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PHARMACOGNOSTIC STUDY AND HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHIC FINGERPRINTING OF *TERMINALIA ARJUNA* (ROXB. EX DC.) WIGHT & ARN. BARK

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ABSTRACT

Background: Arjuna (*Terminalia arjuna* (Roxb.) Wight & Arn.), widely recognized in Ayurveda for its cardioprotective and wound-healing properties, is a rich source of bioactive phytoconstituents including triterpenoids, flavonoids, glycosides, and tannins. Objective: To establish detailed pharmacognostic, physicochemical, and phytochemical profiles of Arjuna bark, supported by high-performance thin-layer chromatography (HPTLC) fingerprinting for authentication and standardization. Methods: The bark was macroscopically and microscopically evaluated, including transverse and powder microscopy to document diagnostic features. Physicochemical analysis (total ash, acid-insoluble ash, extractive values, pH and moisture content) was performed according to Ayurvedic Pharmacopoeia and WHO guidelines. Preliminary phytochemical screening of alcohol and aqueous extracts was conducted using standard chemical tests. HPTLC analysis was performed on silica gel plates using a validated mobile phase and scanned densitometrically at 254 and 366 nm. Results: Macroscopic and microscopic analyses revealed characteristic cork tissue, phloem fibres, and abundant calcium oxalate crystals. Physicochemical parameters were within pharmacopeial limits, indicating appropriate quality raw material. Phytochemical screening confirmed the presence of saponins, tannins, glycosides, flavonoids, phenols, and steroids. HPTLC analysis produced a consistent chromatographic pattern with distinct bands at Rf 0.408, 0.669, 0.785 and 0.956 at 254 nm, and 0.408, 0.668, 0.966 at 366 nm. Conclusion: The integrated pharmacognostic, physicochemical, and chromatographic findings provide reliable diagnostic markers for authenticating Arjuna (*T. arjuna* (Roxb. ex-DC.) Wight & Arn bark and may serve as a reference standard for quality control and further pharmacological research.

Keywords: HPTLC fingerprinting, Arjuna, *Terminalia arjuna*, Standardization

INTRODUCTION

Terminalia arjuna (Roxb.) Wight & Arn., commonly known as Arjuna, is a prominent deciduous tree of the family Combretaceae. In Ayurvedic literature, it is renowned for its cardioprotective and restorative benefits and constitutes an important component of several classical formulations. The species is identified by diverse regional names, such as Arjun, Kahu, Kahua, Arjan, Khawa, Anjani, Jamla in Hindi; Arihan, Arjun, Kahu in Bengali; Asun, Arjuna, Anjan, Sadura in Marathi; Sadado, Ariunsadada in Gujarati; Maddi, Vaidairya, Billi matti, Holematti in Kannada; Nirmarutu, Venmarutu, Affumarutu in Malayalam; and Attumarutu, Irmarutu, Vellaimarutu in Tamil.[1] This large deciduous tree is recognized by its smooth, grey bark that exfoliates in thin sheets, revealing pinkish inner layers, and by its oblong to elliptic leaves arranged sub-opposite with a leathery texture and pale undersides. The tree produces small, white to yellowish flowers clustered in terminal or axillary panicles [Fig. 1], which later develop into woody, fibrous fruits bearing five distinct wings. *Terminalia arjuna* typically thrives along riverbanks and in moist deciduous forests, occurring at elevations up to about 1,200 m in the Indian subcontinent. It is also widely distributed across Sri Lanka, Myanmar, and other regions of Southeast Asia. [2]

Arjuna is esteemed in Ayurveda for its Hṛidya (cardiotonic) and Kasaya (astringent) properties, traditionally used for Hridyarog (cardiac disorders), Raktapitta (bleeding disorders), Prameha (metabolic and urinary disorders), and Vrana (wound healing). Bark preparations such as decoctions, powders, and Ksheerpaka (Medicated milk) strengthen the heart, improve circulation, and reduce ischemic stress, while also aid in the healing of the fractures, ulcers, and skin diseases through antioxidant and anti-inflammatory effects. External application of bark preparations further aids in wound healing and control of localized bleeding, reflecting its broad therapeutic relevance.[3] Arjuna exhibits potent cardioprotective, antioxidant, anti-inflammatory, and hypolipidemic activities. Bark extracts rich in triterpenoids and flavonoids reduce lipid peroxidation, enhance endogenous antioxidants, and attenuate myocardial damage in ischemia–reperfusion and isoproterenol-induced injury models.[4,5] Arjunolic acid demonstrates strong free-radical scavenging and myocardial protective effects, while aqueous extracts protect against doxorubicin-induced cardiotoxicity.[6] Clinical studies also report improved endothelial function, lipid profiles, and cardiac performance in chronic stable angina and heart failure. [7]

Arjuna, a member of the Combretaceae family, contains diverse phytoconstituents across its bark, root, fruit, and wood, including triterpenoids (arjunolic acid, arjunic acid, oleanolic acid, arjungenin), glycosides (arjunetin, arjunosides I–IV, arjunglucosides I–III), flavonoids (baicalein, arjunolone), tannins, sterols (β-sitosterol, friedelin), and phenolic acids (ellagic, gallic, terminic). Additional compounds such as mannitol, leucocyanidin, (+)-leucodelphinidin, and various long-chain fatty acid esters have also been reported.[1] This study aims

to investigate the pharmacognostic features of the plant through powder microscopy and transverse section examination, alongside establishing its chemical profile by phytochemical screening, physicochemical evaluation, and high-performance thin-layer chromatography (HPTLC) fingerprinting.

MATERIALS AND METHODS

Plant Materials Collection and Authentication

The bark of Arjuna (*Terminalia arjuna* (Roxb.) Wight & Arn.) was sourced from a local vendor in Medak, Telangana. A herbarium specimen was prepared for reference, and the plant material was subjected to taxonomic identification and authentication at the Botanical Garden of Indian Republic (BGIR), Botanical Survey of India (BSI), Sector 38A, Gautam Budh Nagar, Noida. Authentication was confirmed under reference number BSI/BGIR/1/TECH./2024/159, dated 9 December 2024.

Plant Sample Processing

The whole and powdered drug samples were stored at ambient temperature in airtight, light-protected containers as per the specifications of the Ayurvedic Pharmacopoeia of India.[8] For analytical purposes, coarsely powdered and finely sieved samples (60# mesh) were utilized in powder microscopy, physicochemical characterization, phytochemical profiling, and chromatographic studies, following standard methodologies.

Pharmacognostic Study

Macroscopy of Plant Material

The bark of *T. arjuna* was assessed for macroscopic features including structure, shape, size, and texture by unaided observation and with the aid of a simple microscope (Olympus OIC DM), while its organoleptic characters such as colour, odour, and taste were recorded through sensory evaluation methods. [9,10]

Transverse Section Microscopy

For microscopic analysis, the sample was pre-soaked in water and subsequently sectioned transversely using a sharp diamond-edged blade. Replicate temporary mounts of these sections were prepared and examined under a digital trinocular compound microscope (Olympus CX21i equipped with a Magcam DC14 camera) and photomicrographs were recorded for documentation. [9,10]

Powder Microscopy of Plant Material

Approximately 2 g of finely powdered, oven-dried bark (80# mesh) were prepared for microscopic analysis. Both wet mounts (using 50% glycerine) and dry mounts were made employing a smearing technique to achieve uniform particle distribution. Observations were carried out under a Zeiss Axio Cam ICc5 microscope at 10X and 40X magnifications, and diagnostic features were recorded through photomicrography. [9,10]

Physicochemical Evaluation

Physicochemical parameters of the *T. arjuna* bark, including loss on drying, total ash, and extractive values, were determined in accordance with the standard procedures prescribed in the Ayurvedic Pharmacopoeia of India and the WHO guidelines.[10,11] Extractability studies were conducted with ethanol and water employing established solvent extraction methods.

Determination of Moisture Content (Loss on Drying)

The procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used. About 5g of the powder of *T. arjuna* bark was taken in three petri dishes respectively to calculate an average. It was then dried at 105°C for 5 hours and then weighed. It was again dried at the same temperature and weighed at one-hour interval until the difference between two consecutive weights corresponded to no difference between them. The percentage of loss on drying was calculated using the formula.

Wt. of the empty petri dish = W1 gm
Wt. of the drug sample = X gm
Wt. of the petri dish with drug before drying (W3) = (W1+ X)
Wt. of petri dish after drying = W2 gm
Loss on drying in % = [W3-W2 / X] × 100

Determination of Extractive Values

Determination of Alcohol-Soluble Extractive

Approx 10 gm of the powder of *T. arjuna* bark was weighed and macerated with 100 ml of ethanol. It was then kept in a closed flask for twenty-four hours, shaken frequently during the first six hours and then allowed to stand for the rest of the eighteen hours. It was then filtered rapidly. Precautions were taken against loss of solvent. Then filtrate was evaporated to dryness in a tarred flat-bottomed shallow petri dish, and dried at 105°C, to constant weight and weighed. The percentage of alcohol soluble extractive with reference to the air-dried drug was calculated using the formula.

Wt. of the drug material = X gm
Wt. of the empty petri dish = W1 gm
Wt. of the petri dish with dried extract = W2 gm
Percentage of extractive value = [W2-W1 / X] × 100

Determination of Water-soluble Extractive

Proceeded as directed for the determination of alcohol-soluble extractive, using distilled water instead of ethanol.

Determination of Ash Values

Determination of Total Ash

About 2 gm of the powder of *T. arjuna* bark was weighed in tarred silica crucible and incinerated at a temperature approx. 500–600°C until free from carbon. It was then cooled under desiccator and weighed. The percentage of ash with reference to the air-dried drug was calculated using the formula.

Wt. of Empty Silica Crucible = A1 gm
Wt. of Sample (X) = X gm
Wt. of the Crucible with Ash = A2 gm
Percentage of Total Ash = [A2 - A1/X] × 100

Determination of Acid-Insoluble Ash (AIA)

25 ml of dilute HCl was added to the respective crucibles containing total ash of *T. arjuna* bark. Then these crucibles were kept in heating mantle until they started to boil after which the solution was filtered using the ashless filter paper (Whatman 41). The insoluble matter was collected on the filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried in muffle furnace and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 minutes and weighed without delay. The content of acid insoluble ash with reference to the airdried drug was calculated using formula.

Wt. of drug sample = X gm
Wt. of Crucible = G1 gm
Wt. of Crucible with insoluble Ash = G2 gm
Wt. of insoluble ash (G3) = G2-G1
Percentage of acid insoluble ash = G3/X × 100

Determination of pH range
Approx. 3 gm of powder of *T. arjuna* bark was weighed and immersed in 30 ml of distilled water. It was then kept in a closed flask and was shaken frequently for five hours and then allowed to stand overnight. It was then filtered into another beaker and the pH of the filtrate was determined using a pH paper by noticing the colour change.

Preliminary Phytochemical Analysis
Approximately 5 g of the powdered *T. arjuna* bark was taken in a conical flask, and solvents (ethanol and distilled water) were added in a 1:10 ratio (50 mL per sample). The mixtures were agitated on a rotary shaker for 6 hours, allowed to settle, and subsequently filtered through Whatman filter paper No.1 to obtain the respective extracts. The filtrate was taken for screening of phytochemicals by using standard chemical tests. [12]

Test for Alkaloids
Dragendorff's Test – 2 ml alcoholic / aqueous extract of powder of *T. arjuna* bark was taken separately in the test tubes and a few drops of diluted hydrochloric acid (dil. HCl) were added to them respectively. The solution formed was then filtered and 1ml of Dragendorff's reagent (potassium bismuth iodide solution) was added to each. Formation of orange precipitate indicates the presence of alkaloids.

Test for Saponins
Foam Test – 2.5 ml alcoholic / aqueous extract of powder of *T. arjuna* bark was taken separately in the test tubes and 10 ml distilled water was added to them. The mixture was vigorously shaken and kept for two minutes. Formation of honeycomb-like froth indicated the presence of saponins and vice-versa.

Test for Tannins
Ferric Chloride Test – 1ml alcoholic / aqueous extract of powder of *T. arjuna* bark was taken separately in the test tubes and 5ml distilled water was added to them. Then, a few drops of 10% $FeCl_3$ solution were added to the respective test tubes. A bluish-black or greenish-black precipitate is formed indicating the presence of tannins.

Test for Glycosides
Keller-Kiliani test – 1 ml alcoholic / aqueous extract of powder of *T. arjuna* bark was taken separately in the test tubes after which the extracts were dissolved in approx. 1 ml each of glacial acetic acid, 10% ferric chloride solution and conc. H_2SO_4 (Sulphuric acid). A reddish-brown color ring at the junction of two layers indicated the presence of glycosides.

Test for Flavonoids
Alkaline Reagent Test- In a test tube containing 1 ml of alcoholic / aqueous extract of powder of *T. arjuna* bark was taken separately, a few drops of 20% NaOH (sodium hydroxide) solution were added, and a yellow color precipitate was formed which after adding a few drops of dil. HCl (Hydrochloric Acid) becomes colorless confirming the presence of flavonoids.

Test for Carbohydrates
Molisch's Test- About 1 ml of the aqueous / alcoholic extract of *T. arjuna* bark was taken in a test tube, to which 2–3 drops of Molisch's reagent were added. Subsequently, concentrated sulphuric acid was carefully poured along the side of the tube to form a separate layer. The appearance of a violet ring at the interface confirmed the presence of carbohydrates.

Test for Phenols
Ferric Chloride Test- Approximately 1 ml of the aqueous / alcoholic extract of *T. arjuna* bark was treated with a few drops of 5% ferric chloride solution. The development of a bluish-green to deep blue coloration indicated the presence of phenolic compounds.

Test for Steroids
Salkowski test– 2 ml alcoholic / aqueous extract of powder of *T. arjuna* bark, was taken separately in the test tubes to which 2 ml of chloroform along with equivalent quantity (2 ml) of conc. H_2SO_4 was added, respectively. The formation of the pink/ red ring indicated the presence of steroids.

Fingerprint Analysis by High-Performance Thin Layer Chromatography (HPTLC)
The filtrate was concentrated (c = 1 g/10 mL) and taken for HPTLC profiling. The extract (4 µL) was spotted on a pre-coated silica gel 60 F254 aluminium plate using a Camag Linomat V sample applicator equipped with a 100 µL Hamilton syringe. The plate was developed in a pre-saturated twin trough chamber using the mobile phase as Ethyl Acetate: Toluene: Formic Acid (5:4:1) (v/v/v) to a distance of 90 mm and dried for 5 min in ambient air. After development, a densitometric scan was done with a Camag TLC scanner III in reflectance- absorbance mode at UV detection wavelengths of 254 nm and 366 nm using winCATS software (version 1.2.1, Camag).

Table 1: Organoleptic features of Arjuna (*Terminalia arjuna* (Roxb.ex DC.) Wight & Arn) Bark and its Powder

Character	Arjuna (*Terminalia arjuna* Roxb.) Bark	Bark Powder
Shabda (Sound)	-	-
Sparsha (Touch)	Smooth	Smooth / Fine
Roopa (Colour)	Outer surface- pink or flesh Inner surface- reddish brown	Reddish Brown
Rasa (Taste)	Kashaya (Astringent)	Kashaya (Astringent)
Gandha (Odor)	None	None

Table 2: Physicochemical observations of Arjuna (*Terminalia ar*juna (Roxb.ex DC.) Wight & Arn) Bark

Test	Observations
Foreign matter	Nil
Extractive Value (Alcohol soluble)	53.38%
Extractive Value (Chloroform water-soluble)	32%
Total Ash	17.81%
Acid Insoluble Ash	0.95%
Moisture Content (LOD)	3.16%
pH value	6

Table 3: Phytochemical Observations of Arjuna (*Terminalia arjuna* (Roxb.ex DC.) Wight & Arn) Bark

Phytochemical	Alcohol extract	Aqueous extract
Alkaloids- Dragendorff's Test	-	-
Saponins- Foam test	+	+
Tannins- $FeCl_3$ test	+	+
Glycosides- Keller–Killiani test	+	+
Flavonoids- Alkaline Reagent Test	+	+
Carbohydrates- Molisch's test	+	+
Phenols- $FeCl_3$ test	+	+
Steroids- Salkowski Test	+	-

Note: (-): Absent, (+): Present

Figure 1: Flowering Twig of Arjuna (*Terminalia arjuna* (Roxb.ex DC.) Wight & Arn)

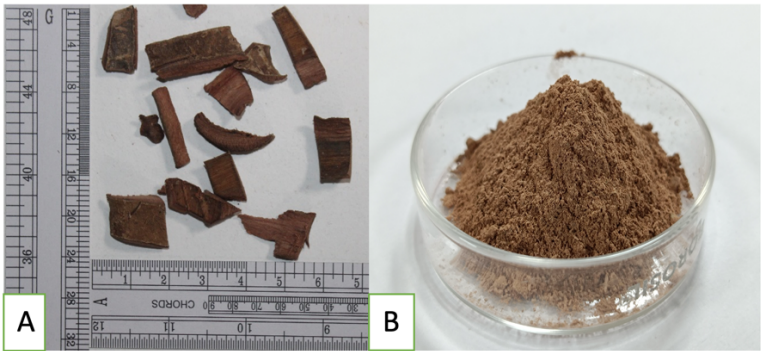

Figure 2: A. Crude Arjuna (*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. bark B. Bark Powder

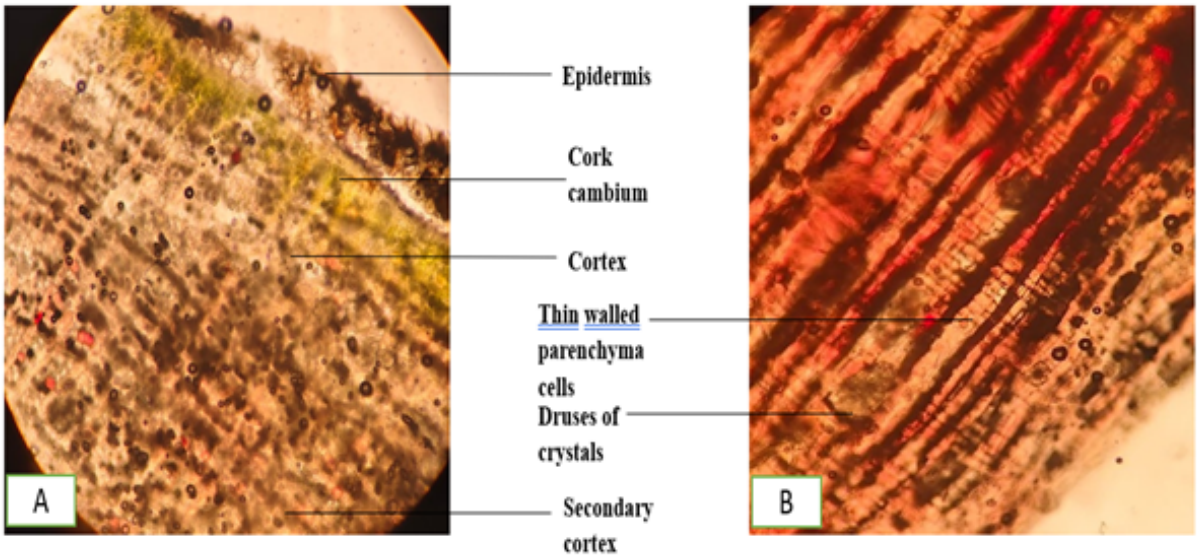

Figure 3: T.S of Arjuna (*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn Stem Bark, A&B: Bark at 10X showing epidermis, cork cambium, cortex, rosette crystals, thin walled parenchyma cells, secondary cortex.

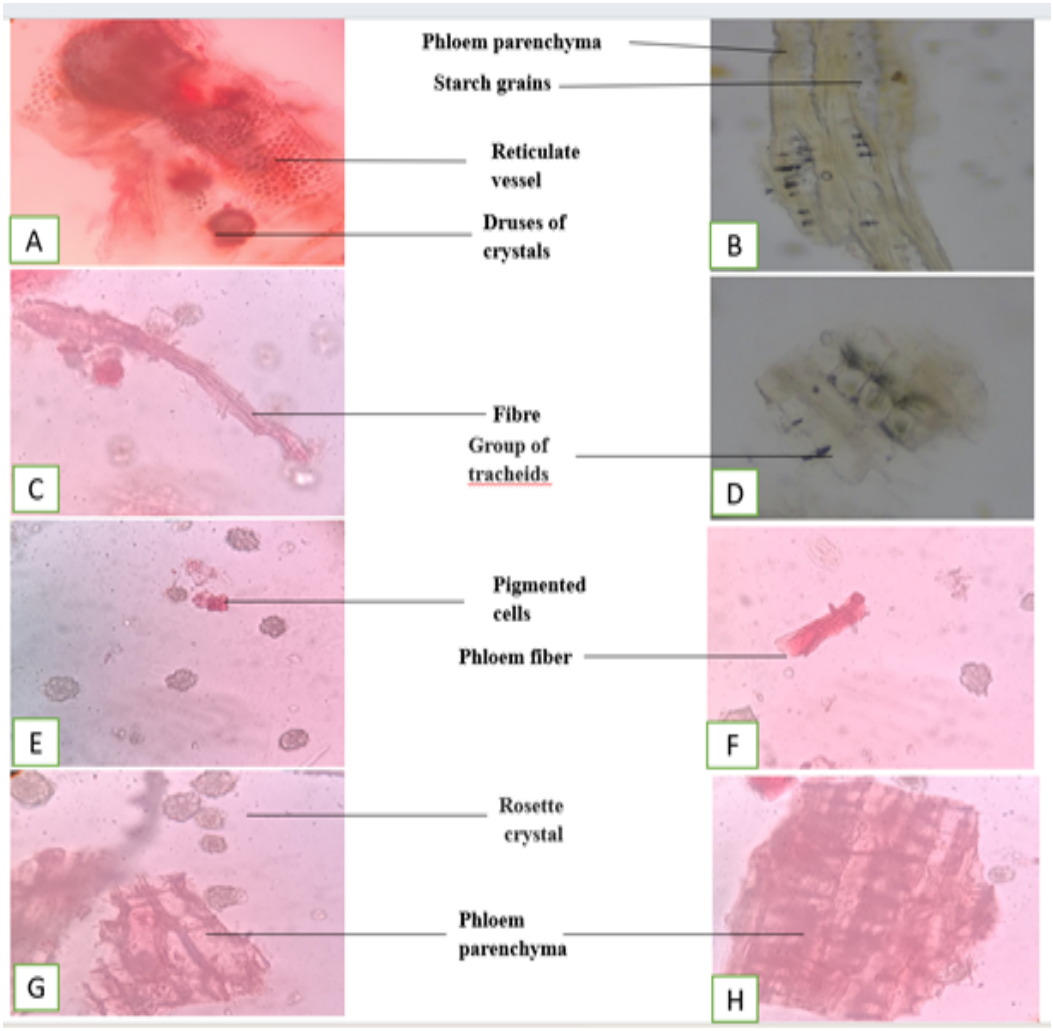

Figure 4: Powder Microscopy of Arjuna (*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn; Stem Bark powder stained in safranine at 40X showing A) Reticulate vessel and Druses of crystals B) Phloem parenchyma & Starch grains C) & D) Fibre Group of tracheids E) Pigmented cells F) Phloem fiber G) Rosette crystal G) & H) Phloem parenchyma

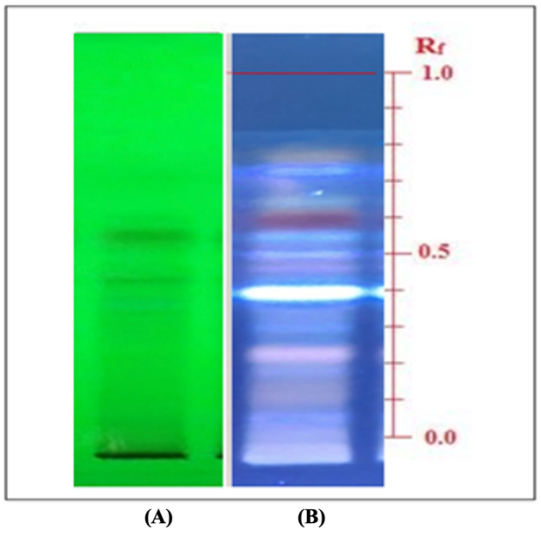

Figure 5: Photography Of HPTLC Plate- (A) Visualization at 254 Nm; (B) Visualization at 366 Nm

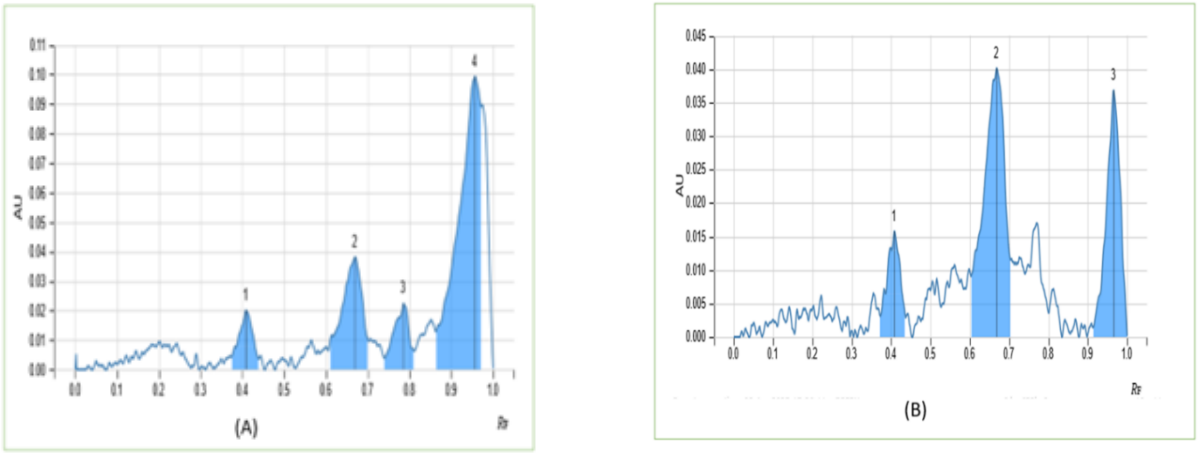

Figure 6: Densitometric Fingerprint Profiles At (A) 254 Nm, (B) 366 Nm of Bark of Arjuna (*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn

RESULTS AND DISCUSSION

Macroscopic characters

T. arjuna stem bark consists of pieces that are generally flat, curved, or recurved in shape. The dimensions vary, with lengths ranging from 10 to 15 cm, widths up to 8 cm, and a thickness between 0.5 to 2 cm. The outer surface is smooth and exhibits a pink or flesh coloured, while the inner surface is reddish-brown, marked by fine longitudinal striations, and tends to peel in thin layers. On breaking, the inner region fractures into small, brittle pieces, whereas the outer layer displays a laminated fibrous structure. [Figure 2; Table 1]

Transverse section microscopic view of Arjuna (*Terminalia arjuna* (Roxb.) Wight & Arn.) Bark

The transverse section (T.S.) of the stem bark of *T. arjuna* observed at 10X magnification revealed distinct histological features. The outer protective layer was represented by the epidermis, consisting of compactly arranged cells. Beneath the epidermis, a well-defined cork cambium was present, contributing to the secondary growth of the bark. The cortex region showed the presence of thin-walled parenchymatous cells, interspersed with rosette crystals of calcium oxalate, which are important diagnostic characters. The secondary cortex was differentiated and exhibited characteristic parenchymatous tissue organization. [Figure 3]

Powder microscopy

The powdered stem bark of *T. arjuna* was examined under the microscope at 40X magnification after staining with safranin. The diagnostic characters revealed the presence of reticulate vessels along with druses of calcium oxalate, which serve as important identification features. Phloem parenchyma cells were distinctly observed, frequently associated with starch grains. Elongated fibres and well-defined groups of tracheids were also present, reflecting the lignified nature of the tissue. Additionally, pigmented cells were recorded, which contributed to the characteristic appearance of the bark powder. Prominent phloem fibres were found along with rosette crystals of calcium oxalate embedded in the parenchymatous matrix. These diagnostic characters confirm the authenticity of *T. arjuna* stem bark and provide reliable pharmacognostic markers for quality evaluation. [Figure 4]

Physicochemical parameters

The bark powder was evaluated for foreign matter, loss on drying, total ash, acid-insoluble ash, pH, and extractive values in water and alcohol [Table 2]. All analyses were performed in accordance with the standard procedures specified in the Ayurvedic Pharmacopoeia of India and WHO guidelines.

Preliminary Phytochemical Analysis

Aqueous and ethanolic extracts, prepared as outlined in the preceding section, were subjected to preliminary phytochemical screening using approximately 2 mL of each extract for individual tests. The findings are presented in Table 3.

HPTLC Fingerprinting Profile of Arjuna (*Terminalia arjuna* (Roxb.) Wight & Arn) Bark

The HPTLC procedure was standardized using pre-activated, precoated silica gel 60 F254 aluminium plate along with various combinations of polar and non-polar solvent systems as mobile phases. Optimal resolution was achieved with ethyl acetate: toluene: formic acid in the ratio (5:4:1) (v/v/v). Under these conditions, the bark extract of *T. arjuna* exhibited four distinct bands at Rf values of 0.408, 0.669, 0.785 and 0.956 under UV light at 254 nm; three bands at 0.408, 0.668 and 0.966 at 366 nm respectively.

The pictorial representation of the developed plate of root ethanolic extract is given in Figure. Densitometrically scanned pictures at 254 nm and 366 nm of the developed plates as fingerprint profile is shown in Figure 6.

CONCLUSION

This study established the pharmacognostic characteristics and HPTLC fingerprint profile of the dried bark of Arjuna (*Terminalia arjuna* (Roxb.) Wight & Arn.) contributing to its standardization and authentication and aiding in the detection of adulteration. The microscopical and physicochemical features documented in this work provide valuable diagnostic markers for the development of a monograph. Phytochemical screening of the aqueous and ethanolic extracts confirmed the presence of flavonoids, saponins, tannins, glycosides, carbohydrates, phenols, and steroids through positive reactions with specific reagents, each associated with medicinal properties and distinct physiological effects. Furthermore, the phytochemical profile suggests that *T. arjuna* bark extracts could be explored for pharmacological screening owing to their potential therapeutic activity. The HPTLC profile developed in this work serves as a benchmark tool for ensuring the authenticity and quality control of herbal preparations prepared from *T. arjuna* bark. This investigation also strengthens the evidence base for the standardization of Arjuna (*Terminalia arjuna* (Roxb.) Wight & Arn.) bark, supporting its validated and safe application in traditional medicine.

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