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Comparative Standardization of Terminalia Arjuna Plant and Marketed Arjuna Product by Using TLC and HPTLC in Measurement of Ellagic Acid

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ABSTRACT:

The standardization and quality control of herbal medicines are the process involved in the physicochemical evaluation of crude drugs covering aspects such as selection and handling of crude material, safety, efficacy, and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumers, and product promotion. Terminalia arjuna (Roxb.) is an ethnomedicinally important plant that has been used by ancient Indian physicians to treat "Hritshool" (angina) and other cardiovascular diseases. It is a member of the Combretaceae family. The plant's medicinal qualities include arjunine, arjunetine, arjunetin arjunolone, and arjunone & Ellagic acid.

This review study focuses on the use of thin-layer chromatography (TLC) & high-performance thin-layer chromatography (HPTLC) in the qualitative and quantitative measurement of ellagic acid in the Arjuna plant. By comparative study was conducted between the marketed Arjuna product and a synthically collected sample of Ellagic acid. The results showed that the analysis marketed arjuna powder had the presence of ellagic acid

KEYWORD: Terminalia Arjuna, Thin-layer Chromatography, High-Performance Thin Layer Chromatography, Visualization, Ellagic Acid, And Arjunic Acid,Arjunine, And Arjunetin Arjunolone And Arjunone

I. INTRODUCTION OF TERMINALIA ARJUNA PLANT

Standardization and quality control of herbal crude medicines - According to WHO (1996a and b, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drugs covering aspects such as selection and handling of crude material, safety, efficacy, and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumers, and product promotion all these aspect covered under the standardization according to WHO.

Figure 1: Tree of Arjuna

Terminalia arjuna powdered tree bark was used by ancient Indian physicians to treat "Hritshool" (angina) and other cardiovascular diseases. Since ancient times, all parts of the plant have been employed for their therapeutic benefits. T. arjuna promotes heart health and reduces the effects of stress and anxiety.

Arjuna is a member of the Combretaceae family, is a possible cardioprotective agent. It is an ayurvedic treatment that has been referenced in several ancient Indian medicinal literature dating back to the Vedic period, including Charaka Samhita, Sushruta Samhita, and Astang Hridayam. Vagabhatta was the first to recommend the use of stem bark powder in cardiac problems. Major chemical ingredients can be found in all portions of T. arjuna. The bark contains important chemical constituents such as 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 a large amount of calcium salts.

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Figure 2: Leaf and Flower of Arjuna

The process of standardizing the herbal remedy made from the Arjuna plant (Terminalia Arjuna) includes a thorough set of requirements in order to guarantee a constant level of quality and effectiveness. This technique entails using a regulated extraction method, precisely identifying the plant portion (leaves or bark, for example) that is being used and rigorous botanical identification of the Arjuna plant. To further enhance the plant's medicinal qualities, it also involves the measurement of important phytochemical components such as tannins, flavonoids, and arjunolic acid. Chemical profiling creates distinct indicators for verification, and quality standards, such as moisture content, ash value, and microbiological contamination, are strictly outlined. To guarantee constant efficacy, the herbal extract's active ingredient concentration is standardized. Bioassays are used to measure biological activity, and stability studies look at storage and shelf life.

Figure 4: Fruit

Ellagic acid, arjunic acid, and ß-sitosterol are the main constituents. Owing to the high demand, the quality of the marketed sample must be guaranteed. Additionally, self-naturalized samples must undergo various analytical tests to ensure their quality. Therefore, in order to assure quality through pharmacogenetic evaluation, a comparative study between the marketed product and a self-naturally collected sample of arjuna bark has been designed for this study.

Figure 5: Stem of Arjuna

An essential step in determining the effectiveness and quality of a marked formulation is identifying the bio-transformed phytochemicals and quantitatively evaluating them. Using high-performance thin-layer chromatography (HPTLC) and thin-layer chromatography (TLC), the quantitative measurement of ellagic acid in T. arjuna was conducted.

II. METHOD AND MATERIAL:

The information on various plants traditionally used for cardiovascular disorders was gathered through systematic literature searches, library searches for articles published in peer-reviewed journals, as well as locally available books, and electronic searches (using PubMed, Sc Finder, Scopus, Scirus, ScienceDirect, Google Scholar, and Web of Science).

1. Botanical Description:

Terminalia arjuna is a huge, fluted, deciduous tree that can grow up to 30 meters tall and 2.5 meters deep. Its trunk is frequently buttressed. Its weak, superficial root system extends radially along the sides of streams. Branches drop from the broad, spreading crown. Bark is thick, smooth, and exfoliates in thin, uneven sheets. It might be grey or pinkish-green in color. Terminalia arjuna bark On the outside, it is sleek, drab, and basic. The interior of the shell is red, supple, and thick.

2. Occurrences Arjuna:

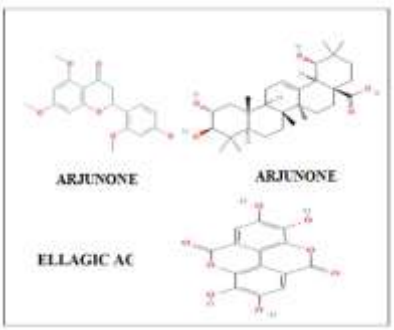

Figure 3: Chemical Structure Of Phytochemicals Of Arjuna

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A plant that grows on the banks of rivers or next to dry riverbeds in the Indian Subcontinent. It may be found growing in Uttar Pradesh, Bihar, Maharashtra, Madhya Pradesh, West Bengal, Odisha, and south and central India. It is also found in Pakistan, Karachi, Sri Lanka, and Bangladesh. Additionally, Malaysia, Indonesia, and Kenya have planted it.

Taxonomic Classification:

- Kingdom: Plantae
- Sub-kingdom: Tracheobionta
- Divisioni: Magnoliophyta
- Subdivision: Spermatophyta
- Class: Magnoliopsida
- Order: Myrtales
- Family: Combretaceae
- Genus: Terminalia
- Species: T. arjuna
- Zoological name: Terminalia arjuna

3. Macroscopic Analysis:

Several morphological traits were observed, such as the bark's color, form, and diameter (cm) and area (cm^). The bark's color and form were visually observed.

The bark's inner surface was pinkish in hue and had minute longitudinal striations, while its exterior surface was smooth and pale greenish yellow. There are sections of bark that were flat, curved, and recurved. While the exterior section was laminated, the interior part had a brief crack. Additionally, a sample of arjuna bark measuring 8.5 cm in length and 6.3 cm in width was noted.

4. Phytochemical Constituents:

Arjuna's chemical ingredients are found in its root bark, stem bark, leaves, seeds, and fruits. The root includes triterpenoids and glycosides, the fruit contains triterpenoids and flavonoids, and the leaves and seeds contain flavonoids and glycosides. From Terminalia arjuna, phytochemicals are considered one of the best heart tonics.

Arjuna contains about 15 per cent of tannins (hydrolysable). It also contains triterpenoid saponins, arjunolic acid, arjunic acid, arjungenin. In addition, it contains B-sitosterol, ellagic acid, and arjunic acid. The crystallizable compounds reported are arjunine and arjunetine. Arjunetin arjunolone and arjunone are the flavonoids reported in Arjuna bark. Calcium, aluminum and magnesium salts, along with coloring matter and sugar are the other constituents of Arjuna

From a medicinal standpoint, the bark was thought to be the most essential portion of the plant Arjuna. Initially, the bark was found to contain 34% ash, which was totally composed of pure calcium carbonate. The aqueous extract included 23% calcium salts and 16% tannins, whereas the alcoholic extract contained tannins and minimal coloring matter

Poly phenols (60–70%) tannins (20–24%)

5. Pharmacological Effect:

T. arjuna is commonly used in the treatment of cardiovascular disorders, such as heart disease and accompanying chest pain, high blood pressure, and high cholesterol. It is also used to treat earaches and urinary tract disorders. T. arjuna's usefulness as an anti-ischemic drug and as a potent antioxidant in preventing LDL, reperfusion ischemic heart injury, and its ability to lower atherogenic lipid levels has been amply proven in many experimental and clinical trials.

6. Physico-Chemical Analysis

(A)Total Ash%:

In a tarredchinadish, 2gm of powdered T. arjuna bark was collected. And that is treated to a muffle furnace at 450 C; weight was collected when it became red-hot and then cooled. A two-hour period of constant reading was observed. Standard ash % should be not more than 25%

(B) Acid Insoluble Ash:

The total ash obtained from 2g of bark and leaf sample were boiled with 25ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on a Gooch crucible, washed with hot water and ignited to obtain constant weight. The percentage of the amount of acid insoluble ash was calculated with reference to air-dried drugs Standard Acid insoluble Ash % Should be less than 1%

(C) Water-Soluble Extractive Values

Macerated 5 g of the air-dried drug, coarsely powdered, with 100 ml chloroform water (2.5 ml chloroform in purified water to produce 1000 ml) of specified strength in a closed flask for 24 h, frequently shaking during 6 hours and allowing to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish and dried at 1050, to constant weight and weight. Then calculate the percentage of water-soluble extractives with

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reference to the air-dried drug Standard water-soluble extractive values should be not less than 20%

(D) Alcohol Soluble Extractives

2 g powdered drug was placed in a conical flask and macerated with 100 ml of water for 6 hours, with frequent shaking, and then allowed to stand for 18 hours filtered through Whatman filter paper. 10 ml of filtrate was transferred to an evaporating dish and the solvent was evaporated in a water bath. Cooled it in desiccators for 30 minutes and finally weighed. The content of extractable matter air-dried material was calculated standard alcohol soluble extractive should not be less than 20%

Identity, Purity, And Strength as Per:

API Foreign matter	NMT 2%
Total ash NMT	NMT 25%
Acid insoluble ash	NMT 1%
Alcohol soluble extractive	NLT 20%
Water soluble extractive	NLT 20%

Table 1 Physicochemical Analysis Of Arjuna

7. Preliminary Phytochemical Analysis Of T. Arjuna Bark Extract:

Phytoconstituents	Tests	Conclusion
Phytosterol	Salkowski reaction	++
Triterpenoids	Liebermann Burchard's test	+
Saponins	Foam test	+
Alkaloids	Dragendroff's test	+
Carbohydrates	Molisch's test	+
Flavanoids	Lead Acetate test	++
Lactones	Legal's test	++
Phenolic Compounds and Tannins5%	Fecl3 Test	++
Proteins	Ninhydrin test	++
Glycosides	Keller-Killiani test	+

Table 2 Preliminary Phytochemical Analysis Present in low concentration; ++: Present in high concentration

III. ANALYTICAL VALIDATION :

It's a critical need for assessing and proving the identification of herbal drugs by using Thin Layer Chromatography, High-Performance Thin Layer Chromatographic methods are utilized in a variety of applications ranging from simple screening tests to separating substances in a mixture &TLC is a highly adaptable separation technology that is commonly used for both qualitative and quantitative sample analysis. quantitative assays using instruments in complicated matrices.

THIN LAYER CHROMATOGRAPHY:

TLC enables the quick identification of phytochemicals in herbal medicines. This device successfully delivered real-time information on the high-resolution mass number of compounds on the TLC plate. TLC is an open separation technique that leads to result scattering, yet it is also an analytical approach with no significant systematic mistakes.

Thin-Layer Chromatography Consists Of Two Phases:

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The Stationary Phases: A thin adsorbent material layer, such as silica, alkyl-silica (C8 or C18), cellulose, and monolithic polymer coated on metal, plastic, or glass sheets, are commonly used in TLC.

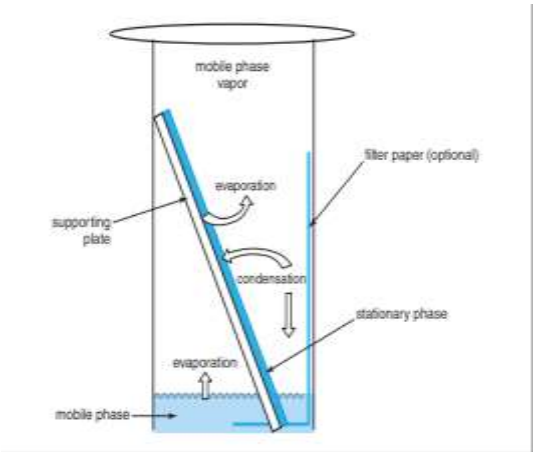


Figure 4 Thin Layer Chromatography

The Mobile Phase:The mobile phase is one in which the solvent mixture or solvent travels. Organic solutions with a wide range of hydrophobicity are often analyzed.
During development, molecules alternate between the free and adsorbed states. The position of equilibrium, and hence the ability of the solvent to drive the solute along the plate, is determined by a balance of intermolecular forces.
A balance of intermolecular forces controls the position of equilibrium and hence the solvent's capacity to move the solute along the plate.
This balance is determined by
(1) the polarity of the TLC coating material,
(2) the polarity of the developing solvent, and
(3) the polarity of the sample molecule(s).

R*f* values:
The behavior of an individual compound in TLC is characterized by a quantity Known as R*f* and is expressed as a decimal fraction.

$$Rf = \frac{\text{Distance of solute spot from starting point}}{\text{Distance of solvent front from starting point}}$$

TLC Identification Test:

Test Solution: 0.5g of powdered drug was extracted with methanol (3 x 15 ml) under reflux in a water bath. The methanolic extract was filtered and concentrated and made up the volume to 25ml with methanol
Standard Solution: Dissolve the 5 mg of Ellagic acid in methanol and make up the volume with methanol to 50 ml in a Volumetric flask and concentrate and make up the volume

Solvent System:

Toluene: Ethyl Format: Formic Acid

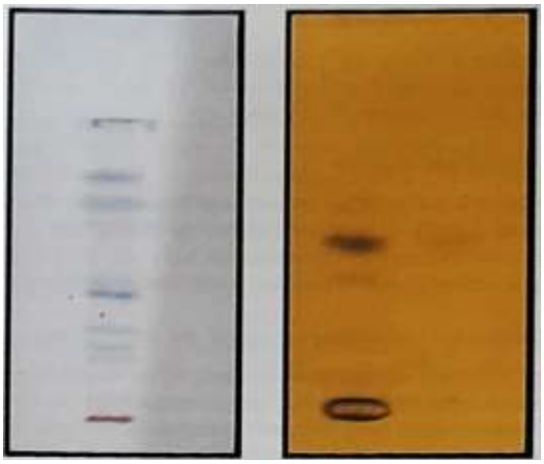
(5:5:2)

Toluene: Ethyl Acetate: Formic Acid: Methanol

(6:3:0.1:1)

Procedure:
Applied 10ml each of the test solution and standard solution on a precoated Silica Gel 60 F254 plate of uniform thickness of 0.5mm. The plates were developed in the solvent system

PLATE PROFILE =
A TLC PLATE =Test solution of marketed arjuna bark powder
B TLC PLATE = standard solution of ellagic acid



EVALUATION: A band (Rf. 0.42) corresponding to ellagic acid is visible in standard and test solution tracks.
A TLC PLATE was standard ELLAGIC ACID = Rf value is 0.42
B TLC PLATE was sample of arjuna bark = Rf value is 0.42

IV. HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY:

Chromatographic methods such as High-performance thin-layer chromatography (HPTLC) is specifically exercised for the standardization of herbal products. and its also called a planar chromatographic technique.

HPTLC is a novel idea that focuses on repeatability and separating power. It employs

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well-defined procedures with optimized and standardized parameters that are validated and produce reliable analytical results with strong intra- and inter-laboratory consistency. it's very useful to determine the quantitively as well as qualitatively of herbal drugs

Various Steps Involved in HPTLC/Planar Chromatography**:**

1. Selection of TLC/HPTLC plates and sorbent.
2. Sample preparation including any clean-up and pre-chromatographic derivatization.
3. Definition (quantitative)
4. Application of sample Development (linomate)
5. Development chamber (chromatogram immersion)
6. Visualization device
7. Detection including post-chromatographic derivatization (scanner)
8. Quantitation and documentation

HPTLC ESTIMATION:

The high-performance thin-layer chromatography technique (HPTLC) was used for the quantitative determination of ELLAGIC ACID in stem bark extracts of Terminalia Arjuna.

HPTLC INSTRUMENTATION:

Chromatography was performed on 10 x 5 cm HPTLC plates coated with 0.25 mm layers of silica gel 60 F254. Before use the plates were washed with methanol and activated at 110ºC for 5 min. [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 [46]

Samples were applied on the plate as a band with 4 mm width using a Camag 100 µl sample syringe with a Linomat 5 applicator.

10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 40 min saturation conditions and toluene : ethyle acetate: formic acid : methanol : of mobile phase was used per run, migration distance was 80 mm. Densitometric scanning was performed using CAMAG TLC scanner III operated by win CATS software

Reagents And Other Materials: Ellagic acid (Sigma Aldrich), Toluene, Ethyl acetate, Formic acid, and Methanol (all Reagents of analytical grade, E-Merck) and silica gel F254 precoated TLC aluminum plates (E-Merck).

PREPARATION OF STANDARDS:

Accurately weighed arjunetin and arjungenin (1 mg) each was separately dissolved in 1 mL methanol and sonicated for 15 min. The stock solution was further diluted to prepare a 1 µg/mL concentration of reference standard, stored at 4 °C for chromatographic analysis.

PREPARATION OF TEST SOLUTION:

0.5g of powdered drug was extracted with methanol (3 x 15 ml) under reflux on a water bath. Methanolic extract was filtered and concentrated and made up the volume to 25ml with methanol.

Mobile Phase: Various solvents were tried depending on their polarity and constituents of interest to be separated. The chromatographic profile in each solvent system was observed and recorded. The selected mobile phases were as follows:

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

HPTLC CHROMATOGRAM:

HPTLC is a standard technique for chemical identification of herbal drugs it represents the identity of a sample and consists of a sequence of separated zones with a certain intensity of peaks. That HPTLC plate contains information regarding other samples and standards, the quality of the chromatography and the chromatographic conditions during all steps.[43]

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A. STANDARD HPTLC CHROMATOGRAM:

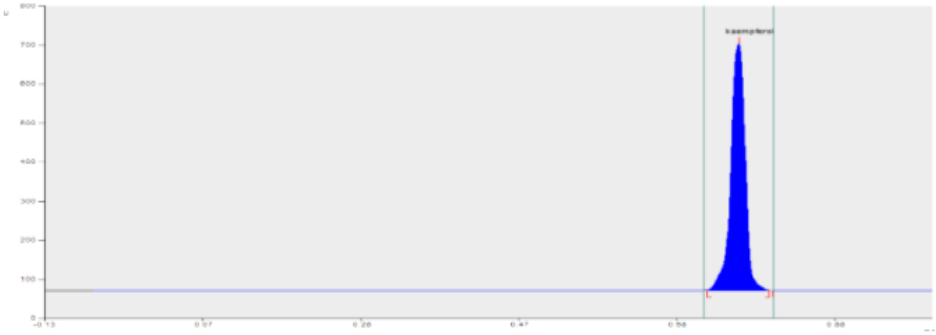

Figure 6 HPTLC Chromatogram of Arjuna plant phytochemicals

HPTLC Graph of Ellagic Acid

As a standard sample represented by TLC scanner 3(CAMAG) in which the X and Y-axis represent the height of the peaks and Rf 0.41 of each detected spot respectively at 254nm [44], [45]

B. SAMPLE HPTLC CHROMATOGRAM:

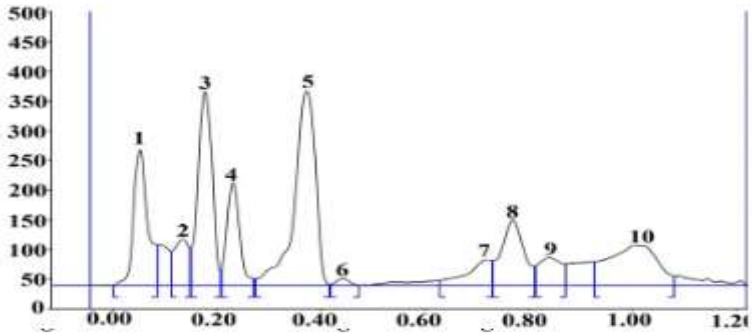

Figure5 HPTLC Chromatogram of Ellagic acid Rf0.41±0.03

HPTLC Graph of Arjun Bark Extract Sample:

This graph represented by TLC scanner 3(CAMAG) in which the X and Y-axis represent the height of the 1,2,3,4,5,6,7,8,9,10. peaks and peak no.5 represents Rf value 0.41 i.e Ellagic acid of each detected spot respectively at 254nm [44], [45]

Sample marketed	R_F 0.148	Concentration Quantity	20.58 µg/ml 205.8ng

CONCLUSION:

The standardization of Terminalia arjuna through Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) has been effectively achieved, enabling a comprehensive qualitative and quantitative comparison with marketed samples. The qualitative analysis, utilizing TLC, revealed the presence of key phyto constituents Ellagic acid by matching the Rf values 0.42 with standard markers, affirming the authenticity and consistency of the botanical samples. Quantitative analysis via HPTLC further quantified these phyto constituents, highlighting variations in the concentration levels between the experimental and marketed samples.

These analytical techniques demonstrated their efficacy in distinguishing genuine Terminalia arjuna samples from sub standard or adulterated products available in the market. The data obtained serve as robust bench mark for ensuring the quality and therapeutic efficacy of Term in alia arjuna, underpinning its standardization and facilitating regulatory compliance. This study underscores the importance of advanced chromatographic techniques in herbal drug standardization, contributing to improved consumer safety and product reliability.

In conclusion, TLC and HPTLC analyses have proven to be indispensable tools in the phytochemical evaluation and standardization of Term in alia arjuna, offering a reliable

methodology for quality assurance in herbal medicine.

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