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Original Article

Comparative Pharmacognostic Evaluation of *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn. and *Terminalia elliptica* Willd.

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ABSTRACT

Background: *Terminalia arjuna* (Arjuna) and *Terminalia elliptica* (Asna or Saj) are two closely related medicinal tree species known for their therapeutic applications. Despite their everyday use in Ayurveda, detailed comparative pharmacognostic studies on their barks remain limited. This study aimed to perform a systematic comparison of the barks of *T. arjuna* and *T. elliptica* using macroscopic and microscopic analyses, physicochemical profiling, phytochemical screening, and advanced instrumental characterisation to identify distinguishing features and assess quality parameters. **Materials and Methods:** Bark samples from both species were evaluated for their macroscopic and microscopic characteristics, followed by assessments of physicochemical parameters, including total ash, extractive values, and moisture content. A qualitative phytochemical analysis was conducted to identify primary and secondary metabolites. Spectral and chromatographic fingerprinting was performed using Fourier-Transform Infrared Spectroscopy (FTIR) and High-Performance Thin-Layer Chromatography (HPTLC). Microbial testing was conducted to evaluate total viable counts and identify potential pathogenic microorganisms, including *E. coli*, Staphylococcus aureus, Pseudomonas, and Salmonella spp. **Results:** While both species displayed similar anatomical features and FTIR profiles indicative of polyphenolic compounds, notable differences emerged in their physicochemical and phytochemical properties. *T. elliptica* had a higher total ash content, indicating more minerals, and contained alkaloids, which *T. arjuna* lacked. Conversely, *T. arjuna* had a higher extractive value and a distinct arjunolic acid band on HPTLC. Both powders met acceptable microbial safety standards. **Conclusion:** The findings provide clear diagnostic features for distinguishing *T. arjuna* from *T. elliptica*, confirming their traditional uses and highlighting the quality and safety of these species.

Keywords: FTIR Spectroscopy, HPTLC, Pharmacognostic Evaluation, Phyto-chemical Profiling, *Terminalia Arjuna* and *Terminalia Elliptica*.

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INTRODUCTION

The genus *Terminalia* (Combretaceae) comprises numerous tree species widely used in traditional medicine. In the Indian subcontinent, the bark of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. is a venerable Ayurvedic remedy, particularly acclaimed for cardiovascular support. It contains diverse bioactive constituents, notably flavonoids, polyphenols, triterpenoid glycosides, tannins, and minerals, that underlie its antioxidant, anti-inflammatory, and cardioprotective effects. *Terminalia elliptica* Willd. (syn. *T. tomentosa*), commonly known as Asna or Saj, is a related Combretaceae tree native to India that is also used medicinally (for wound healing, diarrhoea, and as an astringent) (Amalraj & Gopi, 2016). Recent reviews note that *T. elliptica* exhibits broad pharmacological activities, including antimicrobial, anti-inflammatory, anticancer, antidiabetic, antioxidant, hepatoprotective, and neuroprotective effects, likely reflecting its content of phenolics and other metabolites (Das *et al*., 2020). Although both species share a family lineage and some similar traditional uses, detailed comparative analyses of their bark chemistry and pharmacognosy are lacking.

The present study was undertaken to enable a side-by-side comparison of *T. arjuna* and *T. elliptica* bark and to evaluate key pharmacognostic parameters: macroscopic and microscopic characteristics, physicochemical parameters, and qualitative

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phytochemical profiles of the two barks. Advanced analytical techniques, Fourier-Transform Infrared (FTIR) spectroscopy, and High-Performance Thin-Layer Chromatography (HPTLC), were applied to profile and fingerprint the bark extracts. Additionally, the powdered bark samples were examined microscopically for diagnostic anatomical features. The microbial quality of each bark powder was also tested by determining total viable aerobic counts, fungal counts, and the presence of specific contaminating bacteria. The aim was to identify similarities and distinguishing features that could inform their pharmacological activities, ensure correct identification and quality control in herbal preparations, and provide a scientific basis for their safe medicinal use.

MATERIALS AND METHODS

Specimen Collection and Authentication

Bark samples of *Terminalia arjuna* and *Terminalia elliptica* were collected from their natural habitats in June 2023 (Figure 2). The botanical identification and authentication of the plant specimens were carried out by the Taxonomist and Herbarium Laboratory of the Regional Raw Drug Repository (RRDR) at the All India Institute of Ayurveda (AIIA), New Delhi, India. Voucher specimens have been deposited for future reference under the authentication numbers RRDR/AIIA/173 for *T. arjuna* and RRDR/AIIA/174 for *T. elliptica*.

Macroscopic Evaluation

The raw bark pieces of *T. arjuna* and *T. elliptica* were examined for macroscopic characteristics. Each specimen's outer surface and inner surface were observed for colour, texture, shape, size, and any distinguishing features. Notable traits (such as surface smoothness or fissuring, thickness of bark, and exfoliation patterns) were recorded for comparison.

Preparation of Bark Powder

The collected bark from both species was dried in a ventilated oven at 40 °C for 5-7 days to reduce moisture content. Once fully dehydrated, the bark was coarsely crushed and then grind into a fine powder using a laboratory grinder. The resulting powders were sieved through a #80 mesh to ensure uniform particle size. The prepared *T. arjuna* and *T. elliptica* bark powders were stored in airtight containers and used for all subsequent analyses.

Microscopy

Transverse Section

Mature bark were initially soaked in water to soften the tissues. Thin cross-sections were prepared using a sharp blade and mounted on glass slides with glycerine. These sections were then observed under a light microscope to study the internal anatomical features, Including typical anatomical characteristics numerous rosette-shaped crystals of calcium oxalate (Evans, 2009).

Powder Microscopy

A small amount of bark powder was placed on a glass slide with glycerine as the mounting medium, covered with a coverslip, and examined microscopically. The diagnostic characteristics noted included xylem tracheids, annular thickenings, starch grains, stone cells, ramified pits, oil globules, fibres, and crystals (CBSPD, n.d.).

Organoleptic Evaluation

The organoleptic properties of the *T. arjuna* and *T. elliptica* bark powders were noted by sensory evaluation. This included observing the powder colour and texture, smelling the aroma, and tasting a minute sample to assess flavour using standard organoleptic techniques. The overall appearance and consistency of the powders were also recorded (Edo *et al*., 2024).

Physicochemical Parameters

Several physicochemical quality control parameters were determined following standard procedures described in the Ayurvedic Pharmacopoeia of India (API). The pH of a 10% (w/v), (Aspect and pH Determination of 10% w/v Aqueous Solution of Semisolid... | Download Scientific Diagram, n.d.) Moisture content (loss on drying),(Determination of Moisture Content of Herbal Drug by Loss on Drying Method (LOD): Experiment Findings, n.d.) Total ash, (Total Ash, Acid Insoluble Ash, Water Soluble Extractive Value and... | Download Scientific Diagram, n.d.) Water-soluble ash, (Total Ash, Acid Insoluble Ash, Water Soluble Extractive Value and... | Download Scientific Diagram, n.d.) and the acid-insoluble ash, (Total Ash, Acid Insoluble Ash, Water Soluble Extractive Value and... | Download Scientific Diagram, n.d.) Alcohol-soluble extractive and water-soluble extractive

Phytochemical Screening

Qualitative phytochemical screening was performed on the aqueous and methanolic extracts of *T. arjuna* and *T. elliptica* bark to identify the presence of major secondary metabolite classes. Standard colorimetric and precipitative tests were conducted for alkaloids (e.g. Dragendorff's test), flavonoids (Shinoda test or alkaline reagent test), tannins and phenolics (Ferric chloride test), glycosides (Bornträger's test for anthraquinones, and general glycoside tests), saponins (foam test), coumarins, steroids/terpenoids (Salkowski test for sterols), and proteins/amino acids (Biuret test or Xanthoproteic test). A positive result in each test was recorded based on the appearance of the expected color change or precipitate, and results for each phytochemical class were noted as present (+) or absent (–) for both species.

HPTLC Analysis

High-Performance Thin-Layer Chromatography (HPTLC) was used to develop chemical fingerprints of the bark extracts. **Chemicals and reagents:** All solvents and reagents were of

analytical grade. Methanol (99.8%, Qualigens, India), chloroform (99%, Merck-Emplura), acetic acid (99.5%, Qualigens), toluene (99.8%, Prayog Fine Chem, India), ethyl acetate (99.8%, Merck), and formic acid (85%, Qualigens) were used in the preparation of mobile phases and samples.

Instrumentation and chromatographic conditions

HPTLC was performed on silica gel 60 F254 precoated plates (0.2 mm layer thickness; E. Merck). Sample application was done with a CAMAG Linomat 5 semi-automatic applicator (CAMAG, Switzerland) using a 100 µL syringe, applying bands ~8 mm wide of each sample solution. The plate development was carried out in a CAMAG Twin-Trough chamber pre-saturated with the mobile phase for 30 min. A mobile phase of chloroform:toluene:ethanol in 4:4:1 (v/v/v) ratio was used to resolve the constituents. After sample application, the plate was developed in the chamber to a distance of ~8 cm. Upon development, the plate was removed, dried at 60 °C on a CAMAG TLC Plate Heater, then derivatized by spraying with anisaldehyde-sulfuric acid reagent. The derivatized plate was heated at 105 °C for 5 min to develop coloured bands. Visualization of the resolved bands was performed under Ultraviolet (UV) light (254 nm and 366 nm) and in visible light after derivatization. Documentation was managed through CAMAG Vision CATS software (version 3.2). Densitometric scanning of the plate was carried out with a CAMAG TLC Scanner (deuterium and mercury lamps) in absorbance mode at 540 nm (post-derivatization), with a scanning speed of 20 nm/s.

Preparation of extracts for HPTLCFor each species, 5 g of the dried bark powder was Soxhlet-extracted with 100 mL of methanol for 6–8 hr. The extracts were filtered through Whatman No. 1 paper and concentrated to dryness under reduced pressure at 40 ± 2 °C using a rotary evaporator. The dried extract residues were weighed and then re-dissolved in methanol to obtain a stock solution of 20 mg/mL. These solutions were filtered through a 0.45 µm membrane filter to remove any particulates prior to HPTLC application. The sample solutions were stored in amber vials at 4 °C until analysis. For chromatographic fingerprinting, an appropriate volume of each extract (e.g. 5-10 µL) was applied to the HPTLC plate. A mixed solvent system of Chloroform:Toluene:Ethanol (4:4:1) was utilized as mentioned above. After development and visualization, the Rf values and colour of resolved bands for each extract were recorded for comparative analysis.

FTIR Spectroscopy

Fourier-transform infrared spectra of the bark extracts were obtained to identify functional groups present. A PerkinElmer Spectrum 2 FTIR spectrometer equipped with a Universal ATR (attenuated total reflectance) accessory was used to scan the samples in the mid-infrared range of 4000–400 cm^{-1}. Each dried methanolic extract (powder) was placed directly on the ATR crystal without any further preparation. Spectra were acquired with an appropriate number of scans and resolution (typically 4 cm^{-1} resolution) to ensure a good signal-to-noise ratio. Background correction was applied before each sample run. The major absorption bands in the resultant FTIR spectra for *T. arjuna* and *T. elliptica* were recorded. Spectral data were analyzed using PerkinElmer Spectrum IR software (v10.7.2) to identify characteristic peaks corresponding to various functional groups. The analysis was conducted at the Analytical Chemistry Laboratory, AIIA, New Delhi.

Microbial Analysis

Microbial load and the presence of specific contaminating bacteria were assessed for each bark powder to evaluate their purity and safety.

Total Plate Count (TPC)

A total aerobic bacterial count was performed by the standard plate count method. Each sample (1 g of bark powder) was aseptically suspended in sterile normal saline and subjected to a series of ten-fold dilutions. From appropriate dilutions, 0.1 mL was spread evenly onto nutrient agar plates (in triplicate) for each sample. The plates were incubated at 37 °C for 24-48 hr, after which colonies were counted. The results were expressed as colony-forming units per gram (CFU/g) of sample.

Total Yeast and Mold Count (TYMC)

Similarly, for fungal counts, 0.1 mL aliquots of the sample dilutions were spread onto Sabouraud dextrose agar (SDA) plates (or potato dextrose agar) and incubated at 25-28 °C for 5-7 days. Any yeast or mold colonies that grew were counted, and the results were expressed as CFU/g.

Pathogen detection

In addition to total counts, the bark powders were tested for the presence of specific indicator pathogens using selective culture media and biochemical tests, following pharmacopeial guidelines for herbal materials. *Escherichia coli* detection was carried out by plating the sample suspensions on MacConkey agar (incubated at 37 °C for 24 hr) and observing for characteristic lactose-fermenting colonies (pink colonies). Suspect colonies were further confirmed on Eosin Methylene Blue (EMB) agar, where *E. coli* typically produces metallic green sheen colonies. *Staphylococcus aureus* was tested by plating on Mannitol Salt Agar (incubated at 37 °C for 24-48 hr); yellow colonies with halos (indicating mannitol fermentation) suggest *S. aureus*. Putative *S. aureus* colonies were confirmed by a Gram stain and a coagulase test (since *S. aureus* is coagulase positive) (Kateete *et al.*, 2010). *Pseudomonas aeruginosa* detection was performed on Cetrimide agar, incubated at 37 °C for 24-48 hr; the growth of characteristic green-pigmented colonies indicates *P. aeruginosa*. Suspected colonies were confirmed by an oxidase test (as *P. aeruginosa* is oxidase-positive). *Salmonella* spp. was screened by inoculating on

Xylose Lysine Deoxycholate (XLD) agar and incubating at 37 °C for 24-48 hr. Typical *Salmonella* colonies appear as red colonies with black centers (due to H_2S production). Further confirmation was done using biochemical assays: a Triple Sugar Iron (TSI) agar slant (looking for the characteristic *Salmonella* reaction: alkaline slant, acidic butt with H_2S) and a urease test (Lazarkevich *et al.*, 2024). All microbiological media and reagents were of laboratory diagnostic grade, and proper positive and negative controls were used in each test. The microbiological limits were interpreted according to standard guidelines for herbal products, whereby total aerobic counts should remain below specified thresholds and pathogenic bacteria should be absent for a sample to be considered of acceptable quality.

RESULTS

Morphological Description

T. arjuna is a large deciduous tree reaching 20-30 m in height, characterized by a broad crown and drooping branches. It features smooth, grey to pinkish-grey bark that exfoliates in large flakes. The tree has simple, opposite leaves measuring 10-15 cm, with a cordate base and rounded apex. Its flowers are small, bisexual, greenish-white, and grow in axillary spikes, while the fruit is a fibrous drupe measuring 2.5-5 cm with five distinct wings. In contrast, *T. elliptica* also reaches up to 30 m and has a straight trunk with a spreading crown. Its bark is dark grey to black, vertically fissured, and exfoliates in irregular patches. The leaves are simple, opposite to sub-opposite, and range from 10–18 cm with an unequal base. This species produces small, pale yellow to greenish-white flowers, which can be bisexual or

Table 1: **Macroscopic features of *T. arjuna* and *T. elliptica* bark.**

Feature	*T. arjuna*	*T. elliptica*
Bark colour (outer)	Grey to pinkish-grey exterior; inner surface pinkish (creamy white to pink)	Grey-black exterior (inner surface not well documented; bark is corky and fire-resistant)
Texture (outer bark)	Smooth, thick bark; exfoliates in thin, irregular papery strips or sheets	Rough, hard bark with deep fissures (coarse "crocodile" bark pattern)
Texture (inner bark)	Fibrous and reddish inner bark	Inner bark not extensively reported; appears corky, with fire-resistant quality
Shape/Structure of pieces	Bark pieces flat or slightly curved, sometimes with longitudinal fissures; outer surface relatively smooth	Bark comes off in thick, uneven plates; cross-section shows deeply cracked structure
Other notes	Trunk often fluted and buttressed; outer bark peels away in narrow strips	Bark thickness ~15–20 mm; known for high fire resistance of the outer bark

Figure 1: 1. *Terminalia arjuna*; (a). Habit (b). Flower & (c). Leaf & 2. *Terminalia elliptica*; (a). Habit (b). Flower & (c). Leaf.

unisexual, in axillary spikes, and its fruit is an ovoid to ellipsoid drupe measuring 3-5 cm, also with five distinct wings. *Terminalia elliptica* is commonly found in dry deciduous forests across India (Kirtikar & Basu, 1935) (Figure 1).

Macroscopic and Organoleptic Characteristics

Macroscopic examination revealed clear differences between T. arjuna and T. elliptica bark (Table 1).

Microscopic (Powder) Features

Microscopic examination of the powdered bark of both species revealed typical anatomical characteristics of *Terminalia* (Combretaceae). In both *T. arjuna* and *T. elliptica*, numerous rosette-shaped crystals of calcium oxalate were observed, a diagnostic feature of this genus. Many stone cells (sclereids) were present either singly or in small clusters, and fragments of strongly lignified fibers and vessels were seen (Figure 3 a and b). Cork tissue (periderm) appeared as a few layers of brownish, tangentially elongated cells, consistent with known Terminalia bark structure. Phloem tissue fragments were abundant, characterized by broad medullary rays and plenty of axial parenchyma; thick-walled phloem fibers were interspersed. Parenchyma cells in both samples often contained reddish-brown contents (presumably tannins), which turned blue upon staining with phloroglucinol-HCl, confirming the presence of polyphenolic compounds. Occasional starch granules were also noted in the powders (detected by iodine staining). Overall, the powder microscopy of *T. arjuna* and *T. elliptica* was very similar – both displayed the key elements of bark tissue (calcium oxalate idioblasts, sclerenchymatous cells, fibers, and vessels) that are characteristic of *Terminalia*. The abundance of calcium oxalate crystals in particular correlates with the high ash values measured for these samples (largely due to their calcium content, present as oxalate salts that convert to calcium carbonate upon ignition).

Physicochemical Parameters

The two *Terminalia* bark samples showed some distinct differences in physicochemical constants (Table 2).

Phytochemical Screening

Qualitative phytochemical tests indicated that both *T. arjuna* and *T. elliptica* barks contain a broad spectrum of secondary metabolites (Table 3).

Table 2: Physicochemical parameters of *T. elliptica* and *T. arjuna* bark

Parameter	*T. elliptica* (%)	*T. arjuna* (%)
Total ash	28.97	20.50
Acid-insoluble ash	0.34	0.79
Alcohol-soluble extractive	15.05	25.10
Water-soluble extractive	15.22	25.10
Loss on drying (105 °C)	7.17	5.09

Figure 2: (a). *Terminalia arjuna* Bark (b). *Terminalia elliptica* Bark.

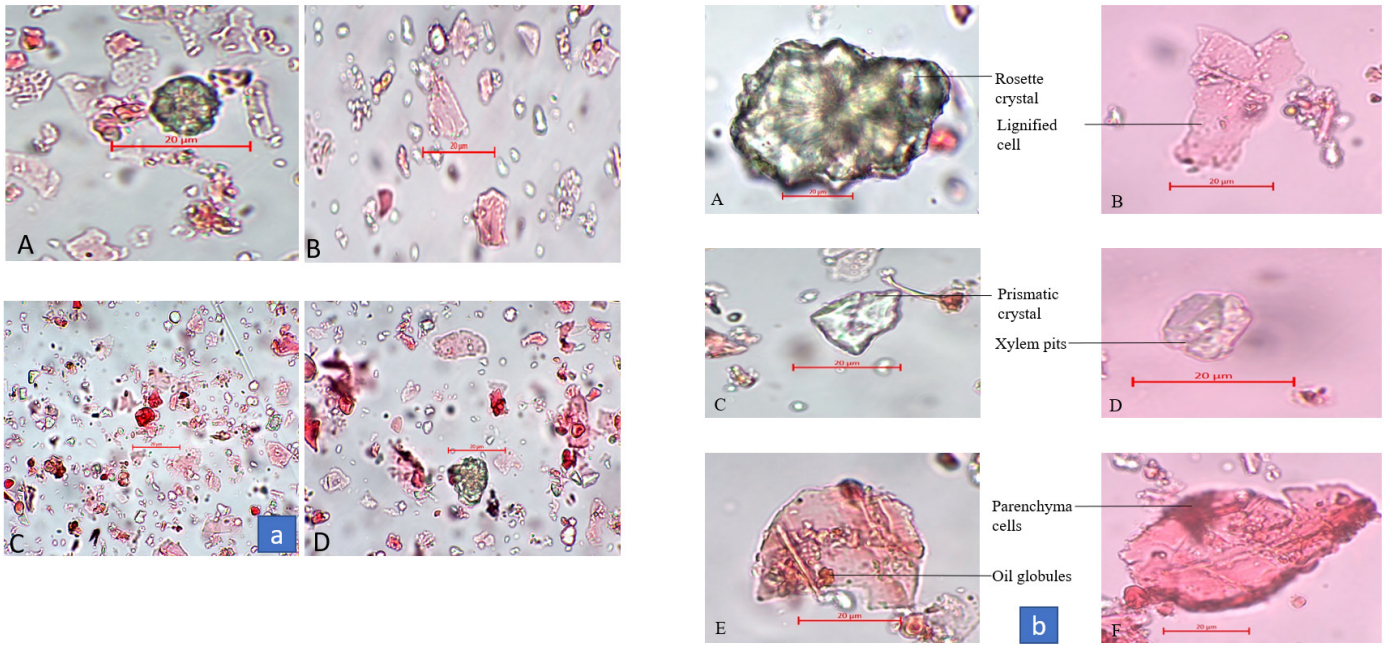

Figure 3a: *Terminalia arjuna*; Stem bark Powder, A: Powder at 40X stained in safranin showing rosette crystals; B: Powder at 40X stained in safranin showing stone cells; C: Powder at 40X stained in safranin lignified cells; D: Powder at 40X stained in safranin showing xylem tracheids. Figure 3b: *Terminalia elliptica*, Stem bark Powder; A.: Powder at 40X stained in safranin showing rosette crystal; B: Powder at 40X stained in safranin showing lignified cell; C: Powder at 40X stained in safranin showing prismatic crystal; D: Powder at 40X stained in safranin showing xylem pits; E: Powder at 40X stained in safranin showing oil globules; F: Powder at 40X stained in safranin showing parenchyma cells.

FTIR Spectroscopy

The FTIR spectra of *T. arjuna* and *T. elliptica* methanolic bark extracts were highly congruent, indicating very similar functional group profiles for the two species. Figure 4 shows the overlaid infrared spectra, and the major absorption bands are listed in Table 4.

HPTLC Fingerprinting

Chromatographic fingerprinting by HPTLC revealed that *T. arjuna* and *T. elliptica* share many common phytoconstituents but also have some distinct spots in their chemical profiles. Figure 5 shows representative HPTLC chromatograms of the methanolic extracts of each species, visualized under UV light after derivatization. Both *T. arjuna* and *T. elliptica* displayed multiple bands of similar Rf values, corresponding to compounds common to both barks (for example, bands attributable to flavonoid glycosides and triterpenoids, which are known to occur in *Terminalia*). A prominent marker band at Rf ≈ 0.44 was observed in *T. arjuna* - this band corresponds to arjunolic acid (a major triterpenoid glycoside in *T. arjuna*), as reported in prior literature. Interestingly, *T. elliptica* showed a band at a similar Rf (~0.44) as well, suggesting that an analogous compound (possibly a related triterpenoid glycoside) is present in *T. elliptica* bark. In addition to the shared bands, *T. elliptica* exhibited a few minor bands that were absent in *T. arjuna*. For instance, weak bands

Table 3: Qualitative phytochemical constituents detected in *T. elliptica* and *T. arjuna* barks.

Phytochemical class	*T. elliptica*	*T. arjuna*
Alkaloids	+	–
Carbohydrates	+	+
Flavonoids	+	+
Glycosides	+	+
Phenols/Tannins	+	+
Coumarins	+	+
Saponins	+	+
Steroids/Triterpenoids	+	+
Proteins/Amino acids	+	+

("+" indicates presence; "–" indicates absence of the compound class in the sample).

at Rf ≈ 0.65 and Rf ≈ 0.95 were detected in *T. elliptica* under 366 nm UV after derivatization, whereas *T. arjuna*'s profile lacked bands at those positions. These unique bands in *T. elliptica* could correspond to compounds like ellagitannins (e.g., chebulinic or chebulagic acid) that have been documented in *T. elliptica* but not in *T. arjuna*. The differences, although subtle, provide a distinct HPTLC "fingerprint" for each species. Thus, HPTLC proved to be a useful tool for differentiating *T. arjuna* and *T. elliptica* bark

extracts based on their chemical composition, in agreement with other studies that emphasize the value of chromatographic fingerprinting for *Terminalia* species identification and quality control.

Microbial Analysis

Microbial quality testing showed that both *Terminalia* bark powders have low microbial loads and are free of dangerous pathogens (Table 5).

DISCUSSION

This comparative study highlights both the shared and distinct pharmacognostic features of *T. arjuna* and *T. elliptica* barks.

Physicochemically, *T. elliptica* was found to have a notably higher total ash content than *T. arjuna* (around 29% vs. 20% of dry weight). Total ash represents the inorganic mineral residue after complete ignition; (*Ash Content - an Overview | ScienceDirect Topics*, n.d.) the elevated ash in *T. elliptica* suggests a greater accumulation of mineral components (especially calcium oxalate, as supported by the microscopic observation of abundant crystals). Classical analyses of *T. arjuna* bark report ash values as high as ~34% (largely calcium carbonate) (9), so the lower ash in our *T. arjuna* sample may reflect specific growing conditions or processing differences. Both barks had very low acid-insoluble ash (<1%), indicating little siliceous or earthy matter contamination, which speaks to good sample cleanliness.

In contrast to the ash content, *T. arjuna* yielded much higher extractive values in both alcohol and water compared to *T. elliptica*. High extractive values correlate with a richer content of soluble phytochemicals (such as polyphenols, glycosides, etc.) (Altemimi *et al.*, 2017). The fact that *T. arjuna* bark gave ~25% extractives in each solvent, versus ~15% for *T. elliptica*, suggests *T. arjuna* contains more readily extractable organic constituents. *T. elliptica*, on the other hand, appears relatively enriched in non-extractable components (like structural polymers or minerals). The moderate moisture (loss on drying) values for both samples (5-7%) confirm that the barks were adequately dried, which is important for stability; these values are in line with WHO guidelines that recommend keeping herbal materials' moisture low to prevent spoilage (*Determination of Moisture Content*, n.d.).

Phytochemical screening demonstrated that both barks contain the key classes of bioactive compounds that likely underlie many of their pharmacological effects. Tannins and flavonoids were strongly present in each, which is consistent with the antioxidant, astringent, and antimicrobial activities historically associated with *Terminalia* barks (Mandal *et al.*, 2013). Steroids/triterpenoids, glycosides, saponins, and even proteins were also found in both species, underscoring their broad chemical complexity. The most striking qualitative difference was the presence of alkaloids in *T. elliptica* (Dragendorff's test positive) contrasted with their apparent absence in *T. arjuna*. Alkaloids have not been widely reported in *T. arjuna*, so this finding for *T. elliptica* may represent a distinguishing chemotaxonomic trait between the species. Similarly, the HPTLC profiles, while showing many common bands, suggested there are minor compounds unique to *T. elliptica*. These unique bands (absent in *T. arjuna*) could correspond to specific ellagitannins or other polyphenols that have been documented in *T. elliptica* but not in arjuna. Previous phytochemical studies of *T. elliptica* indicate the presence of ellagitannins like chebulinic and chebulagic acids, which might account for the extra HPTLC bands observed.(Mandal *et al.*, 2013) By contrast, *T. arjuna* is well known to be rich in oleanane and ursane-type triterpenoid glycosides (such as arjunolic acid, arjunetin, terminic acid) and proanthocyanidin tannins.

FTIR spectroscopy further underscored the overall chemical similarity of *T. arjuna* and *T. elliptica*. Both extracts showed strong broad O–H stretches (~3325 cm^{-1}) and aromatic ring stretches (~1610 cm^{-1}), indicative of polyphenolic compounds, confirming that tannins and flavonoids dominate both barks. The IR spectra of the two species were nearly superimposable, which corroborates the qualitative phytochemical findings that their major functional groups and constituents are alike. Minor differences in the FTIR (such as a detectable C–H stretch

Table 4: Major FTIR absorption bands (in cm^{-1}) observed for *T. elliptica* and *T. arjuna* bark extracts, with probable functional group assignments.

Peak No.	*T. elliptica* (cm^{-1})	*T. arjuna* (cm^{-1})	Assignment (probable functional group)
1	3324.9	3324.5	O–H stretch (H-bonded hydroxyl groups in alcohols/phenols)
2	2920.3	–	C–H stretch (aliphatic, e.g. CH2 groups in fatty acids)
3	–	1614.4	Aromatic C=C stretch (or N–H bend in amines)
4	1610.8	–	N–H bend (primary amines) or C=O stretch (amides)
5	1438.5	1442.3	C–C stretching (aromatic ring bonds)
6	1316.3	1316.2	Phenolic O–H bend (O–H in phenols or tertiary alcohols)
7	1035.0	1035.8	C–N stretch (aliphatic amines)
8	779.8	779.9	C–Cl stretch (alkyl chlorides)
9	513.6	514.3	C–Br stretch (alkyl bromides)

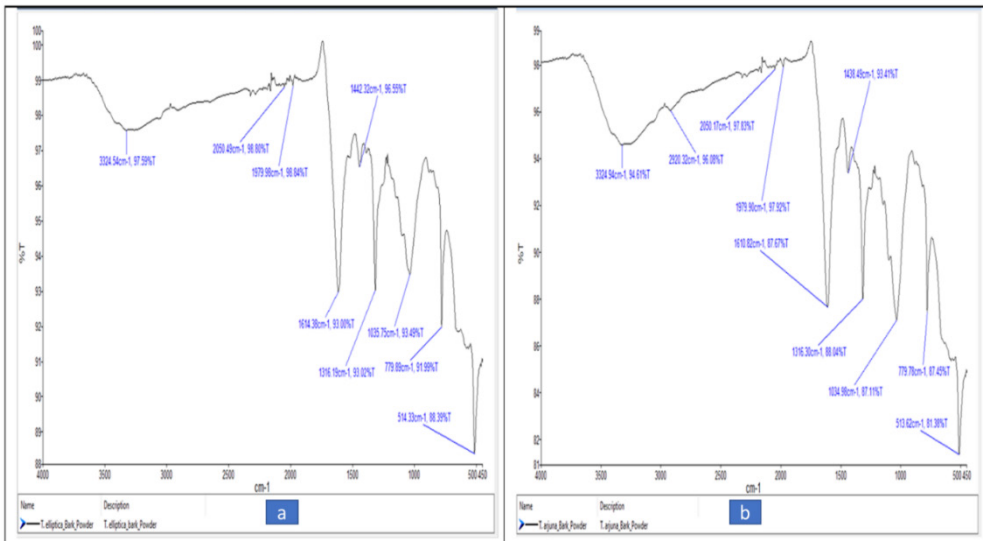

Figure 4: FTIR spectra of methanolic extracts of *T. elliptica* (a) and *T. arjuna* (b) barks, showing common functional group bands.

around 2920 cm^{-1} in *T. elliptica* but not in *T. arjuna*) may point to quantitative differences in certain constituents, but generally the IR results support that both barks are chemically rich in phenolics and share a standard profile. This similarity in chemical makeup aligns with their belonging to the same genus and having analogous traditional uses.

Microscopically, the two barks were almost indistinguishable. Both *T. arjuna* and *T. elliptica* powders exhibited the hallmark anatomical features of *Terminalia* numerous calcium oxalate rosette crystals, sclereids (stone cells), lignified fibers, and vessels. The close similarity in powder microscopy means that relying on microscopic or macroscopic examination alone would make it challenging to tell these two species apart (aside from the noticeable external bark appearance differences in intact samples). This finding underscores the importance of chemical fingerprinting (such as HPTLC or spectroscopic methods) for unambiguous identification and quality control of these herbal materials.

From a pharmacological or medicinal perspective, both *T. arjuna* and *T. elliptica* are considered potent medicinal barks, but their traditional emphases differ somewhat. *T. arjuna* is renowned in Ayurveda as a cardiac tonic and cardioprotective agent (Ramesh & Palaniappan, 2023). Modern pharmacology studies have attributed these benefits to its high content of antioxidants (flavonoids, oligomeric procyanidins) and cardio-active triterpenoids, which can improve cardiac muscle function, reduce blood lipids, and enhance coronary artery blood flow. Arjuna is also used for a variety of other ailments (such as wounds, ulcers, hypertension, and hypercholesterolemia), indicating broad therapeutic utility (Ciumărnean *et al.*, 2020). By contrast, *T. elliptica* is less famous in classical texts but is employed in folk and tribal medicine for its astringent and antimicrobial properties – for example, in treating diarrhea/dysentery, dressing burns and wounds, and

Table 5: Microbial load and pathogen screening results for *T. arjuna* and *T. elliptica* bark powders.

Microbial Parameter	*T. arjuna* result	*T. elliptica* result
Total aerobic plate count (CFU/g)	3.2 × 103 CFU/g	5.6 × 103 CFU/g
Total yeast & mold count (CFU/g)	1.5 × 102 CFU/g	2.0 × 102 CFU/g
Escherichia coli (pathogen test)	Not detected	Not detected
Staphylococcus aureus (pathogen test)	Not detected	Not detected
Pseudomonas aeruginosa (pathogen test)	Not detected	Not detected
Salmonella spp. (pathogen test)	Not detected	Not detected

(CFU = colony-forming units. "Not detected" indicates no colonies grew for that organism in the specified test).

other skin diseases (*IBSD_Antiviral_Compendium_0.Pdf*, n.d.). Our findings of rich tannin and flavonoid content in both barks support these uses, since these compounds are known to confer antimicrobial and anti-inflammatory effects. Recent research on *T. elliptica* has indeed demonstrated potent antibacterial and even anticancer activities for its extracts and isolated constituents (such as chebulinic acid, an ellagitannin also found in related *Terminalia* species). The detection of alkaloids and the presence of unique HPTLC bands in *T. elliptica* hint at additional bioactive compounds in this species that might not be present in *T. arjuna*. These unique constituents could contribute to *T. elliptica*'s specific antimicrobial or other therapeutic effects and warrant further investigation.

In terms of microbial quality, both bark powders showed satisfactory results. Neither *T. arjuna* nor *T. elliptica* had significant microbial contamination; total aerobic counts were

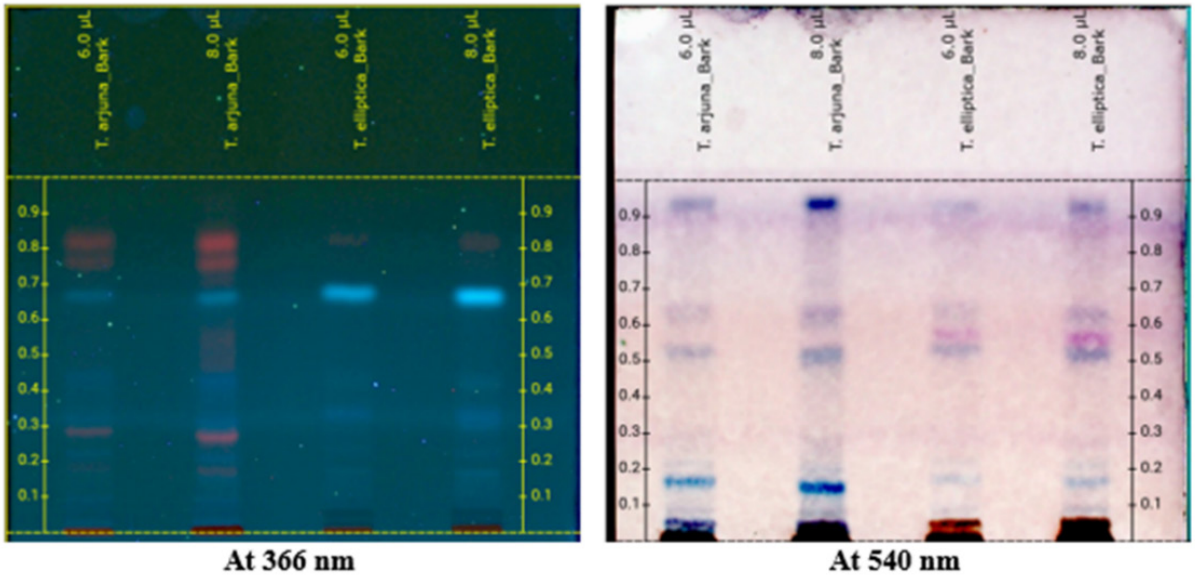

Figure 5: Representative HPTLC chromatograms of *T. arjuna* (A) and *T. elliptica* (B) methanolic extracts (silica gel $60F_{254}$, developed in $CHCl_3$:toluene:EtOH 4:4:1, visualized at 366 nm after anisaldehyde spray). Common bands (arrows) and unique minor bands in T. elliptica (asterisks) are indicated.

low, and no pathogenic bacteria (including *E. coli, S. aureus, P. aeruginosa*, or *Salmonella*) were found. This indicates that the bark materials were processed and stored hygienically and are safe for use in herbal preparations from a microbiological standpoint. Interestingly, the inherently high tannin content of these barks may itself exert an antimicrobial effect, possibly suppressing bacterial growth during storage – a phenomenon that has been noted for other tannin-rich medicinal plant materials. Thus, the microbiological cleanliness of the samples not only reflects good handling but might also be partially due to their chemical composition that deters microbial proliferation. In any case, the absence of harmful microbes ensures that the raw barks meet quality standards for herbal drugs, which is crucial for patient safety.

Comparative analysis shows that while *T. arjuna* and *T. elliptica* share many phytochemical and anatomical features, they can be distinguished by specific quantitative and qualitative markers. *T. arjuna*'s higher extractive values and its well-documented triterpenoid glycosides (like arjunolic acid) align with its established role in cardiovascular therapy. *T. elliptica*'s higher ash content and the presence of alkaloids (along with unique ellagitannin-like compounds) may correlate with its traditional uses as an antimicrobial and anti-inflammatory agent. Both species are rich in polyphenols, contributing to overlapping therapeutic properties. Still, the subtle differences in chemical fingerprints (as visualized in HPTLC and hinted at by phytochemical tests) provide a means to authenticate and differentiate them. These findings can aid in quality control: for instance, distinct FTIR peaks or the presence/absence of an alkaloid reaction, and HPTLC band patterns (with reference standards such as arjunolic acid for *T. arjuna*) could be used to ensure the correct *Terminalia* species is being used in herbal formulations. Overall, integrating classical pharmacognostic evaluation with modern analytical techniques and microbial safety checks offers a comprehensive approach to characterizing and distinguishing these two important medicinal barks.

CONCLUSION

This study compares the pharmacognostic characteristics of the barks from *T. arjuna* and *T. elliptica*, revealing their rich content in polyphenolic compounds and lignified cells, which highlights their medicinal potential. *T. arjuna* shows a higher yield of extractable phytochemicals, particularly valuable for cardioprotection, while T. elliptica has an elevated inorganic ash content and contains unique phytochemical markers like alkaloids. Both species have acceptable microbial purity. The findings emphasize the need for accurate identification due to their differing phytochemical compositions and therapeutic uses. Future research should focus on isolating specific bioactive constituents and validating traditional uses through pharmacological evaluations, laying the groundwork for developing quality standards for these medicinal barks.

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ABBREVIATIONS

HPTLC: High-performance Thin-Layer Chromatography; **TPC:** Total Plate Count; **TYMC:** Total Yeast and Mold Count; **FTIR:** Fourier transform infrared spectroscopy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study compared the barks of *Terminalia arjuna* and *Terminalia elliptica* through macroscopic, microscopic, physicochemical, phytochemical, and instrumental analyses. While both showed similar anatomy and FTIR profiles, *T. elliptica* had higher ash content and contained alkaloids, whereas *T. arjuna* exhibited greater extractive values and a distinct arjunolic acid band on HPTLC. Both met microbial safety standards. The findings establish diagnostic markers to differentiate the two species and affirm their quality and therapeutic relevance.

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