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# COMPARATIVE STANDARDIZATION OF *Terminalia arjuna* PLANT AND MARKETED PRODUCTS USING TLC AND HPTLC FOR ELLAGIC ACID MEASUREMENT

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**ABSTRACT**

The standardization and quality control of herbal medicines encompass critical processes such as the physicochemical evaluation of crude drugs, including selection, handling, safety, efficacy, and stability assessment of the finished product. *Terminalia arjuna* (Roxb.) is a significant plant in ethnomedicine, traditionally used in ancient Indian medicine to treat "Hritshool" (angina) and cardiovascular diseases, belonging to the Combretaceae family. The medicinal components of this plant include arjunine, arjunetine, arjunetin, arjunolone, arjunone, and Ellagic acid.This review focuses on the application of thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) for qualitatively and quantitatively measuring ellagic acid in Arjuna plant samples. A comparative study was conducted between commercially available Arjuna products and synthetically obtained samples of ellagic acid. The analysis revealed the presence of ellagic acid in the marketed Arjuna powder.

## INTRODUCTION

Standardization and quality control of herbal medicines, as defined by the WHO (199a and b, 1992), involves the comprehensive physicochemical evaluation of crude drugs. This process includes the selection and handling of raw materials, as well as the safety, efficacy, and stability assessment of the finished product. It also encompasses documenting safety and risks based on empirical knowledge, providing product information to consumers, and promoting the product. These aspects collectively form the framework of standardization according to WHO guidelines.

**KEYWORDS:**

*Terminalia Arjuna*, Thin-layer Chromatography, High Performance Thin Layer Chromatography, Visualization, Ellagic Acid, And Arjunic Acid, Arjunine, And Arjunetin Arjunolone and Arjunone.

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**Figure 1: Tree of Arjuna**

*Terminalia arjuna*, known for its therapeutic properties, has been utilized since ancient times by Indian physicians to treat "Hritshool" (angina) and various cardiovascular diseases. This plant, belonging to the Combretaceae family, is recognized as a potential cardioprotective agent. It features prominently in Ayurvedic medicine and is extensively documented in ancient Indian texts such as the Charaka Samhita, Sushruta Samhita, and Astang Hridayam. The use of T. arjuna bark powder in cardiac ailments was first recommended by Vagabhatta.

The plant contains a rich array of chemical constituents across all its parts. The bark, in particular, is notable for its flavones, arjunolone, terpenes and their glycosides, arjungenin, friedelin, arjunin, arjunectine, Arjun glycosides I, II, III, arjunoside II, arjunolic acid, oleanolic acid, arjunic acid, ellagic acid, and significant amounts of calcium salts. These components contribute to its therapeutic efficacy in promoting heart health and alleviating the effects of stress and anxiety.

**Figure 2: Leaf and Flower of Arjuna**

Standardizing the herbal remedy derived from Terminalia arjuna, commonly known as Arjuna plant, is a meticulous process aimed at ensuring consistent quality and effectiveness. This procedure involves several essential steps, beginning with the use of a regulated extraction method and precise identification of the plant part used, whether leaves or bark. Botanical identification of Terminalia arjuna is rigorously conducted to maintain authenticity.

Furthermore, the standardization process includes the quantification of key phytochemical components such as tannins, flavonoids, and arjunolic acid, which are crucial for enhancing the plant's medicinal properties. Chemical profiling provides distinct markers for verification, while quality standards such as moisture content, ash value, and microbiological purity are strictly adhered to.

To ensure consistent efficacy, the concentration of active ingredients in the herbal extract is standardized. Bioassays are employed to evaluate biological activity, and stability studies assess the product's storage conditions and shelf life. These comprehensive measures collectively contribute to maintaining the quality, safety, and therapeutic potency of the Arjuna herbal remedy.

**Figure 4: Fruit**

Ellagic acid, arjunic acid, and ß-sitosterol are the primary constituents of interest. Given the significant demand, it is crucial to ensure the quality of marketed samples. Additionally, naturally occurring samples must undergo rigorous analytical testing to verify their quality. Therefore, to ensure quality through pharmacogenetic evaluation, a comparative study has been designed to assess the arjuna bark collected from the market against samples collected naturally.

**Figure 5: Stem of Arjuna**

**METHOD AND MATERIALS**: To compile information on plants traditionally used for treating cardiovascular disorders, a systematic literature review was conducted. This involved searches in peer-reviewed journals, local books, and electronic databases such as PubMed, Sc Finder, Scopus, Scirus, ScienceDirect, Google Scholar, and Web of Science.

1. **Botanical Description**: *Terminalia arjuna*is a large, fluted, deciduous tree that can reach heights of up to 30 meters and a diameter of 2.5 meters. It often has a buttressed trunk and a superficial root system that spreads radially along stream banks. The tree's broad crown supports branches that spread widely. The bark is thick, smooth, and peels off in thin, irregular sheets, appearing grey or pinkish-green in color. Externally, Terminalia arjuna bark is plain and dull, while internally, it is red, flexible, and dense.
2. **Occurrences Arjuna:**

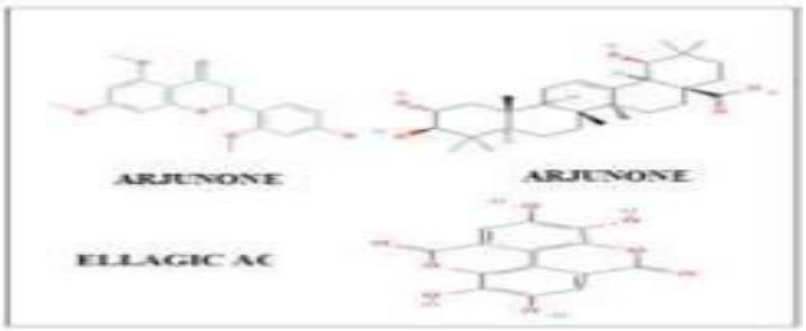


**Figure 3: Chemical Structure of Phytochemicals Of Arjuna**

**HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC):**

High-performance thin-layer chromatography (HPTLC), also known as a planar chromatographic technique, is extensively utilized for the standardization of herbal products.

HPTLC is an advanced method known for its repeatability and high separation efficiency. It employs well-defined procedures with optimized and standardized parameters that are validated, ensuring reliable analytical results with strong intra- and inter-laboratory consistency. This technique is valuable for both quantitative and qualitative analysis of herbal drugs.

Various Steps Involved in HPTLC/Planar Chromatography:

1. Selection of TLC/HPTLC plates and sorbent.
2. Sample preparation, including any necessary clean-up and pre-chromatographic derivatization.
3. Application of the sample and development on the plate.
4. Development in a chromatographic chamber.
5. Visualization of separated components.
6. Detection methods, including post-chromatographic derivatization if needed.
7. Quantitation and documentation of results.

**HPTLC ESTIMATION:**

High-performance thin-layer chromatography (HPTLC) was employed to quantitatively determine Ellagic Acid in stem bark extracts of Terminalia arjuna.

**HPTLC INSTRUMENTATION:**

Chromatography was conducted on 10 x 5 cm HPTLC plates coated with 0.25 mm layers of silica gel 60 F254. Before use, the plates were treated with methanol and activated at 110ºC for 5 minutes. [39]

| Stationary phase | Precoated Silica Gel F $_{254}$ Plates (Merck) |
|---|---|
| Mobile phase | Toluene: ethyle acetate: formic acid: methanol (3: 3: 8: 2 v/v) |
| Saturation | 40 mins |
| Temperature | 25 ± 2 ºC |
| Development chamber | Glass twin trough development chamber |
| Applicator | CAMAG Linomat IV applicator |
| Scanner | CAMAG Scanner III Win Cats (4.06), Switzerland |
| Mode of scanning | Absorption (deuterium) |
| Detection wavelength | 280 nm |
| Scanning Speed | 20 mm/s |

**Table 3 HPTLC Instrument Parameter**

Samples were applied onto the plate using a Camag 100 µl sample syringe with a Linomat 5 applicator, forming a band with a width of 4 mm.

A 10 cm × 10 cm CAMAG twin trough glass chamber facilitated linear ascending development of the TLC plate under saturation conditions for 40 minutes. The mobile phase consisted of toluene: ethyl acetate: formic acid : methanol, with a migration distance of 80 mm per run. Densitometric scanning was conducted using a CAMAG TLC scanner III operated by winCATS software.

Reagents And Other Materials: Ellagic acid (Sigma Aldrich), toluene, ethyl acetate, formic acid, and methanol (all analytical grade, E-Merck), and silica gel F254 precoated TLC aluminum plates (E-Merck).

**PREPARATION OF STANDARDS:** Arjunetin and arjungenin (1 mg each) were accurately weighed and separately dissolved in 1 mL methanol, then sonicated for 15 minutes. The stock solution was further diluted to achieve a 1 µg/mL concentration of the reference standard, stored at 4 ˚C for chromatographic analysis.

**PREPARATION OF TEST SOLUTION:** 0.5 g of powdered drug was extracted with methanol (3 × 15 mL) under reflux on a water bath. The methanolic extract was filtered, concentrated, and adjusted to a volume of 25 mL with methanol.

Mobile Phase: Various solvents were evaluated based on their polarity and the constituents of interest for separation. The chromatographic profiles in each solvent system were observed and recorded. The selected mobile phases included:

| Toluene: Ethyl Acetate: Formic Acid: Methanol (3:3:8:2:) |
|---|

**HPTLC CHROMATOGRAM:** HPTLC is a well-established technique used for the chemical identification of herbal drugs. It displays the sample's identity through a series of separated zones with distinct peak intensities. The HPTLC plate records information about other samples and standards, providing insights into the quality of chromatography and the conditions maintained throughout all steps. [43]

**A. STANDARD HPTLC CHROMATOGRAM:**

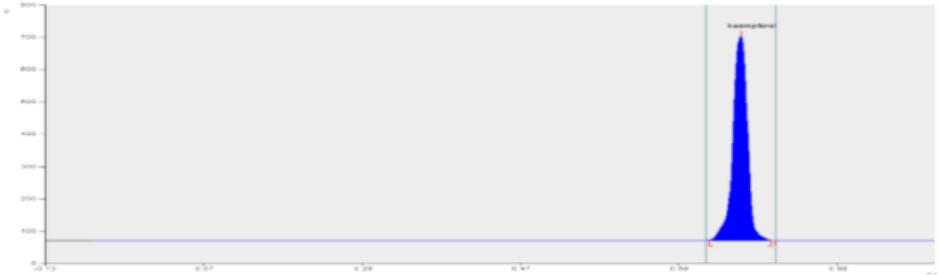

**Figure 6 HPTLC Chromatogram of Arjuna plant phytochemicals**

**CONCLUSION:**

The standardization of Terminalia arjuna using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) has been successfully achieved, allowing for thorough qualitative and quantitative comparisons with marketed samples. Qualitative analysis with TLC confirmed the presence of key phytoconstituents like Ellagic acid, identified by matching their Rf values of 0.42 with standard markers, thereby affirming the authenticity and consistency of the botanical samples. Quantitative analysis through HPTLC further quantified these phytoconstituents, highlighting variations in concentration levels between experimental and marketed samples.These analytical techniques have effectively distinguished genuine Terminalia arjuna samples from substandard or adulterated products available in the market. The data obtained serve as robust benchmarks for ensuring the quality and therapeutic efficacy of Terminalia arjuna, supporting its standardization and

compliance with regulatory standards. This study underscores the critical role of advanced chromatographic techniques in the standardization of herbal drugs, enhancing consumer safety and product reliability.In summary, TLC and HPTLC analyses have proven indispensable in the phytochemical evaluation and standardization of Terminalia arjuna, providing a reliable framework

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