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Research Article

# SCIENTIFIC VALIDATION OF *AMALAKI RASAYANA* THROUGH PHARMACOGNOSTIC AND PHYTOCHEMICAL ANALYSIS

**Dhanisha BK[1]*, Manjusha Rajagopala[2]**

*[1]PhD Scholar, [2]Professor and Head, Department of Shalakyatantra, All India Institute of Ayurveda, New Delhi, India.

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## ABSTRACT

Classical Ayurveda textbooks explain numerous medicinal formulations that have been effectively utilized in wide range of therapeutic conditions. *Rasayana* is the branch of Ayurveda, focuses on rejuvenation, tissue regeneration, modulating immune system, and promoting healthy aging process. *Amalaki rasayana* (AR) is a *Vayasthapana rasayana* explained in Charaka samhitha. AR is extensively reported to influence various biological activities to promote longevity and prevent geriatric symptoms. Considering these factors, AR is planned to administer in age related cataract in its early stage. To improve the acceptance of medicinal preparations globally, it is necessary to revalidate them with the help of advanced scientific techniques, without losing the vital elements of science. **Methods:** The preparation was properly evaluated regarding safety, and quality. In the present study, pharmacognostical analysis of AR was done to ensure the quality standards of the raw drug and the finished product. Organoleptic character analysis, physiochemical analysis, heavy metal analysis, microbial analysis and HPTLC analysis were done. **Result:** Established standards were met in physicochemical, heavy metal and microbial analyses. These validate the formulation's compliance with quality and safety requirements. HPTLC helped in detecting the marker compound gallic acid in the formulation. **Conclusion:** From the observations, the results of this study could serve as a baseline for future research works.

## INTRODUCTION

Herbal formulations have been used safely and extensively for medical purpose in India from ancient period. Approximately 75-80% of the world's population, especially in developing countries, relies on herbal medicine as their primary healthcare provider.[1] Alternative systems of medicine including Ayurveda, Homeopathy, Naturopathy, Siddha, Yoga and Unani has been utilizing the huge diversity of medicinal plants available in India. Classical Ayurveda textbooks explain numerous medicinal formulations that have been effectively utilized in wide range of therapeutic conditions.

*Rasayana* is the branch of Ayurveda, focuses on rejuvenation, tissue regeneration, modulating immune system, and promoting healthy aging process.[2] A review of the recent studies on *Rasayanas* reveals that anti-oxidant and immunomodulation are the most explored actions of the *Rasayana* medicines.[3] Oral intake of *Rasayana* drugs is found to be beneficial in various age-related pathological conditions. *Vatatapika rasayana* is a type of *Rasayana* method in which person can undergo *Rasayana* therapy without disturbing daily routine. *Amalaki rasayana* (AR) is a *Vayasthapana rasayana* explained in Charaka samhitha.[4] In Ayurveda, the concept of *Vayasthapana* focuses on maintaining youthfulness regardless of age, mitigating the progression toward senescence, enhancing longevity, intellect, physical and mental strength, and preventing disease.[3] AR consists of 5 ingredients, dried fruit from *Emblica officinalis (Amalaki),* dried fruits of *Piper longum* (*Pippali),* honey, ghee, and sugar. *Embelica officinalis*, the main ingredient of AR is considered as foremost among the anti-ageing drugs[5] and is *Chakshushya* (ophthalmic)in nature.[6] The fruit of *Emblica officinalis* is known for its strong antioxidant properties and is abundant in

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Vitamin C, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin, quercetin, and various polyphenolic compounds.[7]

AR is extensively reported to influence various biological activities to promote longevity and prevent geriatric symptoms.[8] An in vivo study on Drosophila melanogaster shows that AR feeding significantly enhances tolerance to various cellular stresses by decreasing reactive oxygen species(ROS) and lipid peroxidation on the one hand, and enhanced superoxide dismutase (SOD) activity and Heat shock protein 27 (Hsp27) on the other resulting better homeostasis which improves life span and quality of organism's life.[9] Considering these factors, AR is planned to administer in age related cataract in its early stage.

To improve the acceptance of medicinal preparations globally, it is the need of time to revalidate them with the help of advanced scientific techniques, without losing the vital elements of science. The preparation was properly evaluated in terms of safety, efficacy, and quality. In the current study, pharmacognostical and pharmaceutical analyses of AR was done to ensure the quality standards of the raw drug and the finished product.

## MATERIALS AND METHODS

### Collection/procurement and authentication of drugs

Dried fruits of *Embelica officinalis, Piper longum*, fresh fruits of *Embelica officinalis*, honey, ghee, and sugar were collected from an established drug supplier in New Delhi. Raw drugs were authenticated from Department of Dravyaguna, All India Institute of Ayurveda.

### Preparation of AR

Fresh *Embelica officinalis* fruit were smashed and seeds were removed, then fruit pulp was put in a juicer and juice obtained was filtered and collected using clean cotton cloth. The dried fruits of *Embelica officinalis* were powdered and sifted through a mesh of 80 number. The powder was accurately weighed and was taken into a steel vessel. Enough juice was added to the powder for soaking it properly. The material was covered properly to prevent any contamination or fungal growth. The material was soaked for 21 days by adding enough juice to keep the powder completely immersed in juice. The quantity of juice required for soaking was gradually reduced, and eventually, no additional juice was needed, as the material remained in the same condition as the previous day. After 21 days, the material was collected and spread evenly over steel plates and covered with a layer of thin cotton cloth. Then it was placed under sunlight for drying. Later it was kept in dryer for complete removal of the moisture content. The dried material was collected, powdered, weighed. Honey, ghee, sugar, and powder of piper longum were added in the ratio 1:1:1/4:1/8 and mixed properly. We collected Porcelain pots from local market and was cleaned properly with normal water, then hot water and then with ethanol and dried. Then the pot was fumigated. After proper mixing of the drug, the blend was shifted into porcelain jar and mouth of the jar was sealed properly. Then jar was kept in a dark room for 4 months. After that packing and labelling were done in airtight plastic containers of 100gm.

### Analytical study

The organoleptic characters of AR using sense organs.[10] The common physicochemical parameters mentioned in Central Council for Research in Ayurvedic Sciences (CCRAS) guidelines were analysed adopting methods mentioned in Ayurveda pharmacopeia of India, Inductively coupled plasma-mass spectrometry (ICP-MS) based mass spectrometry was adopted to analyse the presence of lead, cadmium, arsenic and mercury in the given sample, which is highly sensitive and allows for measuring multiple elements simultaneously.[11] To identify the safety of using AR for oral use, microbial limit tests were carried out which include, total microbial plate count (TPC), total yeast and mould count (TYMC) and presence of Staphylococcus aureus, Salmonella species, Pseudomonas aeruginosa and Escherichia coli.

### High-performance thin-layer chromatography-(HPTLC)

The identification and quantification of active constituents and impurities can be performed using HPTLC. HPTLC is a highly flexible, reliable, and cost-effective separation technique, ideally suited for the analysis of botanicals and herbal drugs.[12]

### Sample preparation for HPTLC

Accurately weighed 1.0gm of sample was taken in an iodine flask, and 50ml of methanol was added. The flask was then kept on a water bath to reflux for 1 hour. The solution was then filtered by Whatman No. 1 and made up to a 50ml volumetric flask by adding methanol. After that, the filtrate was again filtered using a syringe filter. This test solution was used for HPTLC fingerprinting.

### Preparation of standard solution of Gallic acid

In chromatographic sciences, the standard solution serves as a benchmark against which the sample under investigation is compared. In preparation of standard solution, a 10mg gallic acid standard was weighed into a 10ml volumetric flask. 5ml of methanol was added to it and sonicated for 10 minutes. The volume was made up to mark with methanol. This standard solution was used for HPTLC fingerprinting and quantification of gallic acid in the AR sample.

**High-performance thin-layer chromatography-(HPTLC)**

High-performance thin-layer chromatographic (HPTLC) analysis HPTLC analysis was performed using CAMAG HPTLC system (Switzerland). 10µL AR sample and 5.0µL of standard gallic acid solution were applied using CAMAG® Linomat 5 on aluminum backed pre-coated silica gel 60F254 TLC plate (Merck, India). The mobile phase was standardized as toluene, ethyl acetate, and formic acid in a specific ratio (5:3.5:0.5). The chromatogram was developed in a saturated Twin Trough chromatographic chamber (CAMAG, Switzerland). The developed plate was visualized under UV at 254nm.

**RESULTS AND DISCUSSIONS**

Organoleptic characteristics of AR were recorded as represented in Table 1.

**Table 1: Organoleptic Analysis**

| S.No. | Parameters | Result | Limit as per API |
|---|---|---|---|
| 1 | Colour | Dark brown | NA |
| 2 | Odour | Characteristic | NA |
| 3 | Taste | Sour and bitter | NA |
| 4 | Consistency | Solid | NA |

Physicochemical parameters of the prepared drug were analysed and mentioned in table 2.

**Table 2: Physico-Chemical Analysis**

| S.No. | Parameters | Result | Limit as per API |
|---|---|---|---|
| 1 | Loss on Drying | 6.65% | NA |
| 2 | Total Ash | 1.36% | NA |
| 3 | Acid Insoluble Ash | 0.0% | NA |
| 4 | Alcohol Soluble Extractive | 61.62% | NA |
| 5 | Water Soluble Extractive | 52. 35% | NA |
| 6 | Total Acidity | 6.36 | NA |
| 7 | pH (10 %) | 3.72 | NA |
| 8 | Specific Gravity | 1.288 | NA |
| 9 | Total Solid Content | 93.35% | NA |
| 10 | Total Fat Content | 15.30% | NA |
| 11 | Total Sugar | 28.81% | NA |
| 12 | Reducing Sugar | 21.46% | NA |
| 13 | Non-Reducing Sugar | 7.35% | NA |

Heavy metal Analysis was done and is compared with the limit of heavy metals as per API (Ayurveda Pharmacopeia of India) and is mentioned in table 3.

**Table 3: Heavy Metal Analysis**

| S.No. | Parameters | Result | Limit as per API |
|---|---|---|---|
| 1 | Lead | 0.208 ppm | NMT 10 ppm |
| 2 | Cadmium | 0.026 ppm | NMT 0.3 ppm |
| 3 | Arsenic | 0.287 ppm | NMT 3 ppm |
| 4 | Mercury | 0.707 ppm | NMT 1 ppm |

To identify the safety of using AR microbial analysis was done and compared with limits as per API and is illustrated in table 4.

| Table 4: Microbiological Analysis | | | |
|---|---|---|---|
| **S.No.** | **Parameters** | **Result** | **Limit as per API** |
| 1 | Total Microbial Plate Count (TPC) | 977 cfu/g | $10^5$ cfu/g |
| 2 | Total Yeast & Mould Count (TYMC) | Absent | $10^3$ cfu/g |
| 3 | *Staphylococcus aureus* | Absent | Absent/g |
| 4 | *Salmonella sp.* | Absent | Absent/g |
| 5 | *Pseudomonas aeruginosa* | Absent | Absent/g |
| 6 | *Escherichia coli* | Absent | Absent/g |

**High-performance thin-layer chromatography-(HPTLC)**

The quality and potency of a formulation are analysed using HPTLC, a rapid and reliable technique that is used for fingerprinting and marker compound quantification of phytochemicals present in the formulation. In this study, The HPTLC profile was developed for methanol extract of AR (Fig. 1). The HPTLC profile of methanol extract, at 254nm, showed a common band of gallic acid (marker compound) with Rf 0.39 (table 5) in the sample. The process of visualizing and quantifying the chromatogram at a wavelength of 254nm, which is suitable for detecting phenolic compounds is critical for identifying and measuring the gallic acid in AR. The Area Under the Curve (AUC) elucidated that the AR sample has 1.75% gallic Acid, indicating the presence of marker compound within the traditional formulation.

**Table 5: Rf value**

| Spot No | Track T1 | Track T2 |
|---|---|---|
| 1. | ------ | 0.22 |
| 2. (Gallic acid) | **0.39** | **0.39** |
| 3. | ------ | 0.48 |
| 4. | ------ | 0.69 |
| 5. | ------ | 0.77 |

**(T1**: Standard Gallic Acid **, T2**: *Amalaki Rasayana*)

**Figure 1**: HPTLC plate at 254nm, Tracks T1: Standard Gallic Acid Track T2: AR.

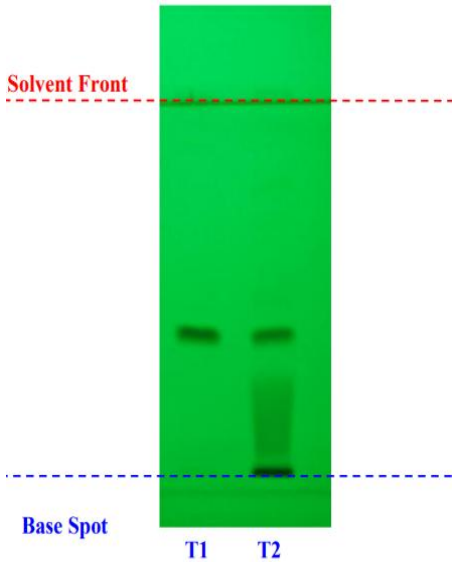


**Figure 2: 3D chromatogram at 254nm**

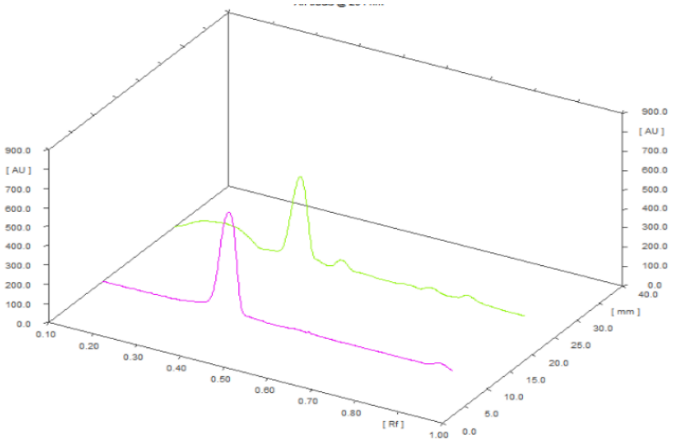



**Figure 3: 2D Chromatogram of Standard Gallic acid**

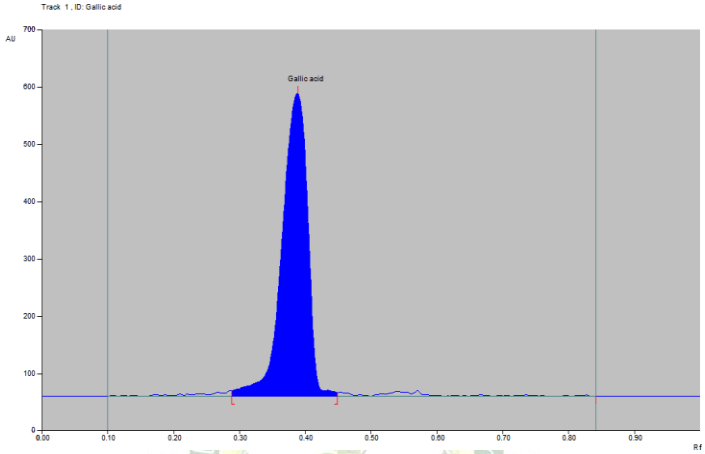
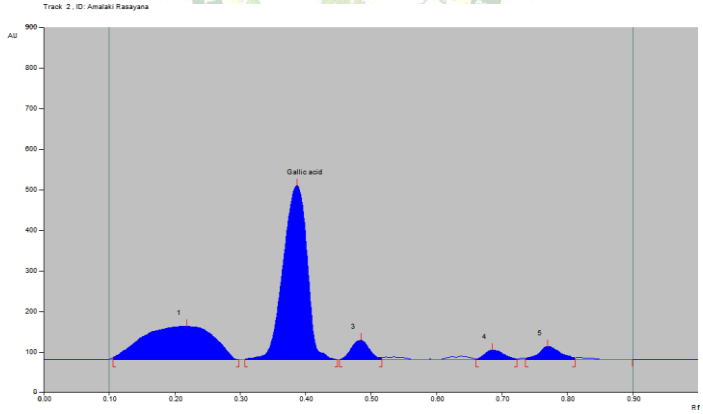



**Figure 4 2D Chromatogram of *Amalaki Rasayana***




## DISCUSSION

AR was prepared following the method mentioned in classical Ayurveda treatise. A comprehensive assessment of the final product for its quality, safety, and efficacy is necessary in the present scenario. Assessing the organoleptic characteristics is essential for evaluating the quality, authenticity, and sensory properties of herbal drugs, thereby ensuring their safety and efficacy. Excessive moisture in drug can promote microbial growth, reduce the shelf-life and degrade the active compounds present in the herbal formulation. Loss on drying of AR indicates the moisture content of drug is less. Alcohol soluble extractive indicates the existence of bioactive compounds in the preparation. pH can affect pharmacological activities of herbal preparations. Acidic pH of the product indicates less chance of bacterial contamination and increased antioxidant potential.[13] Heavy metal analysis showed that lead, cadmium, mercury and arsenic present are less than the permissible limit as per API. The microbial analysis showed that, the drug is free from microbial invasion and is safe to use. The HPTLC profile of methanol extract, at 254nm, showed a similar band of gallic acid (marker compound) with Rf 0.39 in the sample. The process of visualizing and quantifying the chromatogram at a wavelength of 254nm, which is suitable for detecting phenolic compounds is critical for identifying and measuring the gallic acid in AR. The

Area Under the Curve (AUC) revealed that the AR sample contains 1.75% gallic Acid, indicating the existence of marker compound in the traditional formulation.

## CONCLUSION

AR was planned to give orally in patients of age-related cataract in its early stage. Therefore, to guarantee quality control, drug preparation standardization and raw material validation are essential. Physicochemical characteristics of the drugs were determined by standards. The microbiological and heavy metal analyses satisfy the necessary quality requirements. Preliminary analysis of the medication using HPTLC revealed the presence of active ingredients. To fully comprehend the components of the sample, more research is required. From the observations, the outcomes of this study might serve as a baseline for future research works.

## Authors Contribution Statement

The research concept, visualization and design were prepared by DBK. Literature research, manuscript preparation and editing were done by DBK. Manuscript preparation was preparation was supervised, reviewed, and edited by MR. All the authors have gone through and approved the final manuscript.

## Data availability Statement

All data collected are properly stored electronically. Data are available from the corresponding author on request.

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***Address for correspondence**
**Dr. Dhanisha B K**
PhD Scholar,
Department of Shalakyatantra,
All India institute of Ayurveda,
Sarita Vihar, New Delhi.
Email: dhanishabk@gmail.com