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ORIGINAL ARTICLE

Forensic and pharmacognostic studies of the *Terminalia Arjuna* Bark

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TLC

Abstract The Bark of *Terminalia arjuna* is considered as Cardio protective and Hypolipidemic in folklore medicine. In the present investigation, the detailed pharmacognostic study of *T. arjuna* Bark is carried out to lay down the standards, which could be useful in future Forensic identification of unknown plant material. The study includes macroscopic, microscopic, preliminary phytochemical screening and physicochemical evaluation. The objective of this study was to characterize the unknown plant material seized from the scene of crime. In the present communication, a TLC-method was also described for the identification of Arjuna bark.

1. Introduction

Ayurveda is the oldest surviving complete medical system in the world. Derived from its ancient Sanskrit roots – '*ayus*' (life) and '*ved*' (knowledge) – and offering a rich, comprehensive outlook to a healthy life, its origins go back to nearly 5000 years. *Terminalia arjuna* is a large, evergreen tree, with a spreading crown and dropping branches. It has been grown in most parts of India and used in Ayurvedic formulations since ancient times. Besides its wide range of medicinal uses, *T. arjuna* is planted for shade and ornamental purposes. Terminalia's active constituents include tannins, cardenolide, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjun glycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper.[1,2] Improvement of cardiac muscle function and subsequent improvement in the pumping activity of the heart seems to be the primary benefit of Terminalia. It is thought that the saponin glycosides might be responsible for the inotropic effect of Terminalia, while the flavonoids and OPCs provide free radical antioxidant activity and vascular strengthening.[3] A dose-dependent decrease in heart rate and blood pressure was noted in dogs given Terminalia intravenously.[4] Recently, two new cardenolide cardiac glycosides were isolated from the roots and seeds of Terminalia.[5,6] The main action of these cardenolides is to increase the force of cardiac contraction by means of a rise in both intracellular sodium and calcium. In the literature details of morphology, phytoconstituents, medicinal properties and uses of *T. arjuna* are very sparse. Therefore, in the present study an attempt has been made to study the pharmacognostic standards of the bark of *T. arjuna*. These standards are of utmost importance not only in finding out the

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Table 1 Physico-chemical analysis.

Tests	Results (in %)			Interferences (in %)
Physicochemical analysis	S1	S2	S3	
Total ash content	15.76	16.37	16.53	Not more than 27.0
Acid insoluble ash	1.45	0.96	0.94	Not more than 2.0
Moisture content	5.65	6.88	6.64	Not more than 16.0
Test for Extractive Value				
Alcohol soluble extractive	45.03	46.89	46.68	Not less than 16.0
Water soluble extractive	41.42	43.99	43.84	Not less than 17.0

genuinity, but also in the detection of adulterants in marketed drugs as well as in Forensic detection.

2. Experimental procedure

2.1. Materials

T. arjuna bark was procured from the local market of Gwalior. The fresh bark of the plant was dried, and soaked in water for few days. Dried bark of the plant was made into powder. An exhaustive Pharmacognosy was carried out by using the standard methodology.[7]

2.2. Methods

2.2.1. Physico-chemical analysis (Tables 1 and 2)

(a) **Total Ash:** 2 gm of powdered *T. arjuna* bark was taken in a tarred china dish. After that, it was subjected to muffle furnace at a temp. of 450 °C; weight was taken when it becomes red hot and then cooled. Constant reading was taken at an interval of two hours.

(b) **Acid Insoluble Ash:** 2 gm of powdered *T. arjuna* bark was taken and mixed with 25 ml of hydrochloric acid. Total ash was boiled for 5 min and diluted with 25 ml of hydrochloric acid. Insoluble matter was collected on an ashless filter paper. Filter paper was washed with hot water. Crucible was ignited and then cooled. After that it was kept in a desiccator. Residue was weighed and acid in insoluble ash of drug was calculated.

(c) **Determination of Moisture Content (Loss on Drying):** 2 gm of powdered *T. arjuna* bark was taken in a tarred china dish. Then the powder was dried in an oven at a temp. of 100 °C or 105 °C, followed by cooling in a desiccator. After that the loss of moisture content was recorded. The procedure was continued for at least two concurrent readings.

Table 2 Physicochemical evaluations.

Extractive value	Percentage
Ethanol	25.2
Aqueous	20.2
Pet ether	1.4
Loss on drying	5.11
Ash	< 5 w/w
Heavy metal lead	< 10 ppm
Acid insoluble ash	< 3 w/w
Test for pathogen	Nil

Table 3 Phyto-Chemical analysis.

S. No.	Chemical tests	Ethanol extracts	Aqueous extracts
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Phytosteroids	+	–
4	Fixed oils & fats	–	–
5	Saponins	+	+
6	Phenolic compounds tannins	+	+
7	Proteins & amino acids	+	+
8	Gums & mucilages	–	–
9	Volatile oils	–	–
10	Flavonoids	+	+

(+ = Present, – = absent).

(d) **Sulfated Ash:** 2 gm of powdered *T. arjuna* bark was taken in a silica crucible and 3 ml of sulfuric acid was added to it. The powder was incinerated by gradually increasing the heat until it becomes free from carbon. And then the residue was cooled in a desiccator. Ash was weighed and the percentage of sulfated ash was determined.

(e) **Water Insoluble Ash:** 2 gm of powdered *T. arjuna* bark was taken in a silica crucible and 25 ml of water was added to it. The mixture was boiled. After that insoluble matter was filtered on an ashless filter paper. The residue was ignited in a crucible and then cooled. The residue was weighed and water insoluble ash was calculated.

(f) **Determination of Alcohol Content:** 2 gm of powdered *T. arjuna* bark was taken in a tarred silica crucible. The powdered drug was incinerated until it becomes carbon free. The residue was cooled and kept in a desiccator. The ash was weighed and the percentage of total ash was calculated.[8]

2.2.2. Qualitative screening of phytochemicals (Table 3)

Ethanol and aqueous extracts of bark of *T. arjuna* were screened for the presence of alkaloids, carbohydrates, phytosteroids, saponins, flavonoids, phenols, and terpenoids by using standard protocols.

2.3. Preparation of the extract

Ten grams of dried powdered bark material was weighed accurately and placed in a soxhlet extraction chamber, which was suspended above the flask containing 100 mL of 80% ethanol and below a condenser. The flask was heated and the ethanol

evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing ethanol extract was removed and ethanol was evaporated by using a rotary evaporator. The weight of the residual extract was measured and percent yield was calculated. The residue of the extract was dissolved in 25 ml of pure ethanol and stored in air tight glass vials at 40 °C until further use. The same procedure was used to prepare aqueous extract. (Fig. 1)

$$\text{Extract yield } \% = \frac{W1}{W2} \times 100$$

where W1 = net wt of powder in grams after extraction. W2 = total wt of wood powder in grams taken for extraction.

3. Microscopic and macroscopic study[9]

3.1. Macroscopic examination (Fig. 2)

The outer surface of the bark appeared smooth, pale greenish yellow while the inner surface was finely longitudinally striated and pinkish in color. Bark has pieces that were flat, curved and recurved in shape. On the inner part fracture was short while laminated on the outer part. Sample (Arjuna bark) size 8.5 cm in length and 6.3 cm in width was also observed.

3.2. Microscopic Examination (Figs. 3 and 4)

In microscopic examination of the mature bark, a cork consisting of 9–10 layers of tangentially elongated cells was observed. Phellogen was 2–4 cells thick, phelloderm was narrow consisting of 4–6 rows of tangentially elongated and radially arranged cells. Phloem was broad, traversed by uniseriate medullary rays which runs straight and parallel, occasionally becomes slightly curved near the rosette crystals. Group of phloem fibers were lignified, thin walled, tangentially arranged, associated with idioblasts which comprise of clusters and rosettes of calcium oxalate. Some parenchymatous cells of cortex and secondary phloem contain reddish brown pigment while some cells contain starch grains.

3.3. Powder Microscopy (Fig. 5)

Two grams of powdered wooden chips of *T. arjuna* was taken in a test tube and boiled with 50% HNO_3. A pinch of $KClO_3$ was added to it, and then placed in sunlight for three to four days until the chips turn milky white. Wash it with water, then the powder was transferred on a clean microscopic glass slide. Lignified fibers were stained with the staining agent (safranin) and the powder was treated with glycerin and water. The slide was observed under low power. The uniseriate medullary rays running straight and parallel occasionally becoming slightly curved, starch grains, stomata and other characteristics were observed.

4. Thin layer chromatographic analysis

A standard glass TLC plate was coated with the slurry of silica gel G in water to a uniform thickness of 0.25 mm. After that the plate was activated by heating in an oven at 110 °C for about one hour. Aliquots of standard Arjuna bark extract were obtained in Methanol (3.0 gm of powdered drug was

Figure 1 Arjuna Tree (*Terminalia arjuna*).

Figure 2 Bark of *Terminalia arjuna*.

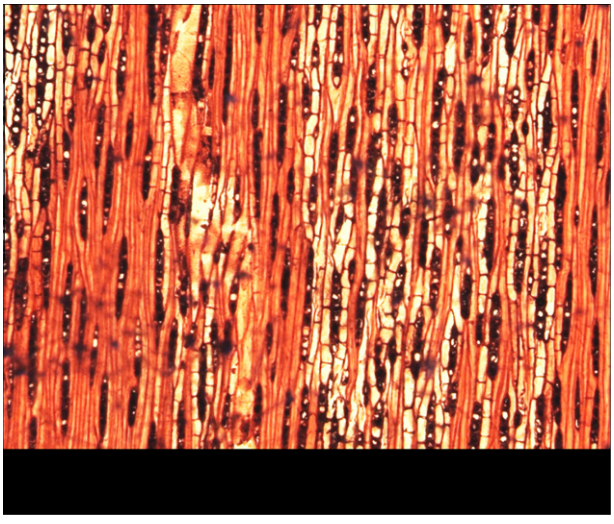

Figure 3 Tangential section of *Terminalia arjuna*.

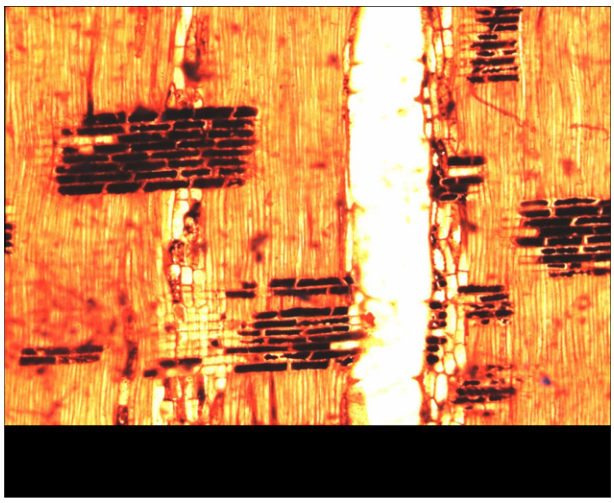

Figure 4 Radial section of *Terminalia arjuna*.

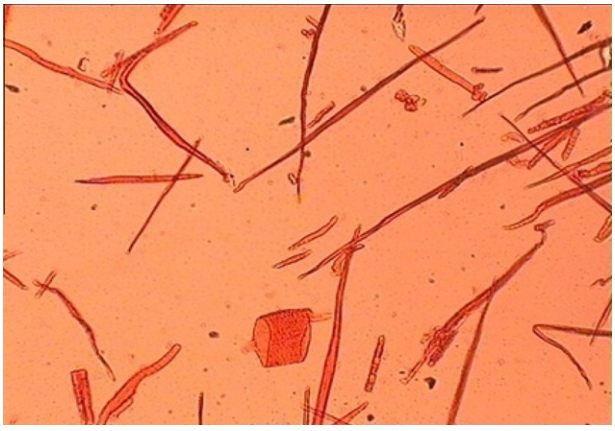

Figure 5 Microscopic slide of powdered *Terminalia arjuna*.

extracted with methanol (3 × 15 ml) under reflux in a water bath). Extract was filtered and the volume was made up to 25 ml with methanol. This extract was spotted onto the plate, and developed with Toluene: Ethyl Formate: Formic Acid (5:5:2) in a pre saturated TLC chamber, to the height of 10 cm. The plate was removed from the chamber, dried in air and visualized under ultraviolet light at 254 nm. A band (R_f 0.42) corresponding to ellagic acid is visible in standard and test solution tracks.

Similarly, TLC was carried out on a pre coated silica gel 60F254 plate by using *arjunic acid*, *arjungenin*, *arjunolic acid* and *arjunetin* as reference standards. Then the plate was developed with *Ethyl Acetate:Toluene:Formic Acid:Glacial Acetic Acid* (6:3:0.5:1). Standard solutions were prepared by dissolving 1 mg each of *arjunic acid RS*, *arjungenin RS*, *arjunolic acid RS* and *arjunetin RS* in 10 ml of *Methanol*. 10 µl each of the test and standard solutions were taken on a TLC plate as bands of 10 mm. The plate was developed to a distance of 8 cm from the line of application. Then the plate was dried in air and sprayed with a solution of *Anisaldehyde Sulfuric acid reagent*. The plate was heated at 110 °C for about 5 min till the bands were clearly visible. The chromatogram obtained with test solution shows a band at R_f 0.80 corresponding to that of *arjunic acid*, and at R_f 0.60 corresponding to that of *arjungenin*. The R_f values of *T. arjuna* in different solvent systems are given in Table 4.

5. Result and discussion

In the present study an attempt has been made to collect and select some Physicochemical, Phyto-Chemical, Macro & Microscopic and TLC examinations, which are found to be very useful tools for the identification and characterization of *T.* arjuna bark. A simple, accurate and precise analytical method is used for the analysis of *T. arjuna* bark, which could be useful in future Forensic identification of unknown plant material. It is found that the Physicochemical, Phyto-Chemical, Macro& Microscopic, and TLC examinations are very useful tools for the identification of *T. arjuna* bark. Phyto chemical studies were carried out for the identification of Arjuna bark with standard plant bark. Thin layer chromatographic studies showed the presence of active principles of *T. arjuna*, this is further suggested that the proposed methods are simple, sensitive and reproducible. The suggested protocol can also be used for the qualitative evaluation of Arjuna bark in laboratory with very less equipments and expenses. These can be employed successfully for routine forensic analysis of *T. arjuna*. As the evaluation expenses are less as compared to other instrumental methods, this could be a method of choice for official monographs in Forensic Toxicology.

Table 4 TLC data of *Terminalia arjuna*.

S.No.	Solvent system used	Detection reagent	Color of spots	R_f value
1.	Toluene:Ethyl formate:Formic acid (5:5:2)	Methanolic	Gray	0.15
		Ferric	Pinkish blue	0.24
		Chloride	Dark blue	0.33
		–	Blue	0.42
2.	Toluene:Ethyl acetate:Formic acid. (07:03:0.5)	Methanolic	Gray	0.81
		Ferric	Pinkish blue	0.10
		Chloride	Dark blue	0.20
		–	Blue	0.39
3.	Ethyl acetate:Toluene:Formic acid:	Anisaldehyde	Blue	0.80
	Glacial acetic acid (6:3:0.5:1)	Sulfuric acid		0.60

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