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**Research Article**

# Standardization and Bioavailability Assessment of Panchashirishadi Agad: An Integrative Analytical Study

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## Abstract

Background: *Panchashirishadi Agad* is a classical Ayurvedic formulation described in Sushruta Samhita for the management of toxic conditions in *Agadtantra*. However, scientific standardization data regarding its pharmaceutical and analytical profile are limited. Objective: To prepare *Panchashirishadi Agad in Churna and Lepa* forms according to classical references and evaluate its physicochemical characteristics, chromatographic profile, and in-vitro bio-accessibility. Materials and Methods: The formulation was prepared as per the classical method described in *Sushruta Samhita (Kalpasthana* 5/81). Raw drugs were authenticated prior to pharmaceutical processing. Physicochemical parameters such as loss on drying, total ash, acid-insoluble ash, pH, and extractive values were assessed using standard analytical procedures. Chromatographic profiling was performed using High Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD). In-vitro bio-accessibility studies were conducted using Franz diffusion apparatus to simulate oral and dermal absorption. Results: The formulations demonstrated satisfactory analytical values with low moisture content, acceptable ash values, and optimum extractive values indicating purity and stability. The pH of the formulations was found to be within a physiologically acceptable range. HPLC-DAD analysis revealed distinct phytoconstituent peaks confirming formulation consistency and authenticity. Franz diffusion studies showed appreciable release and diffusion patterns, indicating favorable bio-accessibility of the active constituents. Conclusion: The present study establishes a comprehensive standardization protocol for *Panchashirishadi Agad* through integration of classical Ayurvedic pharmaceutics and modern analytical techniques, supporting its quality, safety, and therapeutic reliability as an antidotal formulation.

**Keywords:** *Panchashirishadi Agad*, *Agadtantra*, Standardization, HPLC-DAD, Franz diffusion study, Ayurvedic pharmaceutics

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## Introduction

Ayurveda, the ancient Indian system of medicine, emphasizes holistic approaches to health and disease management. Within its diverse branches, *Agadtantra* holds a significant place as one of the *Ashtanga Ayurved* (eight branches), primarily focusing on the diagnosis, management, and prevention of poisoning caused by *Sthavara* (plant-based), *Jangama* (animal-based), and *Krutrima* (artificial) toxins(1). A key therapeutic objective in *Agadtantra* is the administration of formulations designed to neutralize or counteract toxic substances. Among them, *Panchashirishadi Agad*, a classical formulation referenced in *Sushruta Samhita* (*Kalpasthana* 5/81), is prominently described for its efficacy in managing insect and animal poisons through both oral and topical applications (2). Traditionally, such formulations have been evaluated using classical Ayurvedic parameters like *Rasa* (taste), *Guna* (properties), *Veerya* (potency), *Vipaka* (post-digestive effect), and *Prabhava* (specific therapeutic action). However, in the current global pharmaceutical landscape, these qualitative assessments are considered insufficient for standardization, validation, and widespread acceptance. Modern pharma-cognostical and analytical techniques are increasingly essential to ensure the authenticity, reproducibility, stability, and safety of Ayurvedic medicines (3). The World Health Organization (WHO) has also emphasized the importance of scientific validation, proper identification, and standardization of herbal formulations to maintain quality and ensure consumer safety (4).

The *Panchashirishadi Agad* formulation comprises five parts (*Panchanga*) of *Albizia lebbeck*

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*(L.) Benth.(Shirish)* flower buds, roots, leaves, bark, and seeds—combined with *Trikatu* components (*Zingiber officinale* Roscoe ,*Piper nigrum* L., *Piper longum* L.)and *Saindhav lavan*. These ingredients are traditionally known for their *Krimighna* (anti-parasitic), *Vishaghna* (anti-toxic), *Shothahara* (anti-inflammatory), and *Deepana-Pachana* (digestive-stimulant) properties (2). While the pharmacological significance of individual components is supported in both traditional and modern literature, there is a lack of consolidated data on the pharmaceutical standardization and analytical profiling of the compound formulation, especially in its two common dosage forms: *Churna* (powder) and *Lepa* (paste).(4)

Despite the documented therapeutic utility of classical *Agadtantra* formulations, their wider acceptance in contemporary healthcare systems remains limited due to inadequate scientific standardization and quality validation. Traditional Ayurvedic evaluation methods mainly rely on organoleptic and qualitative parameters, which may not sufficiently satisfy modern pharmaceutical requirements such as reproducibility, safety assessment, and dosage uniformity. Therefore, scientific standardization of classical formulations has become essential to establish their quality, authenticity, and therapeutic reliability.

*Panchashirishadi Agad*, an important formulation mentioned in Ayurvedic toxicology, is indicated in various poisonous conditions and is administered in both oral (*Churna*) and topical (*Lepa*) forms. Evaluation of its physicochemical characteristics and bio-accessibility profile is therefore necessary to understand its pharmaceutical behaviour and ensure batch-to-batch consistency.

Modern analytical approaches including physicochemical analysis, chromatographic profiling, and in vitro diffusion studies provide objective and measurable parameters that help correlate traditional knowledge with contemporary scientific standards. Such investigations aid in the identification of phytoconstituents, quality assurance, and preliminary understanding of drug release characteristics. Hence, the present study was undertaken to develop a comprehensive pharmaceutical standardization and bio-accessibility profile of *Panchashirishadi Agad*, which may contribute toward its scientific validation and future pharmacological and clinical exploration.(6,7)

Therefore, the present study aims to prepare *Panchashirishadi Agad* in both *Churna and Lepa* forms, and evaluate them through modern analytical techniques. These include physicochemical testing (ash values, pH, and extractive values), in vitro Franz diffusion studies to assess preliminary bio-availability for oral and dermal application, and HPLC-DAD analysis to identify phytoconstituents and establish chromatographic fingerprinting. This dual approach integrating classical principles with modern methodologies intends to strengthen the scientific credibility of the formulation and set a foundation for its broader therapeutic and clinical utility.

## Material and Methods

### Procurement and Authentication of Raw Materials

All raw herbal ingredients required for the preparation of *Panchashirishadi Agad* were procured from reputed local Ayurvedic herbal markets in Wardha District ensuring good quality and freshness.

### Drug Identification

The identity and purity of each raw drug were authenticated by experts from the Department of Dravyaguna, DMIMS, Salod Wardha using classical Ayurvedic diagnostic criteria and macroscopic and microscopic evaluation in accordance with the Pharmacopoeia of India and WHO guidelines for herbal materials (8,9).


Fig.2.1-*Shirish* Leaves


Fig 2.2-*Shirish Twak*


Fig 2.3-*Shirish Roots*


Fig 2.4-Raw Seeds


Fig 2.5-*Flower bud Churna (powder)*

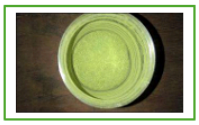


Fig 2.6- Leaves *Churna (powder)*


Fig 2.7-*Twak Churna (powder)*


Fig 2.8-Roots *Churna(powder)*


Fig 2.9-Seed *Churna (powder)*

### Processing and Preparation of Raw Materials

The collected crude drugs were carefully cleaned to remove dust, dirt, extraneous matter, and other foreign material. Each drug was then shade dried at room temperature (25±2°C) for 7– 10 days to prevent degradation of active phytoconstituents due to sunlight or excessive heat(10).After drying, the raw drugs were pulverized individually into fine powders using a mechanical grinder. The powdered drugs were then sieved through a mesh no. 80 sieve to achieve uniform particle size and ensure consistency in the formulation.(9,10)

### Preparation of *Panchashirishadi Agad*

**Place of preparation:** *Panchashirishadi Agad* was prepared at Dattatraya *Rasashala*, MGAC & RC, Salod (H), Wardha.

**Equipment used:** *Khalwa Yantra*, steel vessel, muslin cloth, gas stove.

### Ingredients -Table 1

**Table 1: Showing the ingredients of *Panchshirishadi Agad***

| Sr. No. | Ingredient | Quantity |
|---|---|---|
| 1 | *Shirish (Albizia lebbeck Benth.)*<br>*Family: Fabaceae*<br>*Part used: Stem bark / leaves / seeds (whichever is actually used in your formulation)*<br>*Dosage form: Churna (powder) / Lepa (*flower buds, root, leaves, bark, seeds) churna | 20 gm |
| 2 | *Sunthi* | 5 gm |
| 3 | *Marich* | 5 gm |
| 4 | *Pippali* | 5 gm |

### Preparation of *Panchashirishadi Agad* Extract and *Lepa*

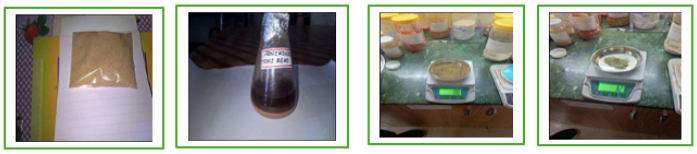
The five parts of *Shirish* (*Albizia lebbeck* Benth.), namely root, stem bark, leaves, flower buds, and seeds, were collected, shade dried, powdered separately, and sieved through a fine mesh. Equal quantities of each powdered drug were mixed thoroughly using a

*Khalwa Yantra* (triturating mortar and pestle apparatus) to obtain homogeneous *Panchashirishadi Agad Churna* (powder formulation).

For preparation of the extract, the prepared powder mixture was transferred to a clean stainless-steel vessel and mixed with 100 mL of sesame oil (*Tila Taila*). The mixture was heated gently over low flame with continuous stirring until the volume was reduced to approximately half (10) and characteristic *Sneha Siddhi Lakshanas* (signs of proper oleaginous processing) were observed. The heated mass was then filtered through sterile muslin cloth to obtain the medicated oil extract.

Subsequently, 2 g of beeswax was added to the filtered medicated oil and heated with continuous stirring until a soft semisolid consistency was achieved(11).The prepared *Panchashirishadi Agad Lepa* (medicated topical paste/ointment) was allowed to cool and stored in an airtight container for further pharmaceutical and analytical evaluation.

| ***Fig-2.3(1)- Panchhirishagad Churna*** | ***Fig 2.3(2)- Panchashirishagad Extract*** | ***Fig 2.3(3 & 4)- Collection of Panchashirishadi Agad for preparation of churna*** |
|---|---|---|



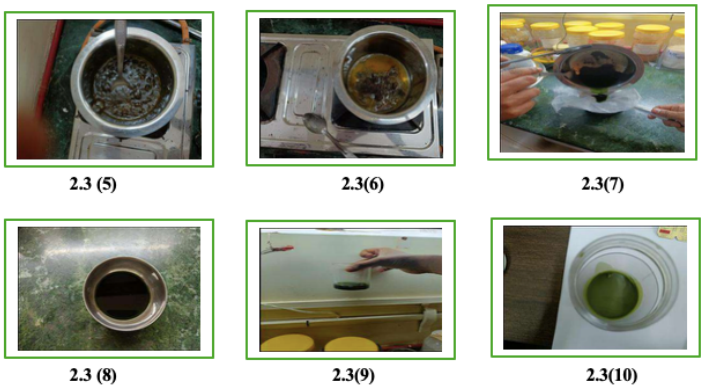



Fig 2.3(5)- *Siddhi Lakshanas of Heating Oil*
Fig 2.3(6)- *Heating of Panchashirishadi Agad Paste with Sesame Oil*
Fig 2.3(7)- *Collection and filtration of heated PA oil*
Fig 2.3(8)- *Panchashirishadi Agad Oil -1*
Fig 2.3(9)- *Panchashirishadi Agad Oil -2*
Fig 2.3(10)- *Panchashirishadi Agad oint. (Lepa)*

**Analytical study**

**Morphological/Organoleptic Characters**

Colour, odor, appearance, texture, and taste (where applicable) were recorded in accordance with WHO guidelines for quality control of herbal medicines (8)

- Colour
- Odor
- Appearance
- Texture
- Taste (if indicated)

**Physicochemical Analysis**

Physicochemical parameters were assessed as per standard protocols (8,9,12) to establish the quality standards of the formulation.

- **Loss on Drying (LOD):** 10 g of the sample was dried at 105°C until constant weight was achieved, as per WHO guidelines (8)
- **Total Ash:** 2 g of sample was incinerated at 450°C in a muffle furnace until ash was obtained with constant weight.(9)
- **Acid Insoluble Ash:** Ash was boiled with diluted hydrochloric acid and the insoluble residue was collected and weighed.(9)
- **Water Soluble Ash:** Total ash was boiled with water; the insoluble fraction was removed, and the difference in weight was calculated.
- **Water and Alcohol Soluble Extractive Values:** 5 g of the powdered sample was macerated separately in water and ethanol, filtered, evaporated, and the residue weighed.(9)
- **pH Determination:** A 10% aqueous solution of the sample was prepared and the pH measured using a calibrated digital pH meter.(12)

All observations were documented, and results were compared with standard limits prescribed in Ayurvedic Pharmacopoeia of India.

**Microbial Limit Tests**

Microbial contamination analysis was carried out to evaluate the safety and microbiological quality of the formulation intended for internal administration. The formulation was tested for specified pathogenic microorganisms including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using standard microbiological procedures(13) In addition, total fungal count and *Enterobacteriaceae* count were assessed as indicators of overall microbial hygiene and possible contamination arising during raw drug handling, processing, or storage(14).Evaluation of *Enterobacteriaceae* is considered important for herbal formulations because members of this group may indicate inadequate sanitary conditions and potential fecal contamination.

**Bio-Accessibility of *Panchashirishadi Agad* (Oral & Dermal) In Vitro Franz Diffusion Test (Oral)**

The in vitro Franz diffusion method described by Bonferoni et al. (15) was employed to assess the oral bio-accessibility of *Panchashirishadi Agad churna*. Goat gastrointestinal epithelial membrane (pore size 0.45 µm) was used as the absorption membrane to mimic human GI physiology. The membrane was carefully mounted between donor and receptor compartments of the Franz diffusion cell apparatus.

A 0.2–2 cm² area of *Panchashirishadi Agad churna* was placed in the donor compartment, while the receptor compartment was filled with buffer solutions of pH 3 and pH 8, simulating stomach and intestinal environments respectively. The system was maintained at 37 ± 0.5ºC. Samples were collected from the receptor compartment at 30, 60, 90, and 180 minutes in pH 3 buffer, and subsequently at 240, 360, 480, 600, and 660 minutes after switching to pH 8 buffer. Collected samples were analyzed to quantify diffused constituents(15).

**In Vitro Franz Diffusion Test (Dermal)**

The Dermal Bio-accessibility of *Panchashirishadi Agad* was evaluated using the same Franz diffusion apparatus and protocol as above, with goat skin membrane replacing the GI membrane. The setup, temperature maintenance (37 ± 0.5ºC), sampling intervals, and buffer solutions (pH 3 and pH 8) remained identical

to the oral test to simulate skin absorption under physiological conditions(15).

### HPLC Analysis of *Panchashirishadi Agad (Churna)* Instrumentation

HPLC analysis was performed on a Shimadzu 10Avp system equipped with a quaternary pump and UV/DAD detector. Chromatographic separation utilized an ACE-AR column (5 µm, 150 × 4.6 mm ID) maintained at 25°C. The mobile phase consisted of Solvent A (water) and Solvent B (methanol-acetonitrile, 30:70 v/v) using gradient elution at a flow rate of 1.0 ml/min.

Detection wavelengths were set at 210 and 254 nm with an injection volume of 20 µl. Total run time was 75 minutes.(16)

### Sample Preparation

A 200 mg sample of *Panchashirishadi Agad* (equal parts of bark, leaves, flower buds, roots, seeds of Albizzia lebbeck) was dissolved in 5 ml acetonitrile-methanol-water (2:2:1, v/v). The solution was kept in an airtight container overnight (12 hours) at room temperature, followed by heating in a water bath at 55°C for 10 minutes. Ultrasonication was applied for 10 minutes to enhance extraction, then filtered through a 0.45 µm nylon membrane filter. A 20 µl aliquot was injected into the HPLC system for analysis.(17)

## Observations and Results

### Raw Material Analysis

All eight ingredients were dried and powdered. Weight loss on drying ranged from 26% (Shirish leaves) to 67% (Shirish root). The final formulation included 30 g of each *Shirish Panchanga* (flower, leaf, bark, seed, root) and 10 g each of *Trikatu* (Sunthi, Marich, Pippali). The observations of weight loss and powdered yield are in agreement with previous analytical studies of *Panchashirishadi Agad* that have documented similar physicochemical variability across raw parts of *Albizia lebbeck* (Shirisha) and associated ingredients (18,19).

**Table 2: Table Showing the Observations of *Panchashirishadi Agad* ingredients**

| Ingredient | Raw Qty (gm) | Powdered Qty (gm) | % Weight Loss | Qty Used in Formulation |
|---|---|---|---|---|
| *Shirish flower bud* | 100 | 67.22 | 32.78% | 30 g |
| *Shirish leaf* | 100 | 78.42 | 21.58% | 30 g |
| *Shirish bark* | 100 | 64.15 | 35.85% | 30 g |
| *Shirish seed* | 100 | 63.14 | 36.86% | 30 g |
| *Shirish root* | 100 | 59.15 | 40.85% | 30 g |
| *Sunthi* | 100 | 60.14 | 39.86% | 10 g |
| *Marich* | 100 | 62.13 | 37.87% | 10 g |
| *Pippali* | 100 | 64.12 | 35.88% | 10 g |

### Pharmaceutical Observation of *Panchashirishadi Agada*

- Final Weight of *Churna:* 180 g
- Form: Fine powder
- Organoleptic Features: The organoleptic properties of *Panchashirishadi Agad* (PSA) were assessed, including colour, odor, taste, and texture. The formulation exhibited a brownish color, a characteristic herbal odour, and a bitter-astringent taste, with a coarse powder texture. These findings align with Ayurvedic textual descriptions, suggesting the formulation retains its intended sensory profile after preparation (20,21)

**Table 3: Showing the Analytical Parameters of *Panchashirishadi Agada (Churna)***

| Parameter | Observed Value |
|---|---|
| Loss on Drying (105°C) | 0.27% |
| Total Ash | 6.2% |
| Acid Insoluble Ash | 0.5% |
| Water Soluble Extractive | 2.3% |
| Alcohol Soluble Extractive | 20.16% |
| Ph | 5 |
| Particle Size | 100 mesh |

### Microbial Load

All tested microbial parameters (TVC, fungus, *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) were absent, demonstrating microbial safety. Similar results were observed in other pharmaceutical evaluations of *Panchashirishadi Agad* and its extracts, which showed negligible contamination and supported its safety profile for therapeutic use (25).

**Table 4: Showing the Microbial Load of *Panchashirishadi Agad and Its Ingredients***

| Microbiological Parameter | Result |
|---|---|
| Total Viable Count (TVC) | Absent |
| Total Fungal Count | Absent |
| *Escherichia coli* | Absent |
| *Salmonella* spp. | Absent |
| *Staphylococcus aureus* | Absent |
| *Pseudomonas aeruginosa* | Absent |

### Comparative Analysis of Key Ingredients

Comparative analysis of *Shirish Panchanga* (bark, leaf, root, flower, seed) showed variation in physicochemical properties including loss on drying, ash values, extractive values, and pH. These findings are in line with previous pharmaco-analytical reports that confirmed the wide phytochemical diversity of different plant parts of *Shirish* (*Albizia lebbeck* (L.) Benth.), correlating with its use in *Agada Tantra* (23,24).

### Bio-Availability of *Panchshirishadi Agad*

### Oral Administration

The bioavailability profile of *Panchashirishadi Agada* following oral administration indicates a rapid absorption phase followed by a gradual decline: The Peak absorption was observed at 30 minutes, indicating rapid onset of action. However, Initial absorption begins at 15 minutes but is significantly lower compared to the peak and Post-peak decline suggests either metabolism, tissue distribution, or excretion. After, 1 hour, a slight reduction in bioavailability is seen, continuing to decrease over 2 to 4 hours, suggesting a relatively short biological half- life. (19,22,25)

### Dermal Administration

The bioavailability trend of dermally applied *Panchashirishadi Agada* closely mirrors that of the oral route: The Peak bioavailability also occurs at 30 minutes, implying efficient transdermal absorption. The Initial lag phase observed at 15 minutes.However, Gradual decline from 1 hour onward, with marked reduction by 4 hours, suggesting rapid systemic distribution or excretion.

**Table 5: Showing the Comparative Analysis of Key Ingredients**

| Parameter | *Shirish* Bark | *Shirish* Leaf | *Shirish* Root | *Shirish* Flower | *Shirish* Seed |
|---|---|---|---|---|---|
| Colour | Brownish | Greenish | Brownish | Yellowish | Greenish |
| Odour | Characteristic | Characteristic | Characteristic | Characteristic | Characteristic |
| Taste | Astringent | Astringent | Astringent | Astringent | Astringent |
| Loss on Dry (%) | 2.8 | 1.6 | 1.27 | 1.3 | 1.6 |
| Total Ash (%) | 5.83 | 3.82 | 6.6 | 4.61 | 3.82 |
| Acid Insoluble Ash (%) | 0.8 | 0.5 | 0.9 | 0.38 | 0.5 |
| Water Soluble Extractive (%) | 25.37 | 21.37 | 18.62 | 15.83 | 21.37 |
| Alcohol Soluble Extractive (%) | 21.34 | 19.36 | 19.72 | 17.77 | 19.36 |
| pH | 5.2 | 5.3 | 4.8 | 5.0 | 5.3 |

**Table 6: Showing the Bioavailability Profile of *Panchashirishadi Agada* (Oral Route)**

| Time Point | Bioavailability Trend | Inference |
|---|---|---|
| 15 minutes | Initial absorption observed (low concentration) | Onset of absorption; drug starts entering systemic circulation |
| 30 minutes | Peak bioavailability achieved | Maximum absorption; indicates rapid onset of Action |
| 1 hour | Slight decline from peak | Possible distribution to tissues or beginning of metabolic clearance |
| 2 hours | Moderate decline | Ongoing metabolism and/or excretion |
| 3 hours | Further decline | Continued reduction due to distribution or Elimination |
| 4 hours | Low bioavailability | Suggests short half-life; most drug cleared or redistributed by this point |

**Table 7: Showing the Bioavailability Profile of *Panchashirishadi Agada (Dermal Route)***

| Time Point | Bioavailability Trend | Inference |
|---|---|---|
| 15 minutes | Initial lag phase; low absorption | Absorption through skin initiated but minimal systemic Availability |
| 30 minutes | Peak bioavailability achieved | Indicates effective and rapid transdermal absorption |
| 1 hour | Slight decline from peak | Suggests onset of systemic distribution or metabolism |
| 2 hours | Moderate decline | Drug likely undergoing further metabolism or tissue Uptake |
| 3 hours | Continued decline | Indicative of progressive elimination |
| 4 hours | Low bioavailability | Most of the drug possibly cleared or distributed to tissues |

### In Vitro Franz Diffusion Test

In vitro dermal diffusion studies using Franz diffusion cells revealed gradual permeation, with steady diffusion at acidic pH and sustained permeation at alkaline pH. Similar experimental models have been used to confirm the effective transdermal absorption potential of herbal formulations, including *Panchashirishadi Agad* (24,25).

**Table 8: Showing In Vitro Franz Diffusion Test Results of Panchashirishadi Agad (Dermal Route)**

| Time (minutes) | pH Level | Observation – Drug Diffusion |
|---|---|---|
| 30 | 3.0 | Initial diffusion observed; low quantity detected |
| 60 | 3.0 | Gradual increase in drug permeation |
| 90 | 3.0 | Steady diffusion continued |
| 180 | 3.0 | Near-maximum diffusion at acidic Ph |
| 240 | 8.0 | Diffusion resumed; moderate levels observed |
| 360 | 8.0 | Sustained diffusion across membrane |
| 480 | 8.0 | Significant drug permeation continues |
| 600 | 8.0 | Almost plateau; slow increase |

### HPLC Analysis of *Panchashirishadi Agad*

High-Performance Liquid Chromatography (HPLC) analysis was performed to identify phytochemical constituents of *Panchashirishadi Agad* in both its *Churna* (oral) and *Lepa* (topical) forms. The analysis was carried out using a Shimadzu 10Avp HPLC system with UV/DAD detection under gradient elution mode.(18,20,26)

**Table 9: Table Showing the HPLC Instrumentation and Chromatographic Conditions**

| Parameter | Specification |
|---|---|
| Instrument | Shimadzu 10Avp, Quaternary Pump, UV/DAD Detector |
| Column | ACE-AR HPLC Column (5 µm, 150 × 4.6 mm ID) |
| Mobile Phase | Solvent A: Water; Solvent B: Methanol-Acetonitrile (30:70, v/v) |
| Elution Mode | Gradient |
| Flow Rate | 1.0 mL/min |
| Detection Wavelength | 210 nm and 254 nm |
| Column Temperature | 25°C |
| Injection Volume | 20 Ml |
| Analysis Run Time | 75 minutes |
| Sample Preparation | Ultrasonication + Filtration (0.45 µm nylon) |

**HPLC Analysis of Panchashirishadi Agad (Churna) Sample Preparation**

200 mg of *Panchashirishadi Agad Churna* was extracted using acetonitrile-methanol-water (2:2:1, v/v). The mixture was kept overnight, water-bath heated, ultra-sonicated, and filtered before HPLC analysis.

**Observation**

Distinct peaks were observed at both 210 nm and 254 nm, suggesting the presence of multiple phytoconstituents. These peaks represent bioactive markers of *Albizzia lebbeck*, the chief ingredient in *Panchashirishadi Agad*.)

**HPLC Analysis of *Panchashirishadi Agad* (*Lepa)* Sample Preparation**

5 g of *Panchashirishadi Agad Lepa* was processed using the same extraction procedure.

**Observation**

Chromatographic patterns were found to be similar to that of the oral formulation, with consistent peaks corresponding to the phytochemical profile of *Albizzia lebbeck*. The results confirm chemical consistency between the two dosage forms. Thus, Both dosage forms of *Panchashirishadi Agad* exhibit a stable and reproducible phytochemical profile, supporting its therapeutic integrity.

## Discussion

The present analytical study of *Panchashirishadi Agad* demonstrates that systematic integration of classical Ayurvedic pharmaceutics with modern standardization parameters can provide a reliable and reproducible profile for traditional formulations. The findings reaffirm the therapeutic relevance of the preparation described in *Sushruta Samhita* (*Kalpasthana 5/81*) while establishing essential quality control benchmarks.

From the classical perspective, the chief ingredient, *Albizia lebbeck* (*Shirisha*), is recognized in Ayurvedic texts as *Vishaghna* (antidotal), *Shothahara* (anti-inflammatory), and *Krimighna* (antiparasitic) (26). The combination with *Trikatu* (*Zingiber officinale*, *Piper nigrum*, *Piper longum*) enhanced *Deepana* and *Pachana* properties, facilitating better absorption and bioavailability (28). *Saindhava Lavana(Rock salt (primarily composed of sodium chloride)* served as a synergist, aiding in *Anupana*-like delivery of active principles to the site of action (29).

Physicochemical analyses revealed values within the permissible limits of the Ayurvedic Pharmacopoeia of India (30). Total ash, acid-insoluble ash, and extractive values indicated good quality and absence of adulterants. Microbial load tests confirmed safety for both internal and external administration critical for antidotal preparations where rapid and safe delivery is essential.

The HPLC-DAD chromatographic profiles of both *Churna* and *Lepa* forms exhibited consistent phytochemical peaks corresponding to *Albizia lebbeck* marker compounds such as saponins, flavonoids, and tannins (31,32). This consistency between dosage forms is crucial for maintaining therapeutic predictability in clinical use (33).Franz diffusion experiments indicated that both oral and dermal routes achieved peak diffusion within 30 minutes, supporting classical claims of *Panchashirishadi Agad's* prompt action in acute poisoning scenarios (27).

Modern literature corroborates the observed bio-accessibility patterns. Studies on *Albizia lebbeck* have reported anti-inflammatory (34), antihistaminic (35), and membrane-stabilizing activities (36), which may explain both local and systemic benefits in venom- or toxin-induced inflammatory responses. *Trikatu's* role in enhancing gastrointestinal motility and permeability

(37) aligned with the rapid oral absorption observed in this study. Additionally, topical formulations containing piperine (from *Piper nigrum* and *Piper longum*) have been shown to improve transdermal permeation (38), supporting the dermal diffusion outcomes.

A key contribution of this study was the dual dosage form evaluation. Many previous works on *Agads* were limited to organoleptic and pharmacognostical studies (39,40), whereas the present investigation extended into bioavailability and phytochemical fingerprinting. Such comprehensive profiling bridged the gap between traditional Ayurvedic quality assessment and WHO-recommended herbal standardization guidelines (41), supporting potential regulatory acceptance. Despite robust findings, in vitro Franz diffusion tests do not fully replicate in vivo pharmacokinetics. Future research should include clinical pharmacokinetic studies, toxicological safety profiling, and efficacy trials against specific poisoning models. Additionally, stability studies under varying storage conditions are necessary to ensure shelf- life reliability.

## Conclusion

The standardization of *Panchashirishadi Agad* validated its safety, quality, and rapid bioavailability. Classical textual claims from *Sushruta Samhita* were substantiated through modern analytical techniques, confirming the presence of bioactive constituents and ensuring therapeutic consistency across dosage forms. The study demonstrated that integrating traditional Ayurvedic knowledge with contemporary analytical methodologies creates a robust framework for *Agadtantra* formulations capable of meeting global regulatory and scientific standards. Future clinical and stability studies will further consolidate its position as a validated antidotal preparation for global healthcare use.

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