

**STANDARDIZATION OF ARJUNARISHTA FORMULATION BY TLC METHOD****Himmat Singh\*, Swatantra Kumar Mishra and Dr Milind Pande**

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**E-mails:** [himmatbpl87@gmail.com](mailto:himmatbpl87@gmail.com), [swatantramishra08@gmail.com](mailto:swatantramishra08@gmail.com)**ABSTRACT**

Ayurveda is the oldest surviving complete medical system in the world. Its origins go back nearly 5000 years. *Arjunarishta* (*Parthadyarishta*) is an important Ayurvedic formulation used for cardiovascular disorders and is prepared by fermenting the decoction of specified plant materials using flowers of *Woodfordia fruticosa*. The objective of this study was to determine the level of alcohol, acidity and pH in commercially available Arjunarisht to establish a routine procedure for standardization of these Ayurvedic preparations. In present communication, a TLC- method was developed for the standardization of *Arjunarishta* by quantitative estimation of major antioxidant compounds, ellagic acid, as markers. The developed method was validated with respect to linearity, precision, accuracy, and robustness. Arjunolic acid and arjunic acid were not detected in the formulation.

**Keywords:** TLC, Parthadyarishta. Ellagic acid, Flavonoids**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 nearly 5000 years. The art of *Ayurveda* had spread around in the 6<sup>th</sup> century BC to Tibet, China, Mongolia, Korea and Sri Lanka, carried over by the Buddhist monks travelling to those lands. Ayurveda therefore is not simply a health care system but a form of lifestyle adopted to maintain perfect balance and harmony within the human existence, from the most abstract transcendental values to the most concrete physiological expressions. Based on the premise that life represents an intelligent co-ordination of the *Atma* (Soul), *Mana* (Mind), *Indriya* (Senses) and *Sharira* (Body). The official Ayurvedic Formulary of India (AFI) lists thirty-seven ashavas and arishtas [1].

Arishta and Asava have been used as medicines for over 3000 years to treat various disorders and are also taken as appetizers and stimulants. Due to their medicinal value, sweet taste, and easy availability people are prone to consume higher doses of these drugs for longer periods. [2] The preparation and sale of 34 varieties of *Arishta* and 25 varieties of *Asava* has been legalized and listed in the official Ayurveda pharmacopoeia of Sri Lanka. [3] Arishtas are an important group of formulations used in Ayurveda. *Arjunarishta* (*Parthadyarishta*) is one of the ancient liquid oral formulations prescribed in Ayurveda for cardiovascular disorders. [4] It nourishes and strengthens heart muscle and promotes cardiac functioning by regulating blood pressure and cholesterol. The plant ingredients in this formulation are *Terminalia Arjuna*, *Madhuca indica*, *Vitis vinifera*, and *Woodfordia fruticosa*. [5] The formulation is prepared by making a decoction of three plants in specified amounts as listed in AFI. Crushed jaggery and the flowers of *Woodfordia fruticosa* are then added and preparation is kept for a specified period of time during which it undergoes fermentation generating alcohol that helps extraction of active principles and also serves as preservative for these formulations [6].

**MATERIAL AND METHODS**

*Terminalia Arjuna*: - Arjuna Consist of the stem bark of *Terminalia Arjuna* W. and A. (Fam. Comretaceae); a large deciduous tree, commonly found throughout the greater parts of the country. In the present study, Arjunarisht was chosen for the research work due to its medicinal properties specially in curing cardiac ailments.[7] The ingredients required for the preparation of the above said formulation are *Terminalia Arjuna* (Arjun), *Vitis vinifera* (Draksha) and *Woodfordia fruticosa* (Dhawai ke phul). Among the above mentioned ingredients the major one is *Terminalia Arjuna* (Arjun) which as per the Ayurveda, have the curative and medicinal properties for various cardiac ailments. While, *Vitis vinifera* (Draksha) and *Woodfordia fruticosa* (Dhawai ke phul) is added in trace quantity in the formulation to induce, support and enhance the process of fermentation. Therefore, in the present study only one ingredient i.e. *Terminalia Arjuna* (Arjun) was subjected to the research and analysis. [8] During the research work, at the very first step it was necessary to identify the raw material required for the preparation of Arjunarisht. Therefore, the raw material was subjected to taxonomical and anatomical characterization. [9]

**Physico-chemical analysis of crude drug *Terminalia Arjuna* (Arjun):**

Physico-chemical analysis was done to as certain the quality of the raw material used in the preparation of Arjunarishta. To check the quality, parameters as mentioned in "Quality Standards of Indian Medicinal Plants" by Indian Council of Medical Research were followed. [10] The parameters and methodology are mentioned as below:

**Physico-chemical Parameters:**

**Total Ash:** 2gm of Powdered (*Terminalia Arjuna*) drug was taken in tarred china dish. After than it was subjected to muffle Furness at 450°C temp. The weight was taken after red hot and cooling at each two hours constant readings.

**Acid Insoluble Ash:** 2gm of Powdered (*Terminalia Arjuna*) drug was taken and mixed 25 ml of hydrochloric acid (HCL). Total ash was boiled for 5 min. and diluted was 25 ml of hydrochloric acid (HCL). Insoluble matter was collected on ash less filter paper (Grade 4T SD'S clear drop, 90mm code- F0401C10, Circuler-100). Filter paper washed with hot water. Crucible was ignited and cools after than keep in dessicator. Residue was weighed and calculated acid insoluble ash of drug.

**Determination of moisture content (Loss on Drying):** 2gm of Powdered (*Terminalia Arjuna*) drug was taken in tarred china dish. Dried in the oven at 100°C or 105°C, cooled in a desiccator and watch. After that the loss was recorded as moisture. The procedure was continued for at least two common readings.

**Sulfated Ash:** 2gm of Powdered (*Terminalia Arjuna*) drug was taken in silica crucible and 3 ml of sulfuric acid was added. Powdered was Incinerated by gradually increasing the heat until free from carbon. And than residue was cooled in the desiccator. Ash was weighed and calculated the percentage of sulfated ash value.

**Water Insoluble Ash:** 2gm of Powdered (*Terminalia Arjuna*) drug was taken in silica crucible and added 25 ml water. The mixture was boiled. After that insoluble matter was filtered on ash less filter paper (Grade 4T SD'S clear drop, 90mm code- F0401C10, Circuler-100). The residue was ignited in crucible and cool. The residue was weighed and calculates water insoluble ash.

**Determination of Alcohol content:** 2gm of powdered (*Terminalia Arjuna*) drug was taken in tarred silica crucible. The powdered drug was incinerated by heating until free carbon. Residue was cooled and kept in desiccator. The ash was weighed and calculated as the percentage of total ash. [11]

**Identification of marker constituents in the crude drugs by TLC**

**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.

**Solvent System:** Toluene: Ethyl Format: Formic Acid (5:5:2)

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

**Visualization:** The plates were examined under ultra-violet light at 254nm.

**Evaluation:** A band (Rf. 0.42) corresponding to ellagic acid is visible in standard and test solution tracks.

**Powder characteristics:**

**Procedure:** 2 gm powdered (*Terminalia Arjuna*) drug was taken in test tube and boiled with clearing agent by using Alcoholic hydrate. Powder was transfer in cleared glass. Lignified fibers were Stain with the staining agent (safferin) powder was treated in glycerin, water and observed the slide under low power. Stained fiber, stomata and other character was observed with the help of stage microscope and calibrate the eyepiece of microscope. [12]

## RESULT AND DISCUSSION

In this chapter the various results obtained from different experiments carried out are compiled.

### Macroscopy

A bark appeared to be, outer surface smooth, pale greenish yellow and inner surface finely longitudinally striated and pinkish in colour, It has bitter and characteristic taste. Bark has pieces, flat, curved, recurved, in shape. Fracture was short in inner and laminated in outer part and Sample (*Arjuna bark*) size 8.5cm in length and 6.3cm.in width was observed.



Figure: 1

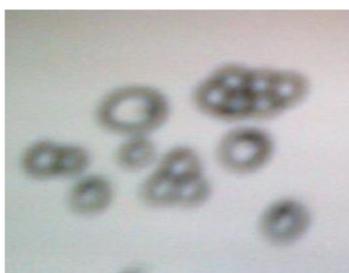


Figure: 2

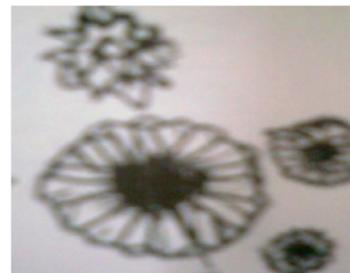


Figure: 3

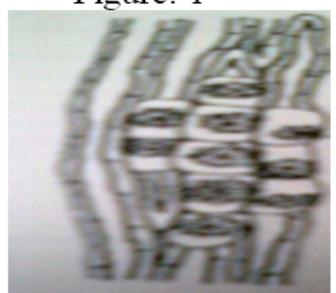


Figure: 4

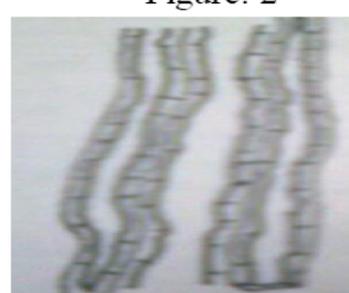


Figure: 5

**Table No.1 - Determination of Proximate Analysis for bark of *Terminalia Arjuna***

| Tests                             | Results (in %) |        |        | Inference (in %)   |
|-----------------------------------|----------------|--------|--------|--------------------|
| Tests for extraneous material     | S1             | S2     | S3     |                    |
| Foreign matter                    | 0.926          | 0.926  | 0.926  | Not more than 2.0  |
| Sand & Silica                     | Absent         | Absent | Absent | Should be absent   |
| Insect infestation                | Absent         | Absent | Absent | Should be absent   |
| Rodent contamination              | Absent         | Absent | Absent | Should be absent   |
| <b>Physico-chemical analysis</b>  |                |        |        |                    |
| Total Ash content                 | 15.762         | 16.367 | 16.534 | Not more than 27.0 |
| Acid insoluble ash                | 1.452          | 0.9568 | 0.9367 | Not more than 2.0  |
| Moisture content                  | 5.653          | 6.885  | 6.644  | Not more than 8.0  |
| <b>Tests for extractive value</b> |                |        |        |                    |
| Alcohol soluble extractive        | 45.01          | 46.88  | 46.66  | Not less than 16.0 |
| Water soluble extractive          | 41.44          | 43.995 | 43.86  | Not less than 17.0 |

**Table No. 2:- TLC Screening of *Terminalia Arjuna***

| Solvent system used                             | Detection reagent          | Colour of spots | Rf value |
|---|----------------------------|-----------------|----------|
| Toluene: Ethyl Format : Formic acid (5 : 5 : 2) | Methanolic ferric chloride | Grey            | 0.15     |
|   |                            | Pinkish blue    | 0.24     |
|   |                            | Dark blue       | 0.33     |
|   |                            | Blue            | 0.42     |

**Figure.6:** TLC profile of test solution of *Terminalia Arjuna* Stem bark (Test solution derivatized with ferric chloride solution Elaagic Acid Standard)**Microscopy:**

**Stem bark:** Mature bark (*Arjuna* bark) was cork consisting with 9-10 layers of tangentially elongated cells observed, Cork cambium (fig.1) starch grain (fig.2) Rosette crystal and rhomboidal type of calcium oxalate crystal (fig.3) was found and secondary cortex was

distinct, a medullary ray was observed transversing (fig. 4-5).

**Physico-chemical analysis of crude drug *Terminalia Arjuna* (Arjun):**

It is shown in table no. 1

**Analysis of *Terminalia Arjuna* bark by TLC method-** TLC densitometry estimation of *Terminalia Arjun*. TLC plates are precoated plates of silica gel 60 F254 (E.merck) of uniform thickness of 0.2 and Solvent system- Toluene: Ethyleformate: Formic acid (5: 5: 2). Results are shown in fig.6 and table 2

**SUMMARY AND CONCLUSION**

Thin layer chromatographic studies showed the presence of active principles like Ellagic acid on 0.42 Rf value for sample with was more close to standard 0.43 Rf with prominent blue coloration in both. This further needed to be subject to HPTLC for exact quantification of Elagic acid. These studies suggested for future because we do not have this facility. Phytochemical chemical studies were carried out for identification of ellagic acid from *Arjuna* bark powder using various chemical tests available for tannins in the reference books also confirm that give bluish black / greenish black with  $FeCl_3$  is found positive indicating that confirm presence of Ellagic acid in given samples of standard and test respectively.

This is further suggested that a percolated TLC plate gives perfect and close results which can be repeated in next

future. This is best method for Qualitative evaluation of arjunaristha in lab scale with very less equipments and expenses.

The all this evaluation needed near about one hour hence time saving also increase utility of this evaluations. The evaluations expenses are less as compared to HPLC evaluations this could be a method of choice for official monographs in Ayurvedic Pharmacopeia.

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