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PHARMACOGNOSTIC EVALUATION AND STANDARDISATION OF THE STEM BARK OF *TERMINALIA TOMENTOSA* ROXB.

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Keywords:

Terminalia tomentosa, Pharmacognosy, Standardisation, Microscopy, Phytochemical screening, Extractive value, Quality control

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ABSTRACT: Background: *Terminalia tomentosa* Roxb. (Combretaceae), commonly known as the crocodile-bark tree, is a large deciduous tree whose stem bark occupies a prominent place in Ayurvedic, Siddha and folk medicine systems across India for the management of diarrhoea, bronchitis, fractures, haemorrhages and inflammatory conditions. Despite this long traditional use, the stem bark has not been subjected to a systematic pharmacognostic and physicochemical evaluation, leaving an important gap with respect to its authentication and quality control. **Objective:** The present study was undertaken to perform a comprehensive pharmacognostic and preliminary phytochemical standardisation of the stem bark of *T. tomentosa*, generating reference parameters for its identification and quality assessment. **Methods:** Authenticated stem bark collected from the Dandeli-Tinaighat forest Karnataka, was evaluated for organoleptic and morphological characteristics, transverse-section and powder microscopy and standard physicochemical parameters including moisture content, ash values, extractive values, fibre dimensions and foaming index. A 70% ethanolic extract was screened qualitatively for major phytoconstituents and quantitatively for total phenolic, flavonoid and tannin content using validated spectrophotometric assays referenced against gallic acid, quercetin and tannic acid standards, respectively. **Results:** The bark was dark grey to black, odourless, bitter and granular in fracture. Microscopy revealed a multilayered cork of 25-30 rectangular cell layers, prismatic calcium oxalate rosettes, simple and compound starch grains, phloem fibres and uniseriate to occasionally biseriate medullary rays. Moisture content was 6.6% w/w; total, acid-insoluble and water-soluble ash were 0.615 g, 0.35 g and 0.585 g respectively. Alcohol-soluble and water-soluble extractive values were 8.2% w/w and 3.8% w/w. Mean fibre length and width were 18.67 mm and 1.58 mm and the foaming index was below 100. Qualitative screening indicated the presence of tannins, triglycerides, flavonoids, saponins, carbohydrates and phenolic compounds, with alkaloids, proteins, steroids and glycosides absent in both extracts. Regression analysis yielded strong linearity for total phenolic (R^2 = 0.956), flavonoid (R^2 = 0.908) and tannin (R^2 = 0.985) content. **Conclusion:** The morphological, microscopical and physicochemical parameters established in this study provide a reliable reference profile for the identification, authentication and quality control of *T. tomentosa* stem bark and may serve as a foundation for future pharmacopoeial standardisation and pharmacological investigation.

INTRODUCTION: Plant-derived medicines continue to occupy a central place in global healthcare, particularly in regions such as India where traditional systems including Ayurveda and Siddha have documented the therapeutic use of botanical drugs for several millennia [1, 2, 3, 4, 5, 6].

Terminalia tomentosa Roxb. (ex-DC) Wight & Arn., synonymously known as *Terminalia alata* Heyne ex Roth and *Terminalia elliptica* Willd., is a large deciduous tree of the family Combretaceae, reaching heights of 20-35 metres and trunk diameters of up to one metre [7].

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It's rough, deeply fissured, dark grey to black bark has earned it the common name 'crocodile-bark tree' and forms the basis of its long-standing medicinal reputation [7, 15].

Classical Ayurvedic texts, including the Charaka Samhita, describe a decoction of the bark for the management of diabetes, rheumatism, fever, and urinary disorders, while folk and Siddha traditions employ bark and leaf preparations for diarrhoea, dysentery, piles, vertigo, constipation, bronchitis, fractures, and haemorrhagic conditions [15, 16, 17]. Phytochemical investigations on related *Terminalia* species have identified triterpenoids such as oleanolic and betulinic acid, the steroid β-sitosterol, and a range of tannins including arjunic acid, arjunolic acid, arjunetin, ellagic acid, and gallic acid, alongside reports of antifungal, antioxidant, anti-hyperglycaemic, anti-diarrhoeal, and antileucorrhoeal activity [9, 10, 11, 12, 13, 14].

Chronic inflammation underlies a wide spectrum of pathological conditions, including atherosclerosis, metabolic syndrome, arthritis, and various autoimmune disorders, for which current therapeutic options are dominated by steroidal and non-steroidal anti-inflammatory drugs and biologic agents such as adalimumab, both of which carry considerable adverse-effect burdens [7, 18]. This has renewed interest in plant-derived alternatives such as *T. tomentosa*, whose polyphenol- and tannin-rich bark may offer a safer therapeutic avenue, provided its identity and quality can be reliably established [8].

Despite the breadth of traditional and pharmacological interest in this species, a systematic literature search indicated that the stem bark of *T. tomentosa* has not previously been subjected to a comprehensive pharmacognostic evaluation encompassing organoleptic, microscopic, and physicochemical standardisation. The absence of such reference data limits the scope for reliable authentication, increases the risk of adulteration, and constrains the development of pharmacopoeial quality standards.

Pharmacognostic characterisation remains an indispensable first step in herbal drug standardisation, since it allows unambiguous identification of the crude drug independent of more resource-intensive analytical techniques, and provides the foundation upon which subsequent pharmacological and toxicological studies can be reliably built [19, 20, 21, 24].

Accordingly, the present study was designed with the primary objective of conducting pharmacognostic and quality-control evaluation of *T. tomentosa* stem bark, and the secondary objectives of characterising its organoleptic and physical properties and performing qualitative and quantitative screening of its principal phytochemical constituents.

MATERIALS AND METHODS:

Plant Material: Fresh stem bark of *T. tomentosa* was collected from the Dandeli-Tinaighat forest range, Karnataka, India. The plant material was identified and authenticated by the Assistant Professor and Head, Department of Botany, Rani Channamma University, Belagavi, M K Ganachari and a voucher specimen was retained for future reference (Annexure No: 10.1).

Chemicals and Reagents: All reagents used, including Mayer's, Dragendorff's, Wagner's, and Hager's reagents, Fehling's solutions A and B, Benedict's reagent, Millon's reagent, ninhydrin, Folin-Ciocalteu phenol reagent, aluminium chloride, sodium nitrite, sodium carbonate, and the reference standards gallic acid, quercetin, and tannic acid, were of analytical grade and procured from standard commercial suppliers.

Organoleptic and Morphological Evaluation: The colour, odour, taste, shape, size, and surface and fracture characteristics of the bark were assessed and recorded according to standard pharmacognostic protocols [19, 20].

Microscopic Evaluation: A transverse section of the stem bark was prepared, mounted, and examined under low- and high-power light microscopy [19, 20]. For powder microscopy, dried bark was coarsely powdered, cleared by boiling with chloral hydrate solution for 5-10 minutes, and stained with a 1:1 mixture of phloroglucinol and concentrated hydrochloric acid. Diagnostic elements including cork cells, cortex, medullary rays, phloem fibres, calcium oxalate crystals, mucilage cells, and starch grains were identified and photographed at 10x magnification.

Determination of Physicochemical (Proximate) Parameters: Alcohol-soluble (**Fig. 5**) and water-soluble (**Fig. 6**) extractive values were determined by cold maceration of accurately weighed, air-dried powdered bark (5.0 g for alcohol, 4.0 g for water) with the respective solvent for 24 and 6 hours followed by 18 hours of standing, after which an aliquot of filtrate was evaporated to dryness, dried at 105°C, and weighed. Moisture content was determined by loss on drying of 2 g powdered bark at 100-105°C to constant weight. Total ash, acid-insoluble ash, and water-soluble ash (**Fig. 7, 8, 9**) were determined by incineration of 2 g powdered bark at 500-600°C, followed by treatment of the resulting ash with dilute hydrochloric acid or water as applicable, in accordance with standard pharmacopoeial procedures [20, 22, 24, 26]. Fibre length and width were measured using a camera lucida on a minimum of twenty-five randomly selected, phloroglucinol-HCl-stained fibres. The foaming index (**Fig. 10**) was determined by serial dilution of an aqueous decoction in stoppered tubes followed by standardised shaking and assessment of foam height after fifteen minutes.

FIG. 1: BARK

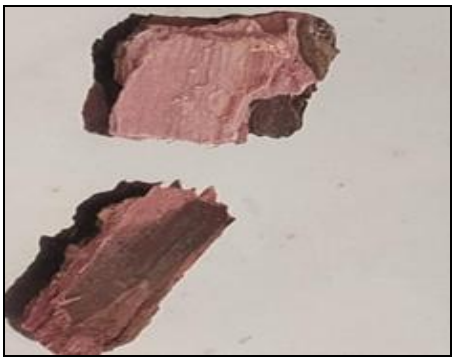


FIG. 2: INNER LAYER OF BARK

FIG. 3: FLOWERS

FIG. 4: LEAVES

Extraction: Five hundred grams of coarsely powdered, air-dried bark was extracted with 70% ethanol. The resulting extract was concentrated under reduced pressure using a rotary flash evaporator, dried over calcium carbonate in a desiccator, and weighed to determine percentage yield prior to phytochemical analysis.

FIG. 5: ALCOHOL SOLUBLE EXTRACTION

FIG. 6: WATER SOLUBLE EXTRACTION

FIG. 7: TOTAL ASH

FIG. 8: ACID INSOLUBLE ASH

FIG. 9: WATER SOLUBLE ASH

FIG. 10: FOAMING INDEX

FIG. 11: PHENOLS, FLAVONOIDS & TANNINS

Qualitative Phytochemical Screening: Aqueous and alcoholic extracts were screened for carbohydrates (Molisch's, Benedict's, Barfoed's, and Fehling's tests), proteins and amino acids (Millon's and ninhydrin tests), sterols and triterpenoids (Liebermann-Burchard and Salkowski tests), glycosides (acid- and water-hydrolysis Fehling's comparison tests), alkaloids (Mayer's, Dragendorff's, Wagner's, and Hager's tests), phenolic compounds (ferric chloride, Shinoda, and zinc-hydrochloride reduction tests), flavonoids (Shinoda test), and tannins (ferric chloride test), following established pharmacognostic protocols.

Quantitative Estimation of Phytoconstituents: Total phenolic content was estimated by the Folin-Ciocalteu method using a gallic acid standard curve, with absorbance read at 725 nm (**Fig. 15**). Total flavonoid content was estimated by the aluminium chloride colorimetric method using a quercetin standard curve, with absorbance read at 510 nm (**Fig. 16**). Total tannin content was

estimated by the Folin-Ciocalteu method using a tannic acid standard curve, with absorbance read at 700 nm (**Fig. 17)**.

All quantitative estimations were performed in triplicate, and results were expressed as the relevant standard equivalents per unit weight of extract.

Statistical Analysis: Quantitative data are expressed as obtained from triplicate determinations. Linear regression analysis was used to construct standard calibration curves for total phenolic, flavonoid, and tannin content, with the coefficient of determination (R^2) used to assess linearity.

RESULTS:

Organoleptic and Morphological Characteristics: The stem bark of *T. tomentosa* was dark grey to black in colour, odourless, and bitter in taste, with an irregular shape, a rough outer surface and a comparatively smooth inner layer, and a granular fracture. Morphological parameters are summarised in **Table 1.**

TABLE 1: MORPHOLOGICAL CHARACTERISTICS OF *T. TOMENTOSA* STEM BARK

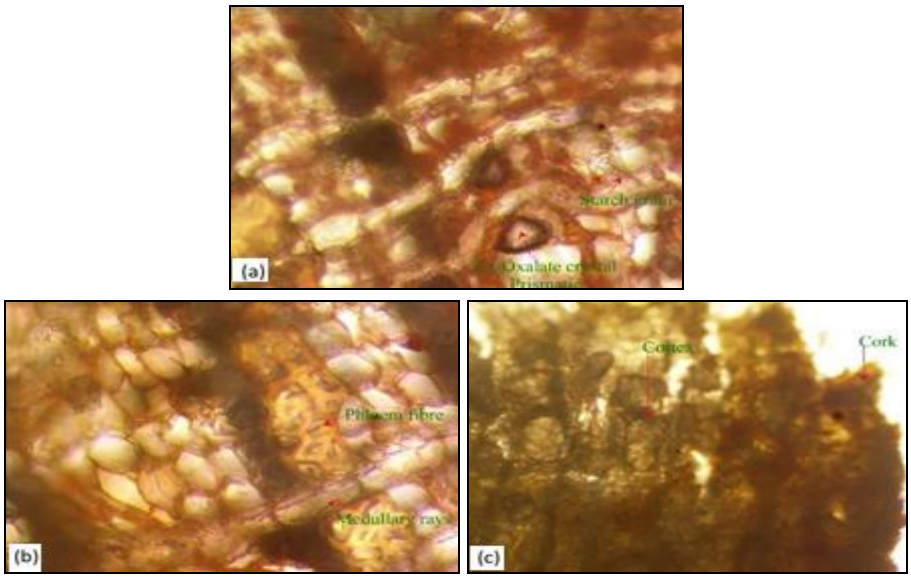
S. no.	Feature	Observation
1	Colour	Dark grey to black
2	Odour	Odourless
3	Taste	Bitter
4	Size	20-35 m height; 1 m diameter
5	Shape	Irregular
6	Inner layer	Slightly smooth
7	Outer layer	Rough
8	Fracture	Granular

Microscopic Characteristics: Transverse-section microscopy of the bark revealed a multilayered cork zone composed of approximately 25-30 layers of rectangular cork cells, an underlying cortex region, prismatic rosette crystals of calcium oxalate distributed through the cortex, simple and compound starch grains, phloem fibres, and medullary rays that were predominantly uniseriate with occasional biseriate arrangement.

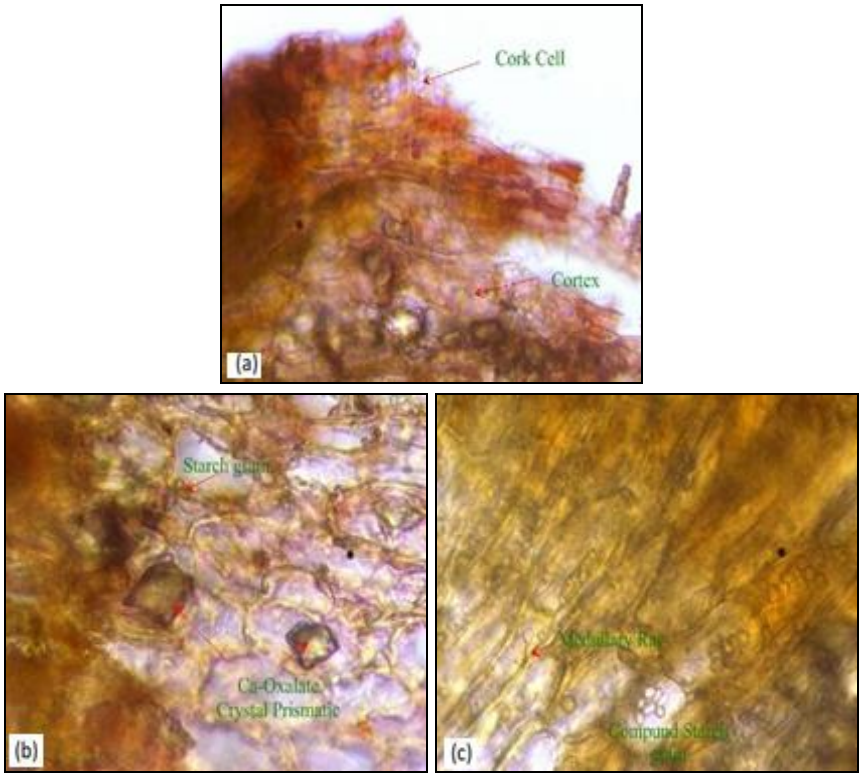
Powder microscopy confirmed the presence of thin, elongated, flexible fibres, thick gummy mucilage cells, prismatic calcium oxalate crystals, and rectangular cork cells, consistent with the transverse-section findings. The coarse powder was light brown in colour, odourless, and exhibited the characteristic taste of the crude drug. These findings are summarised in **Table 2.**

TABLE 2: POWDER MICROSCOPIC CHARACTERISTICS OF *T. TOMENTOSA* STEM BARK

S. no.	Feature	Observation
1	Nature	Coarse powder
2	Colour	Light brown
3	Odour	Odourless
	Microscopic	
4	Fibres	Thin, long, flexible thread-like structures
5	Mucilage cells	Thick, gluey substance
6	Calcium oxalate / starch	Rosette crystals with simple and compound starch grains scattered through the cortex
7	Cork	Multilayered, 25-30 layers of rectangular cork cells
8	Medullary rays	Predominantly uniseriate, occasionally biseriate



(A) T.S. OF *TERMINAL TOMENTOSA* BARK SHOWS: STARCH GRAIN, CA-OXALATE CRYSTAL PRISMATIC, (B) PHLOEM FIBRE, (C) MEDULLARY RAYS CORK & CORTEX REGION



(A) T.S. OF *TERMINAL TOMENTOSA* BARK SHOWS: CORK & CORTEX REGION (B) STARCH GRAIN, CA-OXALATE CRYSTAL PRISMATIC (C) MEDULLARY RAYS, COMPOUND STARCH GRAINS

FIG. 12: TRANSVERSE SECTION OF THE BARK OF *T. TOMENTOSA*

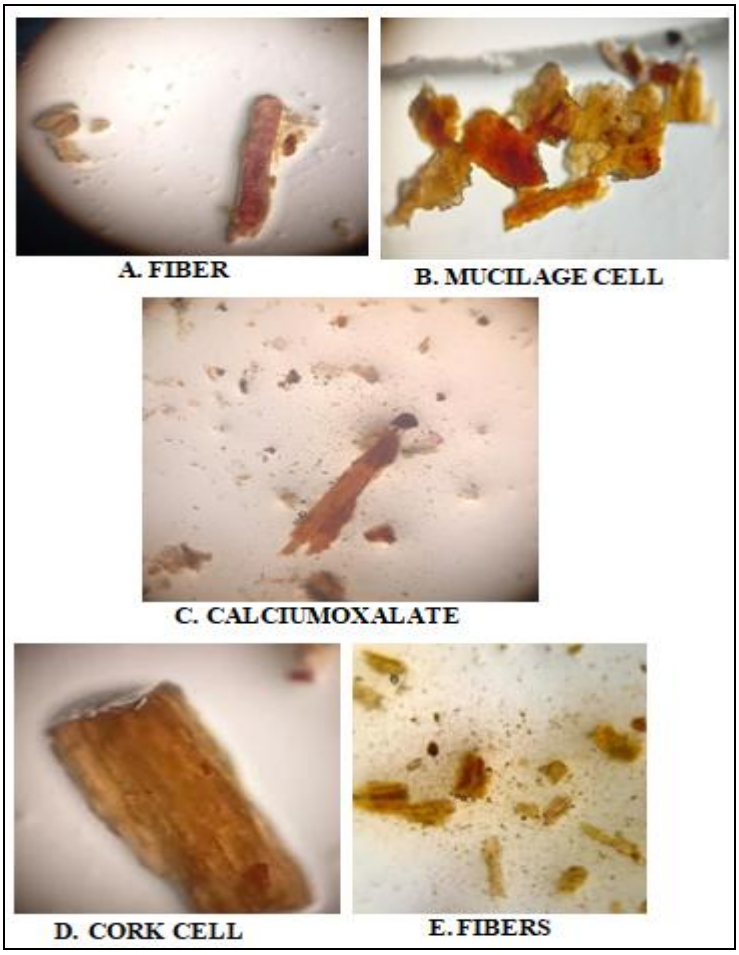


FIG. 13: POWDER CHARACTERISTICS OF BARK OF PLANT OF *TERMINALIA TOMANTOSA* ROXB

Physicochemical (Proximate) Parameters: The alcohol-soluble and water-soluble extractive values of the powdered bark were 8.2% w/w and 3.8% w/w, respectively, indicating a moderate proportion of polar constituents such as phenolics and flavonoids extractable by both solvent systems. Moisture content, determined by loss on drying, was 6.6% w/w, falling within the generally accepted limit for crude plant drugs and suggesting low susceptibility to microbial or fungal degradation on storage. Total ash, acid-insoluble ash, and water-soluble ash were found to be 0.615 g, 0.35 g, and 0.585 g respectively, parameters that are of particular value in assessing the purity of the drug and the presence of inorganic or siliceous contaminants. Fibre dimension analysis showed a mean fibre length of 18.67 mm (range 9.29-46.11 mm) and a mean fibre width of 1.58 mm (range 1.11-2.97 mm). The foaming index of the aqueous decoction was determined to be less than 100, indicating a low saponin-related foaming capacity. These results are summarised in **Table 3-6.**

TABLE 3: EXTRACTIVE VALUES (COLD MACERATION)

S. no.	Parameter	Value (% w/w)
1	Alcohol-soluble extractive value	8.2
2	Water-soluble extractive value	3.8

TABLE 4: ASH VALUES AND MOISTURE CONTENT

S. no.	Parameter	Determined value
1	Total ash	0.615 g
2	Acid-insoluble ash	0.35 g
3	Water-soluble ash	0.585 g
4	Moisture content by loss on drying	6.6% w/w

TABLE 5: FIBRE LENGTH AND WIDTH DETERMINATION

S. no.	Parameter	Value
1	Minimum fibre length	9.29 mm
2	Maximum fibre length	46.11 mm
3	Average fibre length	18.67 mm
4	Minimum fibre width	1.11 mm
5	Maximum fibre width	2.97 mm
6	Average fibre width	1.58 mm

FIG. 14: DETERMINATION OF FIBER LENGTH

Extractive Yield and Physical Characteristics:

TABLE 6: PERCENTAGE YIELD AND PHYSICAL CHARACTERISTICS OF EXTRACTS

Extract	% Dry weight (g)	Colour	Odour	Consistency
Aqueous (40-60°C)	3.8	Brownish red	Characteristic	Powder
Alcoholic	8.2	Brownish red	Characteristic	Powder

Qualitative Phytochemical Screening: Preliminary qualitative screening of both the aqueous and alcoholic extracts demonstrated the presence of tannins, triglycerides, flavonoids, saponins, carbohydrates, and phenolic compounds. Alkaloids, proteins, steroids, and glycosides were not detected in either extract, suggesting that the principal bioactive load of the bark resides in its polyphenolic and tanniferous fraction. The complete qualitative screening profile is presented in **Table 7.**

TABLE 7: QUALITATIVE PHYTOCHEMICAL SCREENING OF *T. TOMENTOSA* BARK EXTRACTS

S. no.	Constituent	Aqueous extract	Alcoholic extract
1	Alkaloids	Absent	Absent
2	Proteins	Absent	Absent
3	Tannins	Present	Present
4	Steroids	Absent	Absent
5	Triglycerides	Present	Present
6	Glycosides	Absent	Absent
7	Flavonoids	Present	Present
8	Saponins	Present	Present
9	Carbohydrates	Present	Present
10	Phenols	Present	Present

Quantitative Estimation of Phytoconstituents: Linear regression analysis of the standard calibration curves confirmed good linearity across the concentration ranges tested for all three assays: total phenolic content ($y = 0.0045x + 0.1885$, $R^2 = 0.956$), total flavonoid content ($y = 0.0064x + 0.7772$, $R^2 = 0.908$), and total tannin content ($y = 0.0067x + 0.6753$, $R^2 = 0.985$). The absorbance values recorded for the test extract across the assayed concentration range increased proportionally with concentration in each assay, consistent with a dose-dependent presence of phenolics, flavonoids, and tannins in the ethanolic bark extract. Detailed absorbance data are presented in **Table 8-10.**

TABLE 8: TOTAL PHENOLIC CONTENT DETERMINATION (GALLIC ACID EQUIVALENT; $R^2 = 0.956$)

Sample	Absorbance at 725 nm
Standard 25 µg/ml	0.543
Standard 50 µg/ml	1.543
Standard 100 µg/ml	2.130
Test 25 µg/ml	0.274
Test 50 µg/ml	0.490
Test 100 µg/ml	0.629

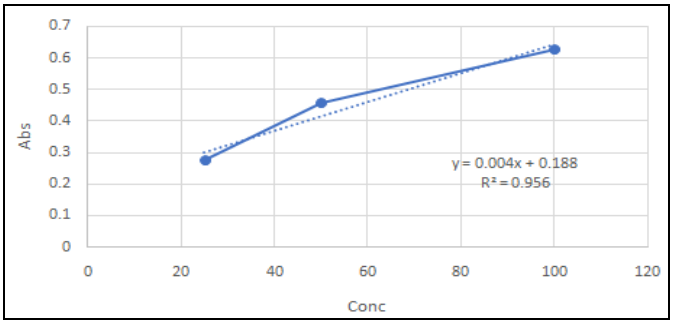


FIG. 15: TOTAL PHENOL

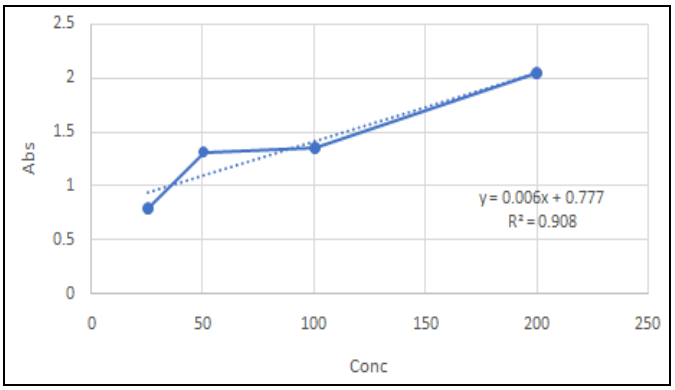


FIG. 16: TOTAL FLAVONOIDS

TABLE 9: TOTAL FLAVONOID CONTENT DETERMINATION (QUERCETIN EQUIVALENT; $R^2 = 0.908$)

Sample	Absorbance at 510 nm
Blank	0.001
Test 25 µg/ml	0.013
Test 50 µg/ml	0.036
Test 100 µg/ml	0.074
Test 200 µg/ml	0.129

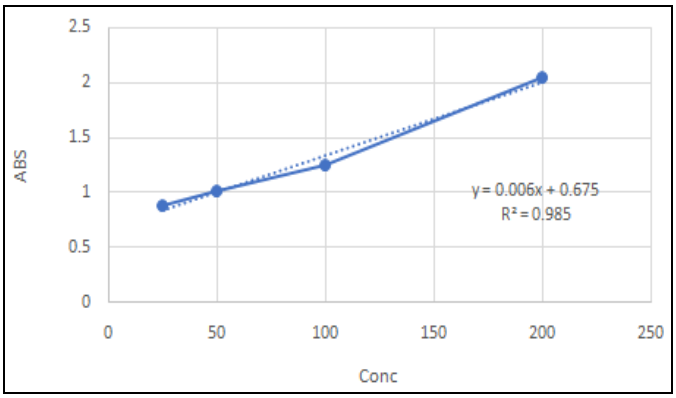


FIG. 17: TOTAL TANNIN

TABLE 10: TOTAL TANNIN CONTENT DETERMINATION (TANNIC ACID EQUIVALENT; R^2 = 0.985)

Sample	Absorbance at 700 nm
Standard 25 µg/ml	0.814
Standard 50 µg/ml	1.486
Standard 100 µg/ml	2.158
Standard 200 µg/ml	2.255
Test 25 µg/ml	0.287
Test 50 µg/ml	0.412
Test 100 µg/ml	0.848
Test 200 µg/ml	1.545

DISCUSSION: This study generated, for the first time, a consolidated set of organoleptic, microscopic, and physicochemical reference parameters for the stem bark of *T. tomentosa*, addressing a notable gap in the pharmacognostic literature on this ethnomedicinally important species. The macroscopic features observed, namely the dark grey to black, deeply fissured, granular-fracture bark, are consistent with field descriptions of the species and support its common designation as the crocodile-bark tree, while providing a practical basis for preliminary field-level authentication. Microscopic evaluation provided more discriminating diagnostic value. The presence of a thick, multilayered cork zone, prismatic calcium oxalate rosettes, simple and compound starch grains, and predominantly uniseriate medullary rays constitutes a characteristic anatomical fingerprint that can be used to distinguish genuine *T. tomentosa* bark from morphologically similar adulterants or substitutes, a recurring concern in the herbal trade given the morphological overlap among several *Terminalia* species [19, 20, 25].

Among the physicochemical parameters, total ash and its acid-insoluble and water-soluble fractions are of particular relevance to drug purity, since elevated values may indicate contamination with sand, soil, or other inorganic material [22, 24]. The moderate ash and extractive values obtained in this study fall within ranges generally regarded as acceptable for bark-derived crude drugs, although direct comparison with pharmacopoeial limits is constrained by the absence of an existing official monograph for this species. The comparatively higher alcohol-soluble extractive value relative to the water-soluble value is consistent with the qualitative screening results, which indicated a predominance of moderately polar constituents, including tannins, flavonoids, and other phenolics, that partition preferentially into hydroalcoholic solvent systems. The low foaming index obtained suggests a comparatively limited saponin-driven surface activity in the aqueous decoction, despite the qualitative detection of saponins, indicating that saponin content, while present, is not the dominant constituent class.

The qualitative phytochemical profile, characterised by the consistent presence of tannins, flavonoids, phenolics, triglycerides, saponins, and carbohydrates alongside the absence of alkaloids, proteins, steroids, and glycosides in both extracts, aligns broadly with prior phytochemical reports on related *Terminalia* species, which have similarly emphasised a polyphenol- and triterpenoid-dominated constituent profile [9, 10, 12, 13, 14]. The strong linearity obtained for the phenolic, flavonoid, and tannin calibration curves (R^2 values of 0.956, 0.908, and 0.985 respectively) lends confidence to the quantitative estimations and indicates that the spectrophotometric assays employed are well suited to future batch-to-batch quality comparisons of this crude drug. Given the established association between polyphenolic and tannin content and antioxidant, anti-inflammatory, and antimicrobial activity in related species, [4, 13, 18] the relatively rich tannin and phenolic content identified here provides a plausible phytochemical basis for several of the traditional indications historically ascribed to *T. tomentosa* bark, including its use in diarrhoea, wound healing, and inflammatory conditions, although this inference requires confirmation through dedicated pharmacological evaluation. This study is not without limitations. The investigation was restricted to a single geographical collection site and a single extraction solvent system for quantitative analysis, and did not extend to chromatographic fingerprinting (such as HPTLC or HPLC) or to isolation and structural elucidation of individual marker compounds. Seasonal and geographical variation in phytoconstituent content, a well-recognised phenomenon in medicinal plants, was also outside the scope of the present work. Future studies incorporating multi-site sampling, chromatographic marker profiling, and correlated pharmacological evaluation would substantially strengthen the standardisation framework proposed

here and support its eventual incorporation into formal pharmacopoeial monographs.

CONCLUSION: The present investigation establishes a comprehensive pharmacognostic and physicochemical reference profile for the stem bark of *Terminalia tomentosa*, encompassing organoleptic, microscopic, and quantitative physicochemical parameters together with a qualitative and quantitative phytochemical profile. These findings provide a scientifically grounded basis for the identification, authentication, and quality control of this ethnomedicinally significant crude drug and may inform the development of future pharmacopoeial standards. Pharmacognostic standardisation of this nature, although more traditional than instrumental analytical techniques, remains a dependable and accessible first step in herbal drug authentication and provides a necessary foundation for the pharmacological and toxicological evaluation of *T. tomentosa* in subsequent research.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest."

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