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, steric, oleic, linoleic, arachidic and benicic acids, chebulnic acid, tannic acid, gallic acid, resin, glycosides of antroquinons 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, antiulcerative, 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)
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 curma 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 F254 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 (10x10 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 reagent followed by heating at 110°C for 7 min the plates 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 Rf 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 Rf 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 Rf 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 Rf 0.95 (Brown) (Figure 4).

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 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
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 Rf of GA in plates were 0.31(Figure 5).

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 Rf 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

<table>
<thead>
<tr>
<th>Colour</th>
<th>Odor</th>
<th>Taste Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellowish brown</td>
<td>Astringent and bitter</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Name of experiments</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
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<tbody>
<tr>
<td>Alkaloids (Wagner’s test)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate (Fehling’s test)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins (Bieuret’s test)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin (Foam test)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Starch test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid (Shinoda’s test)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins (Lead acetate test)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Permissible limits as per WHO/API</th>
<th>Observation / Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp.</td>
<td>none</td>
<td>Absent</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Total Bacteria Count (TBC)</td>
<td>&lt;10⁷ g⁻¹</td>
<td>119 g⁻¹</td>
</tr>
<tr>
<td>Total Yeast &amp; Mould Count (Y &amp; M)</td>
<td>10³ g⁻¹</td>
<td>51 g⁻¹</td>
</tr>
</tbody>
</table>

**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. col

**Figure 4:** HPTLC Fingerprints 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 Fingerprints 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)
References


Author’s Profile

Mr. Pawan Kumar Ahirwar completed his B.Sc. in Biotechnology and M.Sc. in Biochemistry from Jiwaji University, Gwalior (M.P.) Indain the year 2010 and 2012. He is currently pursuing Ph.D. in Biochemistry, Department of Biological Sciences, Faculty of Science and Environment, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalay, Chitrakoot, Satna (M.P.), India since 2016. He has published more than 10 research papers in reputed National and International journals and conferences. His main works focus on Standardization and quality control of herbal drugs. Biochemical analysis, Antibacterial activity, Antioxidant activity and Antidiabetic activity. He has 5 years of research work experience.