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Original Article

Decoding Pharmacognostical and Phytochemical Research Profile of *Tamarindus indica* Linn. - Seed

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ABSTRACT

Background: Raw botanical drugs face frequent market adulteration and batch variation. *Tamarindus indica* Linn. (Fabaceae) seeds represent an inadequately characterised plant rich in therapeutic polysaccharides, tannins, and proteins requiring precise quality control standards. **Objectives:** To establish diagnostic pharmacognostical, physicochemical, and High-Performance Thin-Layer Chromatography quality-control findings for authenticating *Tamarindus indica* Linn. seeds. **Materials and Methods:** Authenticated seeds were evaluated via sectioning (Transverse section, Longitudinal section, and Radial longitudinal section) and 60-mesh powder microscopy. Physicochemical parameters, total tannins, proteins, and swelling index were determined as per Ayurvedic Pharmacopoeia of India guidelines. Methanolic extracts were profiled using High-Performance Thin-Layer Chromatography (Silica gel 60 F_{254}; Chloroform: Ethyl acetate: Acetic acid 5:4:1 v/v/v) under ultraviolet (254/366 nm) and derivatised with Vanillin-H_2SO_4. **Results:** Seeds are sub-rectangular (1-1.5 cm) with a basal hilum groove. Exotesta palisade cells display double bulbous swellings; hypodermis contains sinuous bone-shaped cells. Powder microscopy revealed warty palisade fragments and calcium oxalate crystals. Physicochemical metrics yielded: loss on drying (8.973%), total ash (97.507%), acid-insoluble ash (2.4637%), pH (6.8), and methanolic extractive yield (42.312%). Quantitative assays showed 5.023% w/w tannins, 13.44% protein, and a swelling index of 2.0. Chromatography mapped 23 spots (254 nm), 21 spots (366 nm), and 3 derivatised pinkish red peaks (R_f 0.05, 0.08, 0.10). **Conclusion:** The integrated anatomical findings, physical constants, and High-Performance Thin-Layer Chromatography profiles deliver a definitive standard to authenticate *T. indica* seeds and prevent adulteration. These foundational criteria will facilitate chromatographic isolation of novel bioactive tannins and support the clinical development of standardised herbal formulations.

Keywords: Adulteration, Authentication, HPTLC profiling, Pharmacognosy, Phytochemical screening, Quantitative analysis, Seed, *Tamarindus indica*.

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INTRODUCTION

Since the ancient past, humanity has relied fundamentally on plants and their derivative products to sustain life and manage health. This botanical dependence has driven the survival and adaptation of human civilisation across diverse environmental and historical period (Naeem, 2019). Even among the various sources for medicine, a significant proportion of global therapeutic strategies remains centred on plant-derived drugs to manage acute and complex pathological conditions (Unnikrishnan *et al.*, 2020).

Precise botanical identification remains the foundational step for guaranteeing both the safety and therapeutic reliability of herbal medicines. Accurate identification serves as the primary scientific framework required to identify and to avoid substitution and adulteration within the commercial supply chain (Keshari, 2022). Establishing accurate botanical identity represents both the foundational baseline and the most critical initial phase of natural product evaluation (Shinde *et al.*, 2016).

Because accurate identification is so vital, this research focuses on defining clear identification criteria for *Tamarindus indica* Linn. Although *Tamarindus indica* Linn. is indigenous to the wild tropical savannas of Africa and Sudan, it has been extensively cultivated across the Indian subcontinent since antiquity. The plant's common nomenclature traces back to early medieval

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Arabic physicians and traders who designated the dark, sticky fruit pulp as *tamar hindi*-literally meaning "Indian date"-due to its structural and flavour profile resemblance to local ripe dates. This Arabic designation was subsequently integrated into European and English lexicons as "tamarind." Furthermore, historical records indicate that ancient Egyptian and Greek civilizations were well aware of the plant and its diverse culinary and therapeutic applications as early as the 4th Century B.C. (Morton, 1987).

Tamarindus indica Linn. exhibits a comprehensive, part-specific phytochemical profile. The leaf is characterised with Hordenine, flavonoids, saponins, tannins, malic acid, C-glycosides- orientin, vitexin, *iso*-orientin and *iso*-vitexin. Flower possess Hordenine, also found in bark along with tannins and glycosides. Stem contains saponins, tannins, flavonoids and fruit pulp are enriched with tartaric, malic, lactic, oxalic, succinic and citric acids, protein, carbohydrates, riboflavin, niacin, Vitamin C, Vitamin B, carotene, tamarindinal, calcium, phosphorus, iron, thiamine, riboflavin and niacin, potassium, magnesium, copper, gum, pectin and fibre, saponins, flavonoids, amino acids-serine, beta-alanine, proline, pipecolinic acid, phenylalanine and leucine. Seed is characterised with polysaccharides containing glucose, galactose, xylose, arabinose in ratio of 9: 4: 5: 1, main chain composed of glucose unit with sugars containing branches, cardenolide, uzarigenin-3-0- beta-D-xylopyranosyl (1 to 2)-alpha-L-rhamnopyranoside, contain starch, albumin, and semi-drying oil, protein, amyloids, phytohemaglutinins and flavonoids. A bitter principle-tamarindienal- isolated from fruit pulp and characterised as 5- hydroxyl-2-oxo-hexa-3,5-dienal; isolation of 2-hydroxy-3',4'-Dihydroxyacetophenone (TAO), methyl 3,4-Dihydroxybenzoate (TA1), 3,4-Dihydroxyphenylacetate (TA2) and (-) epicatechin, tannic acid from seeds (Chopra *et al.*, 1986).

Therapeutically, the ripe fruit pulp of *Tamarindus indica* Linn. acts as a cooling, digestive, and carminative, laxative; infusion prescribed in febrile diseases and bilious disorders; used as a gargle in sore throat; applied as a poultice on inflammatory swellings. In contrast, the unripe fruit is indigestible, astringent to the bowels and specifically targets vata imbalances, though it can trigger cough and blood troubles if misused. While ripe fruit is appetizing, laxative, heating, tonic to the heart, anthelmintic; cures vata and kapha; heals wounds and fractures, fungicidal and bactericidal activity against *Aspergillus nigar, Candida albicans, Bacillus subtilis, Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa*. The leaf juice is traditionally used to treat bleeding piles, bilious fever and dysuria, applied to reduce inflammatory swellings, tumours, ringworms; useful in diseases of the blood, smallpox, ophthalmia and other eye diseases, earache, snake bite. Flowers are acrid, sweet, sour, tasty, and appetizing; cure "*vata*" and "*kapha*", urinary discharges, bad odour in perspiration. While the bark is topically used for loss of sensation in paralysis; the ash is given for urinary discharges and gonorrhea. Is also prescribed in asthma and menorrhea, fever and is astringent used for diarrhoea. The ash of the bark is given in colic and indigestion. The ash is also used in gargles and mouthwash for aphthous sores and used in vaginal discharges and ulcers (Pandey, 2002). The polysaccharides are said to possess immunomodulatory activities (Sreelekha *et al.*, 1993) while the tannins are antioxidants (Parvez *et al.*, 2003), used as a supporting tonic in sexual debility in Unani medicine. Seed possess other properties like anti-diabetic (Maiti *et al.*, 2004), anti-snake venom (Ushanandini *et al.*, 2006), antimicrobial (Karthy *et al.*, 2009), antibacterial (Naznin and Islam, 2009).

While *Tamarindus indica* seeds have been widely explored for their phytochemical and pharmacological properties, routine quality testing still benefits greatly from a unified analytical framework. Given the heavy commercial use and market prevalence of tamarind seeds, having a single, synchronized reference helps maintain batch consistency and prevents adulteration in raw drug supplies.

To support this need, our study integrates multi-angle micro-morphological profiles including transverse, longitudinal, and radial sections with detailed powder microscopy, quantitative assays for tannins and proteins, and High-Performance Thin-Layer Chromatography fingerprinting. Gathering these diagnostic parameters into one cohesive reference provides a clear, practical baseline for routine raw material authentication and industrial quality control.

MATERIALS AND METHODS

Collection of Plant Material

The research drug was collected in the month of May, 2025, from the plants growing in the botanical garden of Institute of Teaching and Research in Ayurveda, Institute of National Importance, Ministry of Ayush, Government of India, Jamnagar, India.

Identification and Authentication

It was identified and authenticated by the department of Pharmacognosy of ITRA-Pharmacy, with the help of subject expert. The herbarium specimen was also deposited in the same with Specimen No: ITRA/P'Cognosy/01/2025/02. The seeds were identified by referring various Floras (Cooke, 1901-1908/1997; Hooker, 1879/1999). The ripe fruits were collected, seeds were separated from the pulp, washed with running water and were stored in FAA (Formalin 90: Acetic acid 7: Alcohol 3) solution to be used for section cutting whenever required.

Pulverization

The mature, healthy, ripe fruits were collected, the seeds were manually separated from the pulp and were thoroughly washed under running tap water, then allowed to dry completely at room temperature. Once thoroughly dried, the seeds were ground

using a mechanical grinder and passed through a sieve to obtain a uniform 60-mesh (60#) powder. This processed powder was then preserved and used for the subsequent microscopic evaluations and preliminary phytochemical screenings.

Pharmacognostical evaluation

Macroscopical evaluation

The seed specimens were examined both with the naked eye and under a dissecting microscope (Gunn and Corner, 1976; Dutta, 2007; Khandelwal, 2008; Kokate, 2005). The diagnostic features were determined by analysing its structural and organoleptic characteristics, including fracture, surface texture, odour, taste, and touch.

Microscopical evaluation

The sections were cleared with chloral hydrate and then stained with alcoholic solution of Phloroglucinol (90%) and conc. HCl. Detailed transverse and longitudinal sections, along with key diagnostic features, were illustrated using a Camera Lucida. The digital photographs of the sections were captured using a Canon digital camera.

Powder Microscopy

For the powder microscopic evaluation, the prepared seed powder was examined under a microscope to identify and document its characteristic diagnostic cellular structures.

Analytical evaluation

Physico-chemical constants

Following the Ayurvedic Pharmacopoeia of India (API) guidelines and standard procedures, the seed powder was standardised by determining its foreign matter, loss on drying, ash value, acid-insoluble ash, and pH. Its water-, alcohol-, and petroleum ether-soluble extractive values were also measured (Ministry of Health and Family Welfare, 2001; World Health Organization, 1998).

Preliminary qualitative test

For qualitative screening, distinct test samples were prepared based on the specific solubility profiles of the target metabolites: an ammoniacal-chloroform extraction followed by methanolic dissolution was used for alkaloids; direct sample solutions were prepared in 10% hydrochloric acid, sulfuric acid, or chloroform for flavonoids and steroids; and an acid-hydrolysis ether extraction method was deployed to obtain dried residues for tannins and phenols. Concurrently, aqueous solutions and extracts were prepared to screen for glycosides, saponins, proteins, and carbohydrates, while a direct solution in dry acetic anhydride was prepared for the resin assays.

Quantitative estimation

The seed samples were quantitatively evaluated to determine their total tannin and protein content, along with their swelling index, in accordance with established standard protocols.

HPTLC - High Performance Thin Layer Chromatography

High-Performance Thin-Layer Chromatography study of the methanol extract was carried out by using the same solvent system of Chloroform: Ethyl acetate: Acetic acid (5:4:1). After completion of High-Performance Thin-Layer Chromatography; post chromatographic deprivation was done with vanillin sulphuric acid (Stahl, 1969).

Equipment for High-Performance Thin-Layer Chromatography

A High-Performance Thin-Layer Chromatography system equipped with a sample applicator Linomat V sample applicator (CAMAG), 4132 Muttenz, Switzerland) was used for application of samples. CAMAG Scanner III and Wincats 4.02 were used for scanning the plates. CAMAG twin through glass chamber was used for developing the plates.

Chemicals

Precoated silica gel 60 F254 TLC (Thin Layer Chromatography) aluminium plates (10 × 10 cm, 0.2 mm thick), Analytical Reagent grade methanol and chloroform were obtained from M/S Merck Ltd., Mumbai, India.

Samples for High-Performance Thin-Layer Chromatography

The extract of seed powder for High-Performance Thin-Layer Chromatography were made in same process as mentioned below.

- **Methanolic extract:** 2 g of sample was macerated with 20 mL of methanol for 24 hr and filtered. Filtrate was concentrated and used for spotting.
- **Sample:** Methanolic extract of seed powder.
- **Mobile phase:** Chloroform: Ethyl acetate: Acetic acid (5:4:1).
- **Detection:** Spray with Vanillin-H_2SO_4.

Chromatographic conditions

- **Application mode:** Camag Linomat V.
- **Development chamber:** Camag twin through chamber.
- **Plates:** Precoated Silica Gel GF254 plates.
- **Chamber saturation:** 30 min.
- **Development time:** 30 min.

- **Development distance:** 7 cm.
- **Scanner:** Camag Scanner III.
- **Detection:** Deuterium lamp, Tungsten lamp.
- **Data System:** Win cats software.

RESULTS

Pharmacognostical evaluation

Macroscopical evaluation

Macroscopy of the seed is shown in Figure 1. Seeds are flattened, more or less rounded, sub-rectangular, oval to oblong with raised margin encircling the central flattened portion (oriole) or large patch, which is more brown to brownish black in colour, strongly lustrous and exhibit pale yellowish lines running parallel to each and occasionally getting connected in the central region, 1 to 1.5 cm in length 0.5 to 0.8 cm in width and 0.2 to 0.3 cm in thickness, the edged marginal portion encircling the seed is broad, dull brown, minutely pitted and exhibit centrally located narrow longitudinal groove running throughout the margin, a pale yellowish elevated spot lying in the narrow groove at the basal region of the seed is the hilum, micropyle being located at its opposite end, testa is thick and brittle and easily detachable by roasting or by boiling the seeds in water exposing the inner whitish bulky cotyledon; taste is slightly astringent, odour nil.

Microscopical evaluation

Diagrammatic TS -Transverse section shown in Figure 2A is rectangular in outline, shows centrally located two horizontally placed plano-convex cotyledons lying one above the other, leaving a thin slit in between and occupying almost the whole area of the section, encircled by thick brown coloured outer testa.

L.S. - Longitudinal section shown in Figure 2B is quadrangular in outline shows outer thick testa, exhibiting hilar and micropylar scars, enclosing the white bulky cotyledon and radicle and plumule at the micropylar end. RLS - Radial Longitudinal section shown in Figure 2C is rectangular in shape, shows an outer testa, enclosing a vertically placed cotyledon with a narrow slit in the centre and radicle pointing towards micropylar end.

Detailed Transverse Section shown in Figures 3a and 3b has an outer layer of exotesta of long palisade cells covered with thick cuticle, the cells are narrow, compactly placed, thick lumened which is bulbous at the base and little below the centre of the palisade, both bulbous swellings being embedded with brown contents, underneath this lies a layer of bone shaped cells of hypodermis with brown contents; mesotesta is composed of 4 to 5 rows of radially elongated, thick and sinuous walled parenchymatous

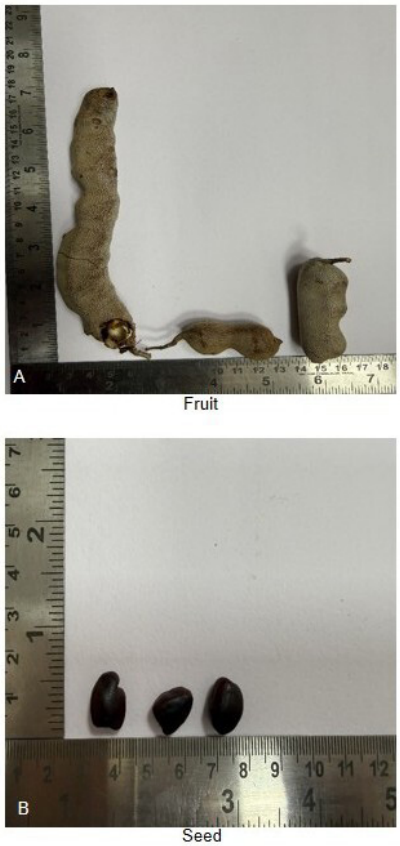


Figure 1: Morphological characters of *Tamarindus indica* Linn. (A) Fruit of *Tamarindus indica* Linn. (B) Seed of *Tamarindus indica* Linn. (Prepared for column width).

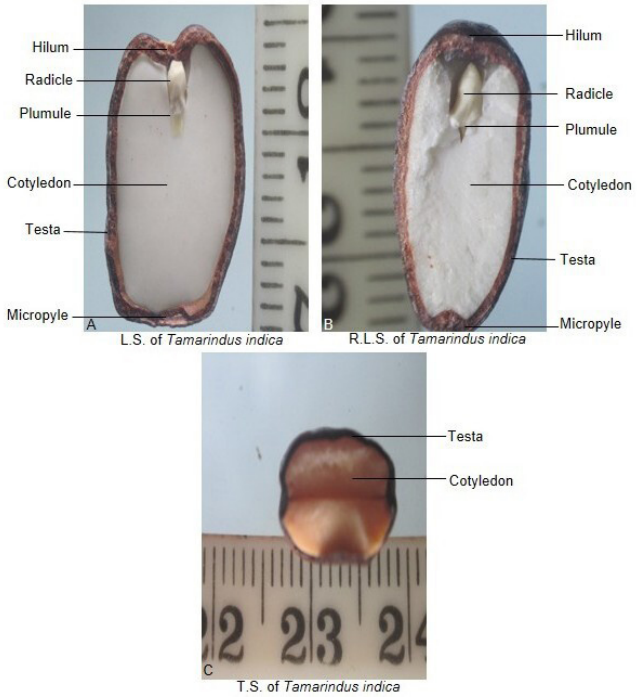


Figure 2: Diagrammatic microscopy of *Tamarindus indica* Linn. seed (A) Longitudinal section of seed (B) Radial Longitudinal section of seed (C) Transverse section of seed. (Prepared for full page width).

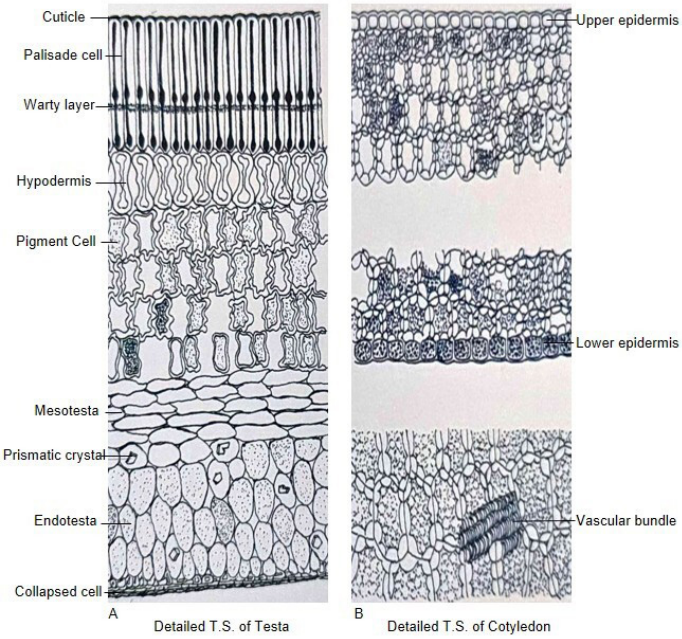


Figure 3a: Detailed microscopy of *Tamarindus indica* Linn. seed (A) Detailed transverse section of testa (B) Detailed transverse section of cotyledon. (Prepared for full page width).

cells embedded with granular brown pigment, and 4 to 6 rows of narrow tangentially elongated cells lying underneath this, endotesta is composed of oval shaped, 5 to 7 rows of thin walled parenchymatous cells embedded with brown granular pigments and prismatic crystals of calcium oxalate, followed by tangentially elongated small sized collapsed celled band of tegmen. The brown content is passing through hypodermis to tegmen. Cotyledon consists of an outer and inner layer of epidermis enclosing a

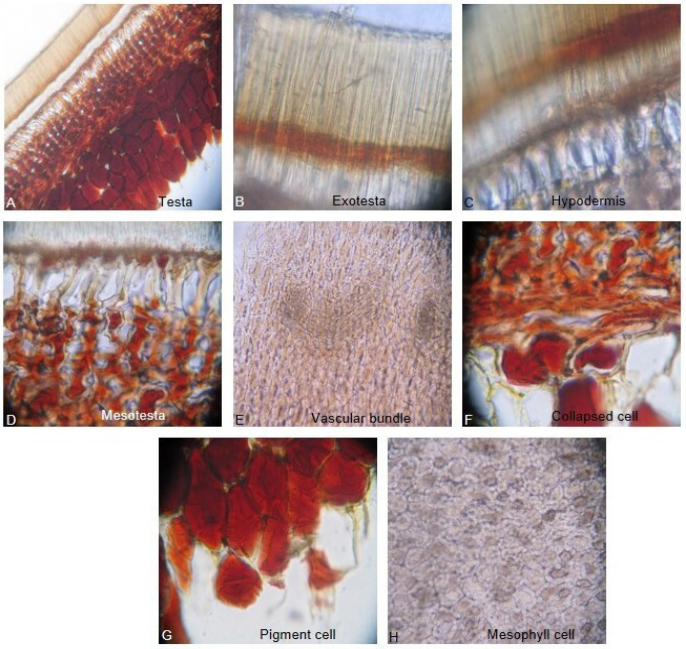


Figure 3b: Detailed microscopy of *Tamarindus indica* Linn. seed (A) Testa (B) Exotesta (C) Hypodermis (D) Mesotesta (E) Vascular bundle (F) Collapsed cell (G) Pigment cell (H) Mesophyll cell. (Prepared for full page width).

wide tissue of the mesophyll. Cells of the outer epidermis are smaller in size, tangentially elongated and are devoid of aleurone grains unlike the cells of inner epidermis which are bigger in size and embedded with aleurone grains, the cells of mesophyll are of different sizes, the centrally located cells being biggest, show characteristically beaded thickened, hemi cellulosic walls which becomes blue with iodine and traversed with vascular strands.

Powder Microscopy

Pinkish pale brown coloured coarse powder with astringent taste and without any characteristic odour.

The diagnostic powder characters shown in Figures 4a and 4b of the powder are fragments of exotesta (palisade cells) in surface view showing circular thick walled cells with brown contents; transversely cut fragments of exotesta showing palisade cells with bulbous swellings and warty line crossing across, bone shaped isolated, thick, irregular, sinuous walled cells of hypodermis of exotesta with brown pigment; isolated or groups of irregular cells of mesotesta with brown pigment; isolated or groups of cells of various sizes and shapes from endotesta; groups of thin walled fibrous cells, sclereids and pitted vessels from the hilar region; fragments of plumule and radical and prismatic crystals of calcium oxalate scattered as such throughout; fragment of cotyledon in surface view embedded with aleurone grains.

Analytical evaluation

Physico-chemical constants

The sample's physicochemical constants including ash values, extractive yields, and loss on drying, ph were systematically determined in Table 1.

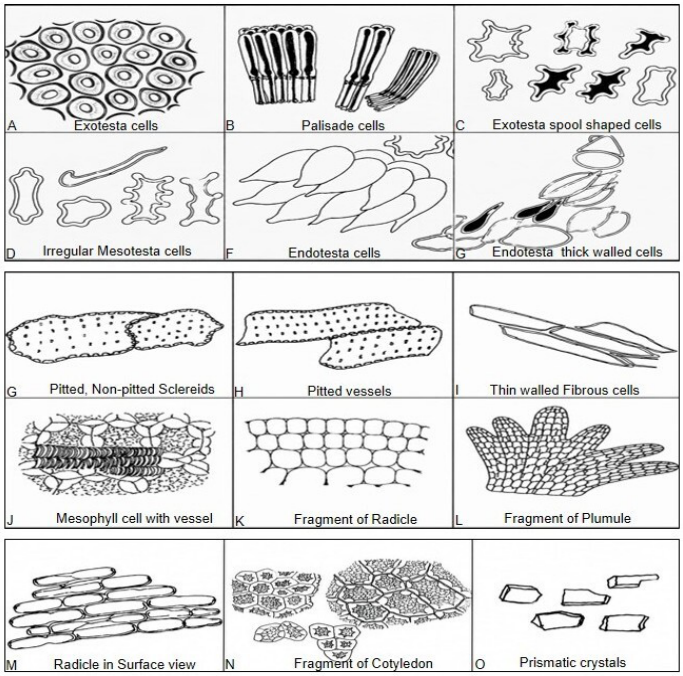


Figure 4a: Powder microscopy of *Tamarindus indica* Linn. seed (A) Exotesta (palisade cells) fragment in surface view (B) Exotesta fragment showing palisade cells with bulbous swellings and warty threads (C) Isolated thick-walled, spool-shaped exotesta cells with brown pigment (D) Isolated irregular mesotesta cells with brown pigment (E) Polymorphic brown endotesta cell groups (F) Thick-walled polymorphic brown endotesta cell groups (G) Pitted and non-pitted sclereids from hilar region (H) Pitted vessel from hilar region (I) Thin-walled hilar fibrous cell groups (J) Mesophyll surface view with embedded annular vessel (K) Fragment of radicle in sectional view (L) Fragment of plumule in sectional view (M) Fragment of radicle in surface view (N) Cotyledon in sectional view showing beaded, porous cells with aleurone grains and oil globules (O) Isolated prismatic crystals of calcium oxalate. (Prepared for full page width).

Table 1: Physicochemical constants of *Tamarindus indica* Linn. - Seed.

Sl. No.	Parameters	*Tamarindus indica* Linn.
1.	Foreign matter % W/W	-
2.	Loss on drying % W/W	8.973
3.	Ash value % W/W	97.507
4.	Acid-insoluble ash % W/W	2.463
5.	Water soluble extract %W/W	25.316
6.	Alcohol soluble extract %W/W	42.312
7.	Ether-soluble extract %W/W	9.872
8.	pH	6.8

% w/w: Percentage weight by weight; pH: Potential of hydrogen.

Preliminary qualitative test

Preliminary qualitative phytochemical screening of the seed extracts revealed the presence of Phenol, Flavones, Steroids, Tannins, Proteins, Amino acid, Carbohydrates as mentioned in Table 2.

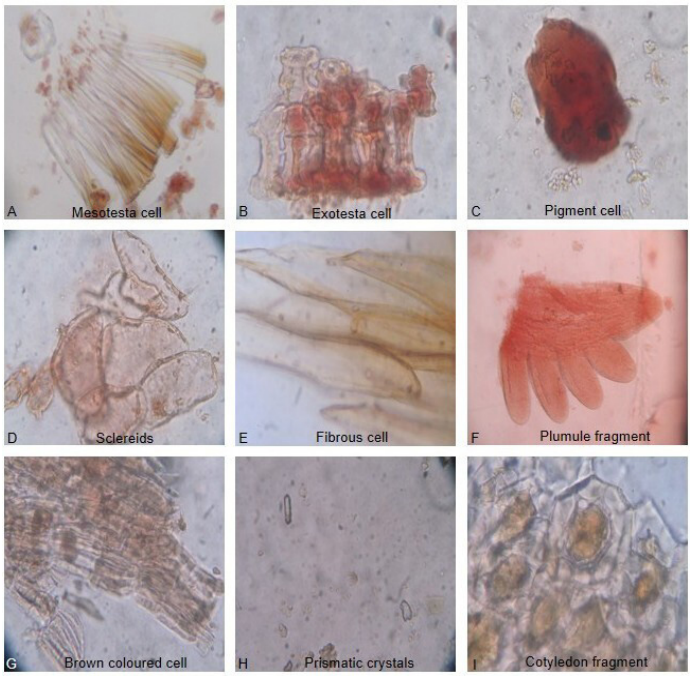


Figure 4b: Powder microscopy of *Tamarindus indica* Linn. seed (A) Mesotesta fragments with brown pigment (B) Exotesta spool shaped cells with brown pigment(C) Pigment cell (D) Pitted, Non-pitted Sclereids from hilar region (E) Group of fibrous cells from hilar region (F) Fragment of Plumule in section view (G) Group of brown coloured cells from endotesta (H) Prismatic crystal (I) Cotyledon fragment showing beaded cells, porous walls, aleurone grains, and oil globules. (Prepared for full page width).

Quantitative estimation

The quantitative values for the total tannin, protein content and swelling index of the seed sample are detailed in Table 3.

HPTLC (High Performance Thin Layer Chromatography)

In sample of methanolic extract of *Tamarindus indica* Linn. seed, 23 spots were seen under 254 nm and 21 spots were seen under 366 nm. After spraying with vanillin sulfuric acid (For detection of Tannin), 3 pinkish red spot were seen in sample of methanolic extract of *Tamarindus indica* Linn. seed detailed in Table 4.

The components were present in such low concentrations that they weren't visible under Ultraviolet illumination, but they were easily detected and recorded *via* scanning densitometry shown in Figure 5.

DISCUSSION

The systematic evaluation of *Tamarindus indica* Linn. (Family: Fabaceae) seed established definitive macro-microscopic and physicochemical benchmarks that are essential for validating the identity and purity of this raw drug. Macroscopically as shown in Figure 1, the flat, subrectangular to oval-oblong dimensions (1-1.5 cm length and 0.5-0.8cm width) paired with the characteristic

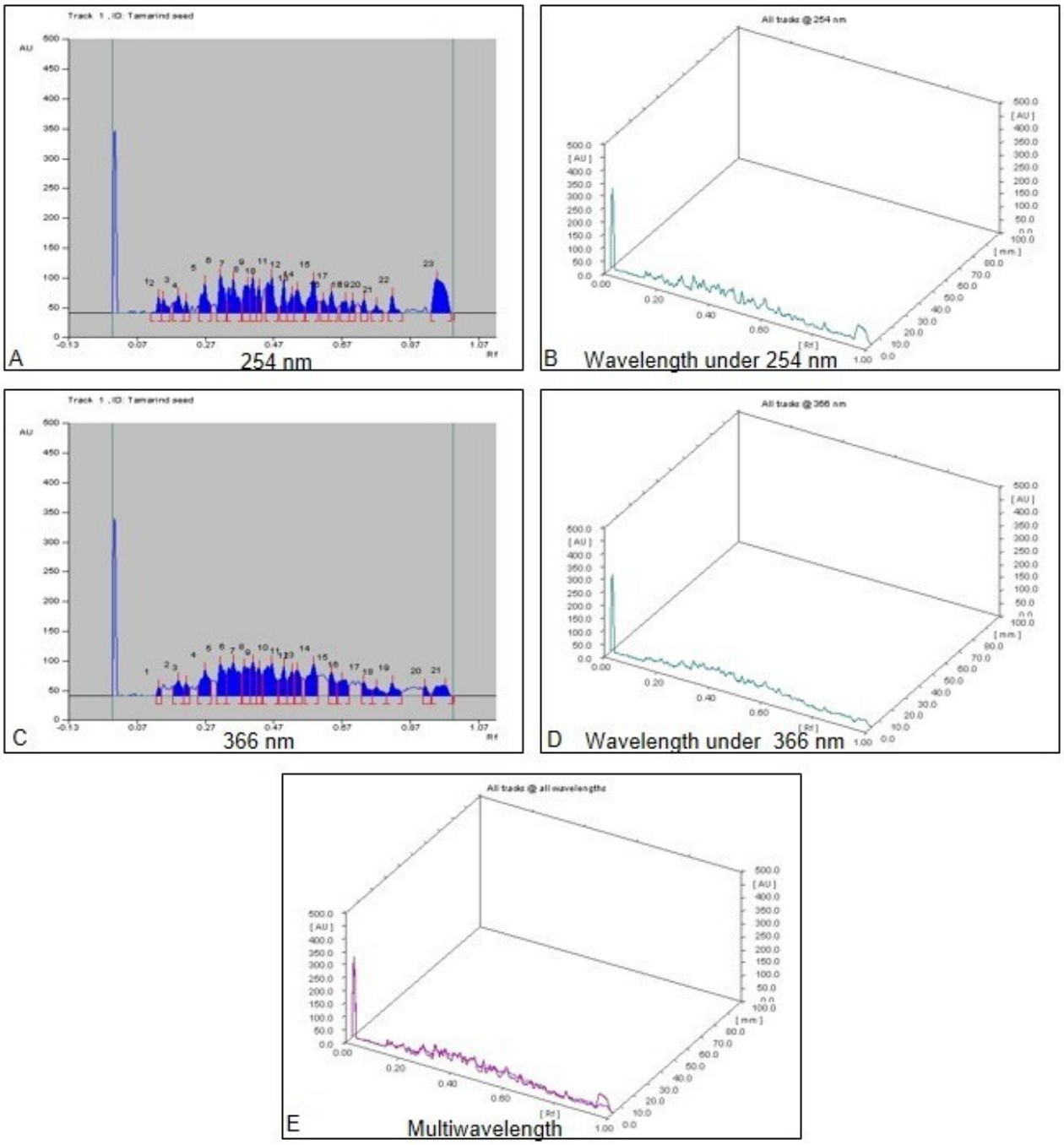


Figure 5: Densitogrammic scanned diagrams of *Tamarindus indica* Linn. (A) Under 254 nm (B) Wavelength under 254 nm (C) Under 366 nm (D) Wavelength under 366 nm (E) Multiwavelength. (Prepared for full page width).

basal hilum groove and bulky, whitish cotyledons serve as reliable macroscopic diagnostic features.

To ensure precision, the anatomical evaluation was confirmed through microscopic profiling (Transverse Section, Longitudinal Section and Radial Longitudinal Section). The prominent, horizontally placed plano-convex cotyledons encircled by thick brown coloured outer testa form the fundamental structural layout of the seed as shown in Figure 2A. The diagrammatic Longitudinal Section as shown in Figure 2B is quadrangular, showing a thick outer testa with hilar and micropylar scars enclosing bulky white cotyledons and the embryonic axis at the micropylar end. In contrast, the rectangular Radial Longitudinal Section as shown in Figure 2C reveals a vertically oriented cotyledon with a central narrow slit and the radicle directed toward the micropyle. At the detailed microscopical level as shown in Figures 3a and 3b, the compact arrangement of very long, narrow palisade cells in the exotesta, protected by a heavily thickened, slightly lignified

Table 2: Preliminary qualitative tests of *Tamarindus indica* Linn. - Seed.

Sl. No.	Constituents	*Tamarindus indica* Linn.
1.	Phenols	+
2.	Flavones	+
3.	Steroid	+
4.	Quinones	-
5.	Tannins	+
6.	Amino acid	+
7.	Protein	+
8.	Starch grains	-
9.	Terpenoid / Fatty acid	+
10.	Resins	-
11.	Alkaloids	-
12.	Volatile oil	-
13.	Glycosides	-
14.	Saponins	-
15.	Carbohydrates	+

'+' = Present, '-' = Absent.

Table 3: Quantitative estimation of *Tamarindus indica* Linn. - Seed.

Sl. No.	Constituents	*Tamarindus indica* Linn.
1.	Tannin %	5.023
2.	Protein %	13.44
3.	Swelling Index	2

%: Percentage by weight.

cuticle, represents a vital structural defensive barrier rich in dark brown pigments. The presence of these brown granular pigments across the bone-shaped hypodermal cells, the radially elongated mesotesta, and the parenchymatous endotesta highlights a highly uniform distribution of secondary metabolites throughout the seed coat layers.

When evaluating commercial raw materials that have been ground or processed, powder microscopy acts as a highly effective analytical tool for authentication. The powder microscopy characters observed in Figures 4a and 4b such as isolated bone-shaped hypodermal cells with sinuous walls, warty crossings on the palisade layers, uniquely bulbous-thickened mesophyll walls embedded with protein, and scattered prismatic calcium oxalate crystals provides an unalterable structural fingerprint. These specific cellular fragments make it possible to quickly identify authentic *T. indica* even within complex polyherbal mixtures or heavily pulverized commercial drugs.

The physicochemical data as described in Table 1 offers deep insights into the quality, purity, and chemical behaviour of the seed sample. The complete absence of foreign matter highlights the high purity baseline of the starting plant material. The observed loss on drying value (8.973%) is safe and typical for mucilage-rich seeds; this level indicates a healthy moisture profile that supports stability while pointing to a high concentration of water-binding hydrocolloids (Singh *et al.*, 2011; Saikia and Zothanpuia, 2019). Interestingly, the total ash value was exceptionally high (97.507%), pointing to an extensive concentration of inorganic mineral components naturally present within the seed matrix. The remarkably low acid-insoluble ash value (2.4637%) relative to the total ash content indicates an negligible presence of silica, thereby confirming that the seed sample is virtually free from external siliceous contaminants such as sand, soil, or earth.

The extractive values demonstrate a clear affinity for polar solvents, as the methanolic extract yielded the highest recovery (42.312%), followed by the water-soluble fraction (25.316%). This pattern suggests that the seed's metabolic profile consists heavily of polar and highly hydrophilic molecules. In contrast, the petroleum ether yield (9.872%) represents the smaller, non-polar lipid and fixed oil fraction of the kernel (Andriamanantena *et al.*, 1983; Razali *et al.*, 2015). The near-neutral pH value of 6.8 is ideal for formulation development, as it minimises the risk of chemical irritation or incompatibility during product manufacturing (Kar and Kar, 2018; Kulkarni *et al.*, 1997).

Preliminary phytochemical screening as described in Table 2 confirmed a rich diversity of bioactive compounds, showing positive reactions for phenols, flavones, steroids, tannins, proteins, amino acids and carbohydrates. Quantitative data as described in Table 3, indicates that the seed exhibits a rich therapeutic and nutritional profile, yielding 5.023%W/W of total tannins supporting its wound healing property (Mohamad *et al.*, 2011), a swelling index of 2 (reflecting its characteristic polysaccharide mucilage content), and a substantial total protein content of 13.44% supporting its anti-inflammatory activity (Lima *et al.*, 2022).

Finally, the High-Performance Thin-Layer Chromatography fingerprinting as described in Table 4 provided deep analytical resolution of the methanolic extract. Under short-wave Ultraviolet light (254 nm), the separation revealed a highly complex chemical profile consisting of 23 distinct spots distributed across the R_f spectrum. Under long-wave Ultraviolet light (366 nm), a similarly dense pattern of 21 fluorescent spots was documented.

When the plate was derivatized using vanillin-sulfuric acid reagent specifically to target tannin, three distinct pinkish-red bands emerged at lower R_f values (0.05, 0.08 and 0.1). Due to the low concentration of these components, they remained below the visual detection threshold under standard Ultraviolet visualisation. However, high-sensitivity scanning densitometry successfully captured and mapped their presence on the resulting densitogram as shown in Figure 5. Together, these High-Performance Thin-Layer Chromatography Retention Factor (R_f) patterns form a reliable, reproducible chemical blueprint

Table 4: HPTLC fingerprinting of methanolic extract of *Tamarindus indica* Linn. - Seed.

Sample name	Mobile phase	Observation under U.V.				Post chromatographic visualisation	
		254 nm.		366 nm.		After spraying	
		No. of spot	Max R_f	No. of spot	Max R_f	No. of spot	Max R_f
Methanolic extract of *Tamarindus indica* Linn. seed	Chloroform: Ethyl acetate: Acetic acid (5:4:1)	23	0.14,0.15, 0.19,0.22, 0.27,0.32, 0.35,0.40, 0.41,0.43 ,0.47,0.50, 0.53,0.54 ,0.59,0.62, 0.64,0.68, 0.70,0.74, 0.77,0.82, 0.95	21	0.14,0.19, 0.22,0.27, 0.32,0.35 ,0.39,0.41, 0.43,0.47 ,0.50,0.53, 0.54,0.59, 0.64,0.67, 0.74,0.77, 0.82,0.92, 0.97	3	0.05,0.08,0.1

HPTLC: High-Performance Thin-Layer Chromatography; R_f: Retardation factor; UV: Ultraviolet light.

that can be used to easily verify the quality and authenticity of *T. indica* seeds in industrial applications.

CONCLUSION

The present study establishes a definitive, multi-dimensional framework for *Tamarindus indica* Linn. seeds, delivering precise structural, physical, and chemical criteria to guarantee botanical identity, purity, and quality control. Macroscopic features like the subrectangular geometry and basal hilum groove are reinforced by distinctive tissue benchmarks, including a dense arrangement of narrow palisade cells in the exotesta, bone-shaped hypodermal cells, and a uniform distribution of brown granular pigments across the seed coat layers. For pulverized seed sample, specific powder microscopy characters namely isolated bone-shaped fragments, warty crossings, and scattered prismatic calcium oxalate crystals ensure reliable authentication even within complex polyherbal mixtures. Physicochemical profiling validates the excellent quality and cleanliness of the raw material, where an optimal moisture profile indicates high hydrocolloid content, and a low acid-insoluble ash value confirms the absence of external siliceous material. The high polar extractive values (of methanol, water) reveal a metabolic profile dominated by hydrophilic components, which aligns with a near-neutral pH that ensures excellent physiological compatibility and minimises the risk of chemical irritation or formulation incompatibility during pharmaceutical processing. Crucially, these quantitative findings directly substantiate the therapeutic profile of the seed matrix; the total tannin content provides a firm biochemical rationale for its wound-healing efficacy, the substantial protein reserve supports its documented anti-inflammatory activities, and a swelling index of 2 physically confirms a functional polysaccharide mucilage layer. Finally, high-resolution High-Performance Thin-Layer Chromatography fingerprinting maps the chemical complexity of the matrix by resolving 23 spots at 254 nm and 21 bands at 366 nm, while post-derivatization densitometric scanning successfully captures three low-migration tannin peaks that sit below the visual detection threshold under standard Ultraviolet light. Ultimately, this research successfully integrates structural, physical, and chemical metrics to create a complete quality control profile for *T. indica* seeds, directly correlating quantitative data with therapeutic logic to validate the seed's clinical utility and offer a reproducible industrial standard to prevent adulteration.

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ABBREVIATIONS

API: Ayurvedic Pharmacopoeia of India; **AR:** Analytical Reagent; **B.C.:** Before Christ; **FAA:** Formalin-Acetic Acid-Alcohol; **HPTLC:** High-Performance Thin-Layer Chromatography; **R_f:** Retardation Factor; **RLS:** Radial Longitudinal Section; **T. indica:** *Tamarindus indica*; **TAO:** 2-Hydroxy-3′,4′-dihydroxyacetophenone; **TA1:** Methyl 3,4-dihydroxybenzoate; **TA2:** 3,4-Dihydroxyphenylacetate; **TLC:** Thin-Layer Chromatography; **TS:** Transverse Section; **UV:** Ultraviolet; **v/v/v:** Volume by Volume by Volume; **w/w:** Weight by Weight.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

Madhavi H. Rabadia: Writing - original draft, Formal Analysis, Conceptualization. **Jignesh K. Kevalia:** Conceptualization, Resources, Supervision. **Krunal A. Doshi:** Formal analysis, Methodology, Supervision. **Manali Chotaliya:** Writing - review and editing. **Sejal Chauhan:** Writing - review and editing. **Vinay J. Shukla:** Conceptualization, Supervision.

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