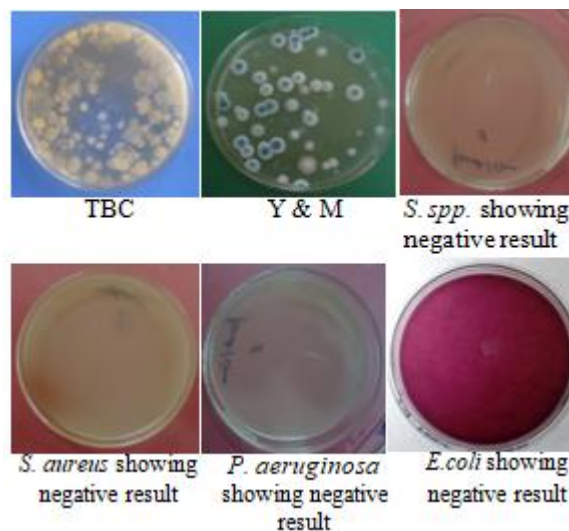
Microorganism	Permissible limits as per WHO/API	Observation / Result
<i>Salmonella spp.</i>	none	Absent
<i>Staphylococcus aureus</i>	Absent	Absent
<i>Escherichia coli</i>	Absent	Absent
<i>Pseudomonas aeruginosa</i>	Absent	Absent
Total Bacteria Count (TBC)	$<10^5 \text{ g}^{-1}$	$119 \text{ g}^{-1}$
Total Yeast & Mould Count (Y & M)	$10^3 \text{ g}^{-1}$	$51 \text{ g}^{-1}$



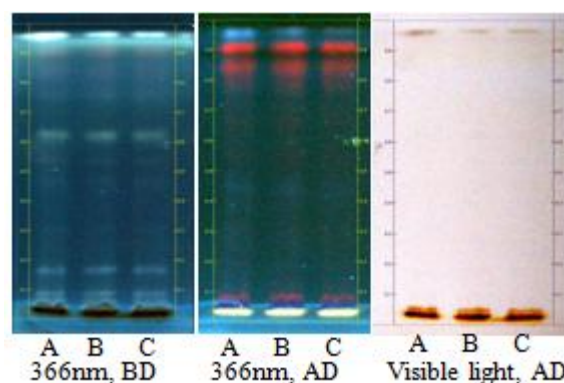
**Figure 1:** Morphological Characters of *T. chebula* (a) Dried Fruits and (b) Powder of Fruits.



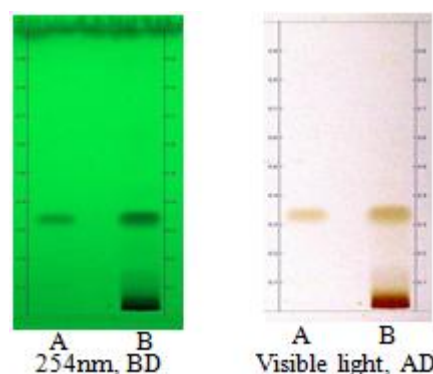
**Figure 2:** Different Powder Characteristics of Fruits of *T. chebula*



**Figure 3:** Microbial Analysis of Fruits Curna of *T. chebula* Against TBC, Y & M, *S. spp.*, *S. aureus*, *P. aeruginosa* and *E. coli*

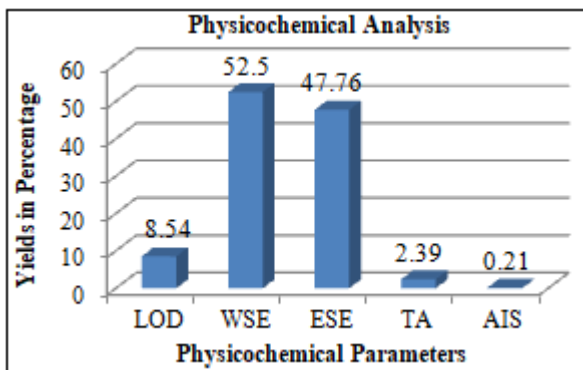


**Figure 4:** HPTLC Finger Prints of Test Solution of *T. chebula* fruit at 366nm (Before Derivatization –BD) and 366nm and Visible Light (After Derivatization-AD). Whereas A, B, C = ethanolic extract of fruits of *T. chebula*.



**Figure 5:** HPTLC Finger Prints of Gallic Acid (Standard) and Test Solution of *T. chebula* Fruit at 254nm (Before Derivatization –BD) and Visible Light, AD. Whereas, Sample A: Gallic Acid (Standard) and sample B: *T. chebula* Fruit (Ethanolic Extract)





**Graph 1:** Percentage Yields of Physicochemical Parameter of Fruits of *T. chebula*

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## Author's Profile



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