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## STANDARDIZATION OF AYURVEDIC FORMULATION “ARJUNARISHTA” IN TERMS OF PHYSICO-CHEMICAL, SPECTROSCOPY AND CHROMATOGRAPHIC TECHNIQUES

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### Keywords:

Standardization, Arjunarishta, High-Performance Thin Layer Chromatography (HPTLC), Fourier transform infrared spectroscopy (FT-IR), Arjungenin

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**ABSTRACT: Objective:** Arjunarishta is an important Ayurvedic formulation with chief ingredient Arjuna (*Terminalia arjuna*), which is excellent “Hrudya” Dravya. It nourishes & strengthens heart muscles & promotes cardiac functioning by regulating blood pressure and cholesterol level. This study aims to established quality parameters for standardization of Ayurvedic formulation “Arjunarishta” on the basis of various techniques viz. Physico-chemical screening, Chromatography & IR Spectroscopy. **Method:** Conventional analysis such as Organoleptic tests, pH, Alcohol content, Brix, and Specific gravity was done. Chromatographic analysis was performed to estimate Arjungenin, Ellagic acid, and Gallic acid using High-Performance Thin Layer Chromatography (HPTLC). IR fingerprint was done using Fourier transform infrared (FT-IR) spectrometer. **Result:** The above analysis showed the pH of tested formulation ranges between 3.0-5.0, Brix 25-30%, and Alcohol content 6-10% v/v. Organoleptic tests confirmed characteristic odor of self-generated alcohol with sweet, astringent & slightly bitter taste. HPTLC analysis confirmed the presence of Arjungenin, Ellagic acid, and Gallic acid. FT-IR reveals unique transmittance spectra in the range of 4000 - 600 cm<sup>-1</sup>. **Conclusion:** This study can be used for qualitative evaluation of Arjunarishta in terms of modern parameters, which may help in the authenticity of the drug and to compile suitable information for the better utility and safe use of this formulation in therapeutics.

**INTRODUCTION:** Ayurveda is the oldest traditional Indian medicinal system that contributes to a healthy life of human being <sup>1</sup>. The word 'Ayurveda' is comprised of two Sanskrit words, 'Ayu' means Life and 'Veda' means knowledge, which is originated in India but now practiced all over the world <sup>2-3</sup>. The World Health Organization (WHO) reported that almost 80% of the world populations rely on medicines mainly of herbal sources in their healthcare <sup>4-7</sup>.

The primary focus of Ayurvedic medicine is to prevent illness and promote good health. Various forms of Ayurvedic medicines such as Churna, Avaleha, Bhasmas, Asavas, Arishtas, Tailas are being used since ancient time to present era. Arishtas are an important group of Ayurvedic formulations used as medicines to treat various disorders in Ayurveda for over 3000 years <sup>8-9</sup>.

Arjunarishta also known as Parthadyarishta, is formulated with key ingredient 'Arjuna twak' (Arjuna Bark) (*Terminalia arjuna*) which is excellent 'Hrudya' Dravya i.e. Cardio-protective; it nourishes and strengthens heart muscles and promotes cardiac functioning by regulating blood pressure and cholesterol, is one of the ancient Ayurveda formulations prescribed in cardiovascular disorder in Ayurveda <sup>10-19</sup>.

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**FIG. 1: ARJUN TWAK (ARJUNA BARK) (*TERMINALIA ARJUNA*)**

Arjun Twak (*Terminalia arjuna*) has anti-hypotensive effects, boosts anti-oxidant activities, and prevents fibrosis<sup>20-23</sup>. It contains hydrolyzable tannins as gallic acid, ellagic acid, triterpenoid glycosides, Sapogenins as arjunic acid, arjunolic acid, arjungenin; flavonoids, mineral salt and sugar<sup>24-27</sup>. The previous studies reveal the effect of arjunolic acid, arjungenin, and arjunetin on the process of respiratory oxidative burst and cardiac fibrosis<sup>28-29</sup>.

The formulation Arjunarishta also contains Mrudvika (*Vitis vinifera*), Dhataki Pushpa (*Woodfordia fruticosa*), Madhuka Pushpa (*Madhuka indica*) which are rich sources of antioxidants<sup>30-31</sup>.

In order to achieve consistent, reproducible results in terms of efficacy and safety of drugs, the quality of phyto ingredients should be standardized and duly validated. In the case of polyherbal formulations, phytochemical screening, fingerprint profiling, and quantification of multiple markers need to be developed and implemented for ensuring quality and batch to batch consistency.

In quality control methods for medicinal plant materials, WHO has clearly specified for quantification of marker compounds and development of fingerprint profile for all botanical preparations<sup>32-33</sup>. Every drug should have complete information related to its organoleptic properties, physico-chemical characteristics & phytoconstituents.

Here an attempt was made to standardize ayurvedic formulation Arjunarishta in terms of physico-chemical, spectroscopy, and chromatographic techniques.

**MATERIALS AND METHODS:** Three batches of Arjunarishta were procured from approved stockist of Shree Dhootapapeshwar Ltd. batch codes designated were as Sample-1, Sample-2, and

Sample-3. These batches were analyzed for physico-chemical parameters, IR spectroscopic, and chromatographic evaluations to standardize the formulation.

All chemicals and reagents (Toluene, Chloroform, Ethyl acetate, Acetic acid, Formic acid, Methanol, etc.) used in the analysis were of analytical reagent grade of Merck. Reference standards (RS) of Arjungenin of purity 98.90% (CAS No, 58880-25-4), Gallic acid of purity 98.50% (CAS No, 149-91-7), and Ellagic acid of purity 99.80% (CAS No, 476-66-4) were used for the chromatographic study.

**Organoleptic Properties:** Various parameters such as color, taste, and odor of the samples of Arjunarishta were observed and recorded.

**Physico-chemical Evaluation:** In Physico-chemical screening, the samples of Arjunarishta were analyzed for various physicochemical parameters such as pH, alcohol content, specific gravity, and Brix and as per the Ayurvedic Pharmacopoeia of India (API)<sup>34</sup> and as per in-house specifications.

**Chromatographic Analysis:** HPTLC Instrument Camag with sample applicator Linomat 5, Densitometer TLC Scanner 4, and Wincat Software was used for chromatographic analysis of a methanolic extract of Arjunarishta. A twin trough chamber was used for the development of HPTLC plates. A photo documentation cabinet fitted with a high-resolution camera was used for capturing images at different wavelengths. Densitometer TLC Scanner 4 equipped with Deuterium (D2) & Tungsten (W) lamps were used to obtain spectra for the quantitative determination of Arjungenin, Gallic acid & Ellagic acid. Precoated silica gel G60-F<sub>254</sub> Aluminium sheets (E. Merck, Germany) 20 × 10 mm, thickness layer 0.2 mm was used as the stationary phase. The solvent systems Toluene: Ethyl acetate: Acetic acid (5: 5: 0.5) v/v for Arjungenin and Chloroform: Ethyl acetate: Formic acid (5: 4: 1.6) v/v for Gallic acid & Ellagic acid was selected for estimation, which gave good resolution. The wavelengths of 600 nm, 290 nm & 278 nm were used for quantification of Arjungenin, gallic acid & ellagic acid, respectively, in the samples.

**IR Spectroscopic Evaluation:** The samples of Arjunarishta were analyzed in the range of  $4000\text{ cm}^{-1}$  to  $600\text{ cm}^{-1}$  using ALPHA FTIR, Bruker with ATR sampling mode and OPUS software

**RESULTS AND DISCUSSION:** The formulation Arjunarishta characterized as a dark brown colored liquid having characteristic fermented odor with sweet taste followed by slight astringent & bitter **Table 1**.

The Physicochemical evaluations showed the pH of the formulation ranges between 3.5 to 5.0, Alcohol content between 7 to 10 % v/v, Brix 25 to 35 %, and Specific gravity 1.0 to 1.2. **Table 1** The pH of the solution provides an indication of the acidity or alkalinity of a solution. The digital pH meter was used for the pH measurement after calibration with buffer solutions. pH was noted for all the samples, and the results have been shown in **Table 1**. The formulation Arjunarishta contains self-generated alcohol.

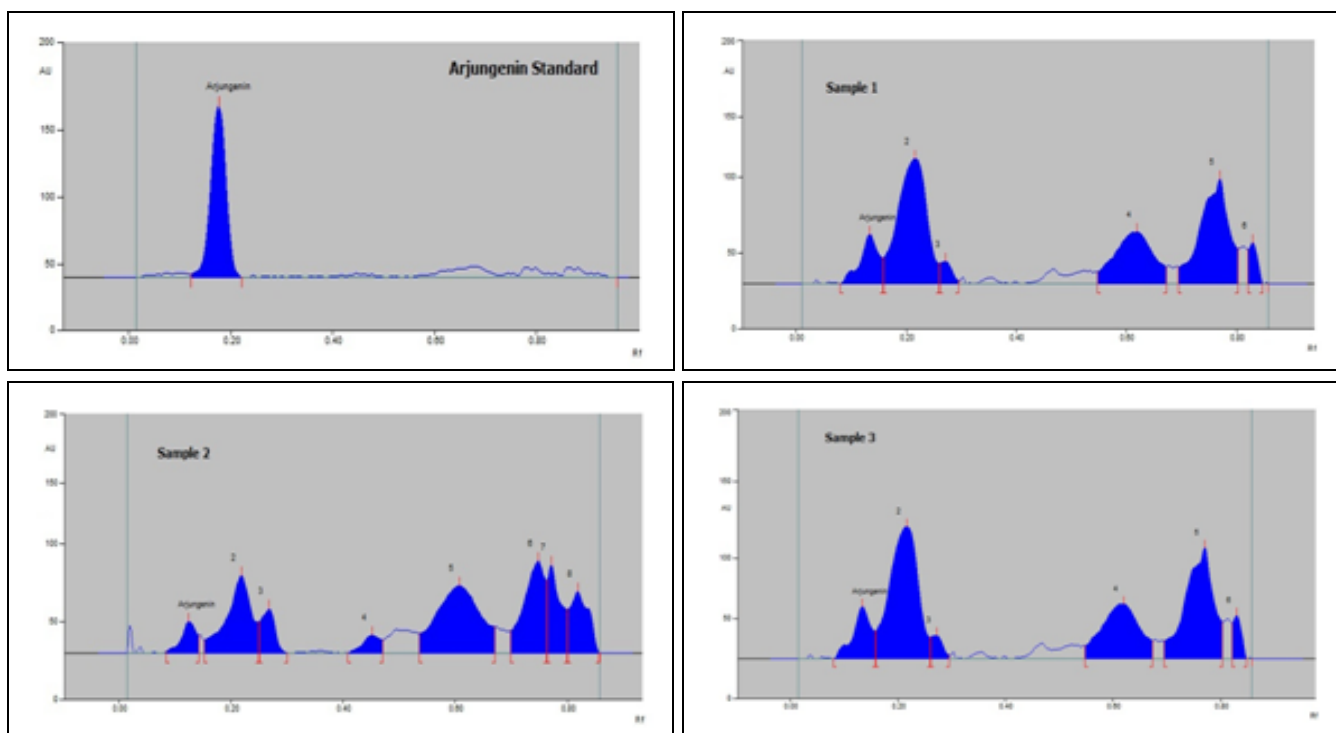
The alcohol content was determined as per the method described in The Ayurvedic Pharmacopoeia of India (API) for all the samples<sup>34</sup>. Brix was determined using a refractometer, and results were recorded. Brix is the sugar content of an aqueous solution. It represents the strength of the solution as a percentage by mass<sup>35</sup>. Specific gravity

is the ratio of the specific weight of the material to the specific weight of the distilled water. Specific gravity was determined as per the method described in The Ayurvedic Pharmacopoeia of India (API) and recorded.<sup>34</sup> **Table 1**.

**TABLE 1: RESULTS OF ORGANOLEPTIC AND PHYSICO-CHEMICAL EVALUATIONS**

Batch code	Sample-1	Sample-2	Sample-3
Colour	Dark brown	Dark brown	Dark brown
Odor	Fermented	Fermented	Fermented
Taste	Sweet with slight astringent & Bitter	Sweet with slight astringent & Bitter	Sweet with slight astringent & Bitter
pH	4.85	5	4.87
Brix %	29	31	30
Alcohol content (% v/v)	8.04	7.77	7.22
Specific gravity	1.09	1.09	1.08

The chief ingredient Arjuna twak (Bark) (*Terminalia arjuna*) contributed to the presence of Arjungenin, Gallic acid, and Ellagic acid in the formulation Arjunarishta. The HPTLC analysis confirms the presence of Arjungenin, Gallic acid, and Ellagic acid at  $R_f 0.15 \pm 0.02$ ,  $0.24 \pm 0.02$ , and  $0.37 \pm 0.02$ , respectively **Fig. 2, 3 & 4**. The content of Arjungenin, Gallic acid & Ellagic acid in Sample-1, Sample-2, and Sample-3 of Arjunarishta was estimated, and the results have been shown in **Table 2**.



**FIG. 2: PEAK DISPLAY OF ARJUNGENIN STANDARD AND ARJUNARISHTA FORMULATION (SAMPLE-1 SAMPLE-2 & SAMPLE-3)**

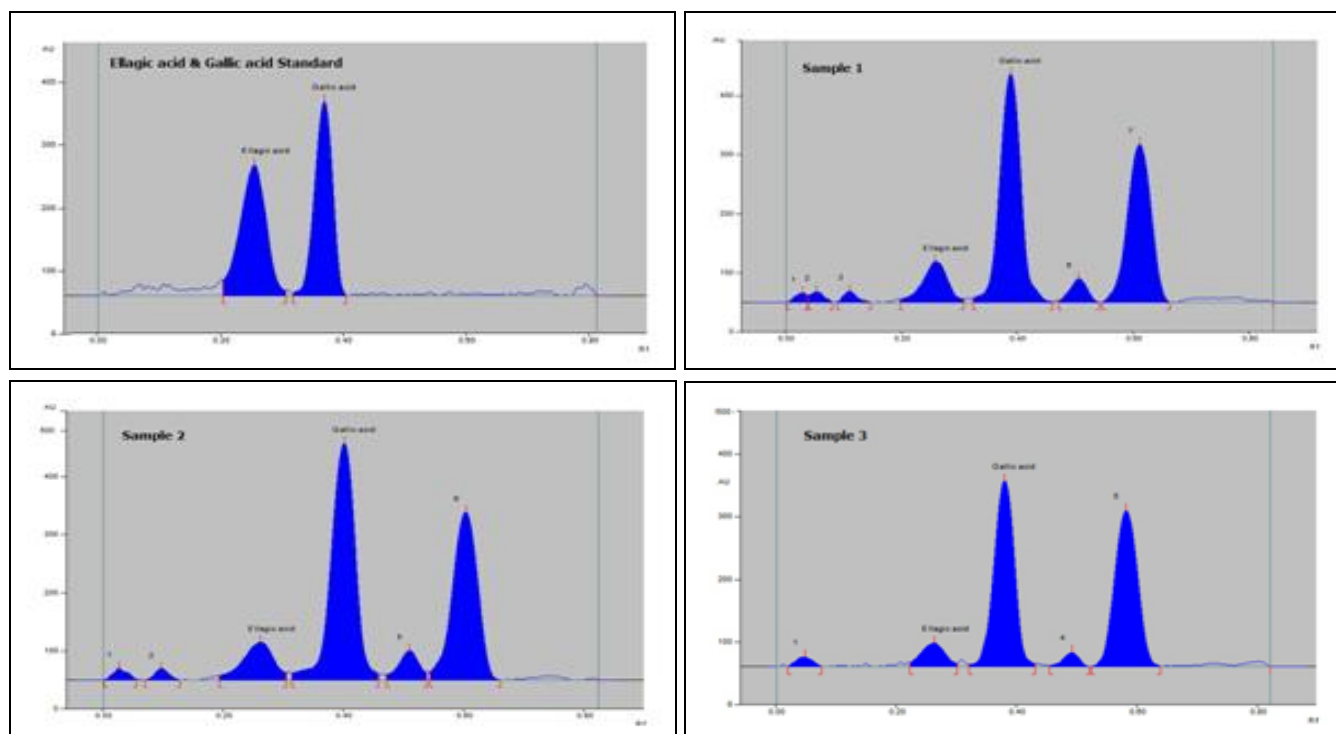


FIG. 3: PEAK DISPLAY OF GALLIC ACID & ELLAGIC ACID STANDARD AND ARJUNARISHTA FORMULATION (SAMPLE-1, SAMPLE-2 & SAMPLE-3)

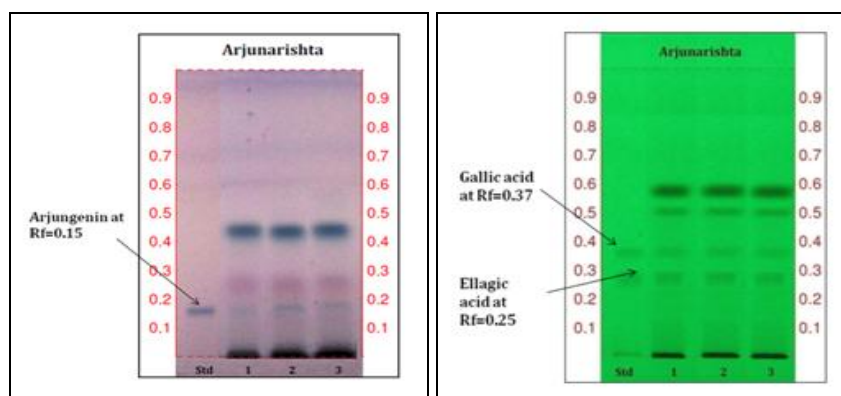


FIG. 4: THE HPTLC ANALYSIS CONFIRMS THE PRESENCE OF ARJUNGENIN, GALLIC ACID & ELLAGIC ACID IN ARJUNARISHTA FORMULATION (SAMPLE-1, SAMPLE-2 & SAMPLE-3)

TABLE 2: PHYTOCHEMICAL CONTENT IN ARJUNARISHTA

Batch code	Sample-1	Sample-2	Sample-3
Arjungenin (%)	0.007	0.006	0.007
Gallic acid (%)	0.04	0.04	0.02
Ellagic acid (%)	0.004	0.003	0.003

This technique of HPTLC has been known for the simultaneous estimation of marker compounds in polyherbal formulations. With the application of a very small quantity of samples, various samples can be run on the TLC plate and quantifications can be performed up to very low concentrations<sup>36</sup>.

The FT-IR spectrum of Arjunarishta reveals the presence of O-H stretching at  $3268\text{ cm}^{-1}$ , C=C stretching at  $1637\text{ cm}^{-1}$  &  $1418\text{ cm}^{-1}$ , C-O stret-

ching at  $1262\text{ cm}^{-1}$  &  $1242\text{ cm}^{-1}$  and C-H bending at  $925\text{ cm}^{-1}$ ,  $871\text{ cm}^{-1}$ ,  $818\text{ cm}^{-1}$  &  $773\text{ cm}^{-1}$ . This is shown in Table 3 and Fig. 5. It confirms the presence of alcohol, carboxylic acid, primary alcohol alkenes, and aromatic groups in Arjunarishta formulation<sup>37</sup>.

TABLE 3: FT-IR FINGERPRINT OF ARJUNARISHTA

Wavenumber ( $\text{cm}^{-1}$ )	Vibrational modes in the IR region	Functional groups
3268	O-H (H-bonded)	Alcohol
1637	C=C (Stretch)	Alkene
1418	C=C (Stretch)	Aromatic
1262	C-O (Stretch)	Carboxylic acid
1042	C-O (Stretch)	Primary alcohol
925	C-H (Bend)	Alkene
871	C-H (Bend)	Alkene
818	C-H (Bend)	Alkene
773	C-H (Bend)	Alkene

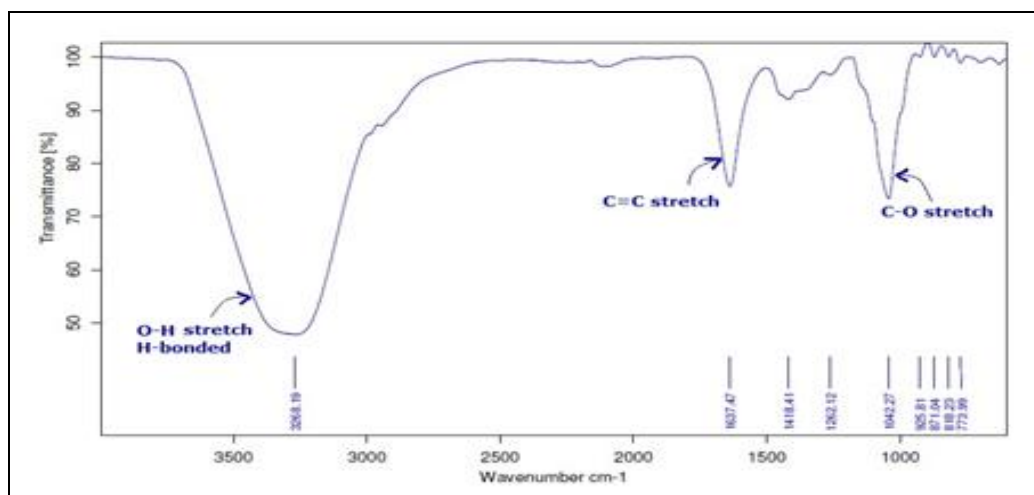


FIG. 5: FT-IR SPECTRUM OF ARJUNARISHTA

**CONCLUSION:** In this study, the formulation Arjunarishta was analyzed according to the standard pharmacopoeia procedures for its physico-chemical screening, spectroscopic and chromatographic evaluations. This study will help to develop a quality control profile for future reference and can be used for qualitative evaluation of Arjunarishta in terms of modern parameters, which may help in the authenticity of the drug and to compile suitable information for the better utility and safe use of this formulation in therapeutics.

With the increasing use of Ayurvedic medicines, there is certainly a need for more advanced techniques that will help drug manufacturer's to follow specifications & justify therapeutic efficacy, safety, and stability of products.

Besides this, quality assessment of Ayurvedic medicines has to be done in an integrated manner, including both traditional and modern analytical techniques. This will help in validating the ancient wisdom of Ayurveda, which has been in practice for many centuries.

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**CONFLICTS OF INTEREST:** Nil

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