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### WORLD JOURNAL OF PHARMACEUTICAL RESEARCH

SJIF Impact Factor 8.453

Volume 14, Issue 13, 1297-1314.

Research Article

ISSN 2277-7105

## ANALYTICAL PROFILING OF PANCHA SHIRISHANAMA AGAD: A HERBAL FORMULATION FOR INSECT BITE

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Article Received on 14 May 2025,

Revised on 03 June 2025, Accepted on 23 June 2025 DOI: 10.20959/wjpr202513-37410



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#### **ABSTRACT**

Pancha Shirishanama Agad is a classical Ayurvedic polyherbal formulation traditionally prescribed for the management of conditions associated with Keet Visha (insect venom and bites), described by Acharya Sushruta in his treatise. Despite its therapeutic relevance, comprehensive pharmacognostical and analytical data to support its standardization and safety are limited. This study aimed to evaluate the pharmacognostical, physicochemical, phytochemical, chromatographic, and microbiological parameters of Pancha Shirishanama Agad to establish its identity, purity, and safety. Materials and Methods: The formulation was prepared using five parts of Albizia lebbeck (Shirish) combined with Trikatu, Pancha Lavana, and honey. Macroscopic and microscopic analysis were conducted to authenticate the raw drugs. Physicochemical parameters including moisture content, ash values, and extractive values were assessed as per API guidelines. Preliminary phytochemical screening was performed on both ethanolic and aqueous extracts to identify the

major classes of bioactive constituents. Thin Layer Chromatography (TLC) was performed using silica gel 60F254 plates and a toluene: ethyl acetate (12:8 mL) mobile phase. Microbial

load and aflatoxins were analysed as per standard API methods. Results: Organoleptic evaluation confirmed acceptable sensory attributes. The powder microscopy revealed characteristic diagnostic elements such as stone cells and starch granules, indicative of authentic plant material. Ash values and extractive yields were within acceptable limits. Phytochemical screening detected carbohydrates, steroids, saponins, tannins, and phenolics. Thin Layer Chromatography (TLC) profiling exhibited multiple distinct Rf values, indicating the presence of diverse phytochemical constituents within the formulation. Microbial counts were within permissible limits, and no aflatoxins were detected. honey used in the formulation satisfied most standard quality criteria; however, moisture content exceeded the acceptable limits. Conclusion: The analytical profile of *Pancha shirishanama Agad* affirms its quality, safety, and therapeutic consistency. The findings support its use as a reliable Ayurvedic formulation and serve as a reference for further pharmacological and clinical research.

**KEYWORDS:** Ayurvedic formulations, Pancha shirishanama Agad, phytochemical analysis, Analytical, TLC, quality control, Honey.

#### INTRODUCTION

Ayurveda, a traditional system of medicine, is increasingly recognized for its holistic approach to disease prevention and management. Its remedies are generally considered safe, cost-effective, and suitable for long-term use, contributing to its rising global demand. However, despite these advantages, Ayurvedic formulations—particularly polyherbal preparations—often face challenges related to quality assurance. Inconsistencies in chemical composition, lack of proper authentication of raw materials, and insufficient standardization pose serious concerns regarding their safety and efficacy. So, establishing quality control measures is crucial to substantiate the safety, efficacy, and reproducibility of herbal medicines. Selection of appropriate plant material and the application of modern analytical methods for standardization are critical steps toward validation of Ayurvedic drugs. Comprehensive quality control, including evaluation of physiochemical, phytochemical, and microbial parameters, plays a pivotal role in confirming their therapeutic reliability.

Pancha Shirishanama Agad is a classical Ayurvedic polyherbal formulation traditionally prescribed for the management of conditions associated with Keet Visha (insect venom and bites), described by Acharya Sushruta in his treatise. [1] Its composition includes five parts of the Shirish plant<sup>[2-4]</sup> - roots, stem bark, leaves, flowers, and seeds—combined with Trikatu<sup>[5-7]</sup>,

Pancha Lavana<sup>[8]</sup>, and Madhu (honey). <sup>[9-11]</sup> These ingredients are known for their diverse pharmacological properties such as anti-inflammatory, analgesic, antioxidant, and immunomodulatory activities.

Despite its traditional relevance and pharmacological promise, standardized analytical data on Pancha Shirishanama Agad remains limited. This study aims to bridge that gap by developing a detailed analytical profile of Pancha Shirishanama Agad using pharmacognostical, physicochemical, phytochemical, chromatographic, microbial, and honey quality assessments.

#### MATERIALS AND METHODS

#### 1. Test formulation

Pancha Shirishanama Agad is traditionally prepared by combining coarse powders of five distinct parts of the Shirish plant—roots, flowers, stem bark, leaves, and seeds (shown in figure 1) with fine powders of Trikatu, comprising Shunthi, Maricha, and Pippali (shown in figure 2). The formulation also includes five distinct salts, traditionally known as *Pancha* Lavana—Saindhava, Sauvarchala, Samudra, Vida, and Audbhida (shown in figure 3) along with honey (Madhu), which serves as a natural adjuvant to enhance therapeutic efficacy. The formulation is conventionally administered in the form of a decoction (Kwatha). Details of all the contents of Pancha Shirishanama Agad are shown in table 1.



Figure 1: Shirish (a) Stem bark, (b) Flowers, (c) Seeds, (d) Root, (e) Leaves, (f) Pancha Shirishanama Agad powder.



Figure 2: (a) Shunthi, (b) Maricha, (c) Pippali, (d) Prepared *Trikatu* powder.



Figure 3: (a) Saindhava, (b) Sauvarchala, (c) Samudra, (d) Vid, (e) Audbhida, (f) Prepared *Pancha Lavana* Powder.

Table 1: Contents of Pancha Shirishanama Agad.

S. No.	Ingredient	Botanical name	Family	Part used	Form used	Ratio
1.	Shirish	Albizzia lebbeck benth	Fabaceae	Roots (moola), leaves (patra), Stembark (twak), Seed (beej), Flower (pushpa)	Yavakuta (coarse powder)	1 pala (~48 grams)
2.	Shunthi	Zingiber offficinale Roscoe	Zingiberaceae	Rhizome (kanda)		1
3.	Maricha	Piper nigrum Linn	Piperaceae	Fruit (phala)		1 <i>yama</i> (~5grams)
4.	Pippali	Piper longum Linn	Piperaceae	Fruit (phala)		
5.	Saindhava lavana	-	-	Salt (namak)		
6.	Sauvarchal lavana	-	-	Salt		1
7.	Samudra lavana	-	-	Salt		1 <i>yama</i> (~5 grams)
8.	Vid lavana	-	-	salt		
9.	Audbhidha lavana	-	-	salt		
10.	Madhu	Apis cerana Fabricius	Apidae			As required

#### 2. Collection and authentication of raw material

The therapeutic efficacy of medicinal substances is significantly enhanced when they are procured with Desha-Sampat (from their natural and suitable habitat) and Kala-Sampat (at the appropriate seasonal time). [12] Accordingly, the leaves of *Shirish* were collected in April 2024 (Vasanta Ritu), the stem bark in September 2024 (Sharad Ritu), the roots in June 2024 (Grishma Ritu), and the flowers in May 2024 (Yatha Ritu) from the botanical garden of Rishikul Campus, Uttarakhand Ayurved University (UAU), Haridwar. The Shirish seeds, along with Shunthi (Zingiber officinale), Maricha (Piper nigrum), Pippali (Piper longum), Pancha Lavana, and Madhu (Dabur Honey, Batch No. BD3804), were procured from the local market in Dehradun, Uttarakhand, in September 2024, ensuring optimal quality. All raw materials utilized in the preparation of Pancha Shirishanama Agad were authenticated by subject experts from the Postgraduate Department of Dravyaguna, Rishikul Campus, Uttarakhand Ayurved University, under the reference number DG/RC/UAU-232 on 04/10/2024.

#### 3. Preparation of the Formulation

Following proper authentication, the five constituent parts of *Shirish* were well washed with running water, shade-dried and coarsely powdered (*Yavakuta*). The *Trikatu* and *Pancha Lavana* ingredients were finely pulverized to achieve a uniform consistency. All powdered materials were stored in clean, airtight, round plastic vacuum-sealed bags containing silica gel sachets to prevent moisture absorption.

For the preparation of the decoction (*Kwatha*), 1 *Pala* (approximately 48 grams) of *Shirish Panchanga* coarse powder was combined with sixteen times its volume of water and subjected to gentle heating, maintaining the temperature between 95°C and 100°C. The mixture was stirred continuously to prevent sedimentation and thermal degradation. Once the volume was reduced to one-eighth (~96 mL), the decoction was filtered through a double-folded, clean cotton cloth into a stainless-steel vessel. Subsequently, *Prakshepak Dravyas* i.e., *Trikatu Churna* (1 *Yava*, ~5 grams) and *Pancha Lavana* (1 *Yava*, ~5 grams)—were incorporated into the decoction. After the mixture cooled, *Madhu* (honey) was added and thoroughly mixed. The final formulation is depicted in Figure 4. As per the *Sharangdhara Samhita*, the recommended dosage of *Kwatha* is 2 *Pala* (~96 mL) per day, which may be administered in two divided doses of approximately 1 *Pala* (~48 mL) each. [13]



Figure 4: Preparation of Pancha Shirishanama Agad Kwatha (Decoction).

#### 4. Place of study

analytical evaluations of Pancha Shirishanama Agad—including organoleptic characterization, physicochemical analysis, phytochemical screening, powder microscopy, TLC profiling, microbial contamination assessment, aflatoxin detection, and honey quality control—were conducted in the month of December 2024 at the Drug Innovation Centre, a unit of Bilwal Medchem and Research Laboratory, Reengus, Rajasthan with Institution Ethical Committee clearance number UAU/RC/IEC/2024/PG/188 on 27/08/2024.

#### 5. Analytical parameters

#### 5.1 Macroscopic evaluation

The collected samples were examined with the naked eye and a magnifying lens. Organoleptic analysis was done to observe colour, odour, taste, texture, and other physical features, following standard pharmacognostical methods to determine the quality and authenticity of the raw materials. [14]

#### 5.2 Physicochemical evaluation

Standardization of the formulation includes physicochemical evaluations such as loss on drying, Aqueous Extractive Value, Alcoholic Extract Value, total ash, Acid Insoluble Ash, Water Soluble Ash that were carried out in accordance with the guidelines prescribed in the Ayurvedic Pharmacopoeia of India (API). [15]

#### **5.3 Powder microscopy**

A small amount of powdered sample was hydrated with a few drops of water, then stained with iodine and potassium iodide to highlight starch granules and carbohydrate-rich areas. Glycerine was added to prevent drying, and the mixture was mounted on a slide for microscopic examination. For microscopic examination, an additional portion of the sample was treated with potassium hydroxide (KOH) and gently heated over a spirit lamp for 3-5 minutes. After cooling, the material was stained with safranine. KOH acted as a clearing agent to remove cellular debris, while safranine, a basic dye, selectively stained plant tissues and internal structures, thereby improving their visibility under light microscopy. [16]

#### 5.4 Preliminary Phytochemical screening

The aqueous and ethanolic extracts of the test formulation Pancha Shirishanama Agad (shown in figure 6) were subjected to qualitative phytochemical screening for major

constituents, including carbohydrates, alkaloids, proteins, amino acids, glycosides, saponins, steroids, tannins, and phenolic compounds, using standard phytochemical protocols. [17]



Figure 6: (a) Aqueous, (b) Alcoholic extracts of Pancha Shirishanama Agad.

#### 5.5 Thin layer Chromatography profiling

Thin Layer Chromatography (TLC) serves as a vital method for the quality assessment and standardization of herbal preparations. In the present investigation, TLC was utilized to identify and characterize the phytoconstituents in the formulation.

Chromatography was performed in following stages:

- Stationary phase: Silica gel 60F254 size 10 X 20 cm
- **Mobile phase:** Toluene (12 ml): Ethyl acetate (8 ml)

Upon completion of the development process, the Retention factor (Rf) values were determined by calculating the ratio of the distance migrated by the analyte to that of the solvent.<sup>[18]</sup>

#### 5.6 Microbial contamination and Aflatoxin Analysis

Pancha Shirishanama Agad was evaluated for total aerobic microbial load, including bacterial and fungal counts, using the plate count method. Screening for specific pathogens and aflatoxins (B1, B2, G1, and G2) was also performed according to API standards. <sup>[19]</sup> For microbial enumeration, 100  $\mu$ L of the sample was mixed with 1 mL of buffered peptone water, and serial dilutions were prepared up to  $10^{-2}$ . A 0.1 mL aliquot from the diluted sample was aseptically inoculated onto nutrient agar (NA) for the enumeration of total aerobic bacteria and onto potato dextrose agar (PDA) to assess fungal growth. Nutrient agar (NA) plates were incubated at  $37 \pm 1^{\circ}$ C for 18 to 24 hours to allow bacterial growth, whereas

potato dextrose agar (PDA) plates were incubated at  $25 \pm 1$ °C for 48 to 72 hours for fungal development. Post incubation, microbial colonies were counted, and the results were recorded as colony-forming units per gram of the test material.

Aflatoxins  $B_1$ ,  $B_2$ ,  $G_1$ , and  $G_2$  were analyzed as per the Ayurvedic Pharmacopoeia of India. About 50 g of the powdered sample was extracted using methanol:water (80:20) with sodium chloride, followed by chloroform partitioning. The chloroform layer was dried, concentrated, and subjected to TLC using silica gel plates and a chloroform:acetone (9:1) solvent system. The results were evaluated against the permissible limits: aflatoxin  $B_1$  (NMT 5  $\mu$ g/kg) and total aflatoxins (NMT 10  $\mu$ g/kg). [20]

#### **5.7 Honey Analysis**

Organoleptic, physicochemical, reducing sugars, and purity test were evaluated for the honey used in the formulation.<sup>[21]</sup>

#### **RESULTS**

#### 1. Organoleptic analysis

Organoleptic characters including appearance, colour, taste, odour and texture of *Pancha Shirishanama Agad* are shown in table 2.

Table 2: Organoleptic characters of Pancha Shirishanama Agad.

S. No.	Parameters	Result
1.	Appearance	Dark brown liquid
2.	Colour	Brownish
3.	Taste	Sweet, bitter
4.	Odour	Sweet pleasant
5.	Texture	Smooth liquid

#### 2. Physicochemical Analysis

Physicochemical parameters including loss on Loss on Drying, Aqueous Extractive Value, Alcoholic Extract Value, Total Ash, Acid Insoluble Ash, Water Soluble Ash of *Pancha Shirishanama Agad* are shown in table 3.

Table 3: Physiochemical parameters of *Pancha Shirishanama Agad*.

S. No.	Name of Tests	Value	Standard values as per API
1.	Loss on Drying (%)	6.95	6.8 (loss in drying)
2.	Aqueous Extractive Value (% w/w)	21.23	Not less than 8%
3.	Alcoholic Extract Value (% w/w)	11.36	Not less than 6%

4.	Total Ash (% w/w)	8.65	Not more than 14%
5.	Acid Insoluble Ash (% w/w)	3.32	Not more than 5%
6.	Water Soluble Ash (% w/w)	5.69	Not more than 10%

#### 3. Powder Microscopy

Powder microscopy showed presence of stone cells, fragment of fibre, starch grain and fragment of pitted cells shown in Figure 7.

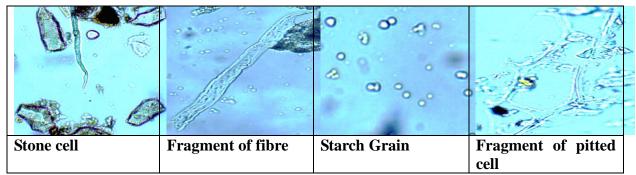


Figure 7: Powder microscopy of Pancha Shirishanama Agad.

#### 4. Phytochemical Analysis

Table 4 and figure 8 depicts the presence or absence of Phytochemical parameters in aqueous and alcoholic extracts of Pancha Shirishanama Agad.

Table 4: Phytochemical parameters of *Pancha Shirishanama Agad*.

Phytochemical Analysis					
Tests for Carbohydrate					
Tests Procedure		Aqueous	Alcoholic		
Molisch's test	2 ml test solution+ 2 ml Molisch's reagent +1 ml Conc. H2SO4	Purple colour ring not formed (absent)	Purple colour ring formed (present)		
Benedict's test  4 ml test solution +  CuSO4 + sodium  hydroxide + heat to boil		Orange red colour formed (present)	Orange red colour formed (present)		
Fehling solution test	2 ml test solution+ Fehling solution A+ Fehling solution B + heat till boil	Not found (Absent)	Found (Present)		
Tests for Alkalo	id				
Dragendroff test	2 ml test solution + 2 ml Dragondroff's reagent	Orange precipitate not formed (absent)	Orange precipitate formed (absent)		
Wagner's test	2 ml test solution + 2 drops of wagner's reagent	Reddish brown precipitate not formed (absent)	Reddish brown precipitate formed (absent)		
Hager's test	2ml Test solution+ saturated solution of	Orange yellow precipitate not formed	Orange yellow precipitate formed		

	picric acid	(absent)	(absent)			
Tests for Amino Acid						
Ninhydrin test	2 ml test solution + Ninhydrin reagent	Deep blue Colour formed (present)	Deep blue Colour not formed (absent)			
<b>Tests for Protein</b>	1					
Biuret test	2 ml test solution +1 ml of 4% Sodium hydroxide + Drop of 1% copper sulphate solution	Violet colour not formed (absent)	Voilet colour not formed (absent)			
Xanthoproteic test	2 ml test solution + 5 ml conc. HNO3 +2 ml water	Yellow colour not formed (Absent)	Yellow colour formed (present)			
Millon test	2 ml Millon's reagent + 2 ml test solution	White Precipitate not formed (absent)	White Precipitate formed (present)			
Tests for saponing	n		<u>-</u>			
Foam test	2 ml test solution + water + sodium bicarbonate + shaken it vigorously	Honeycomb resembling Froth formed (present)	Froth not formed (absent)			
<b>Tests for Glycos</b>	ide					
Borntrager's test	2ml test solution+ 1ml benzene solution + 0.5ml diluted ammonia solution	Reddish pink colour not formed (absent)	Reddish pink colour formed (present)			
<b>Tests for Phenol</b>	ic compound					
Phenolic test	Heat the 2ml test solution with water + 2ml ferric chloride	Green blue colour not formed (absent)	Green blue colour formed (present)			
Tests for Steroid	•					
Salkowski	2ml test solution + 2ml chloroform+ 2ml conc. H2SO4 + shake	Appearance of red colour (present)	Appearance of red colour (present)			
<b>Tests for Tannin</b>	•	T				
Ferric chloride	2 ml test solution + 5% of ferric chloride solution	Deep blue or Dark green colour not formed (absent)	deep blue or Dark green colour formed (present)			
Lead acetate	2 ml test solution + 10% w/v solution of lead acetate	Precipitate Formed (present)	Precipitate formed (present)			
Potassium Dichromate	2 ml test solution + potassium dichromate	Dark colour not appeared (absent)	Dark colour Appeared (present)			

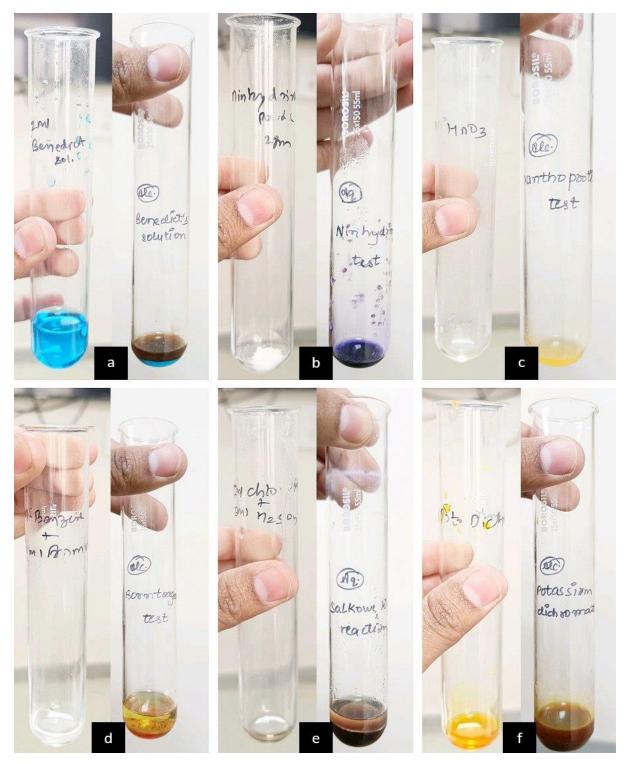


Figure 8: Presence of (a) Carbohydrates (b) Amino Acids (c) Proteins (d) Glycosides (e) Steroids (f) tannin.

#### 5. Thin layer chromatography analysis

The various retention factor values of *Pancha Shirishanama Agad* are shown in table 5.

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Table 5: Thin Layer Chromatography parameters of *Pancha Shirishanama Agad*.

#### 6. Microbial and Aflatoxin Analysis

The microbial contamination analysis and aflatoxins are shown in table 6 and 7 respectively.

Table 6: Microbial contamination parameters of Pancha Shirishanama Agad.

S. No	Microbial contamination	Observation	Reference Value	Test method
1.	Total bacterial count	$10^3/g$	Not More Than $10^5/g$	A.P.I, Part II, Vol-I,
2.	Total fungal count	$10^2/g$	Not More Than $10^3/g$	Appendix - 2.4

<sup>\*</sup>NMT- Not More Than

Table 7: Aflatoxin parameters of Pancha Shirishanama Agad.

S. No	Aflatoxin	Observation	Reference	Test method
1.	Aflatoxin B1	Not Detected	0.5 PPB	A.P.I, Part II, Vol-I,
2.	Aflatoxin B2	Not Detected	0.1 PPB	Appendix - 2.7
3.	Aflatoxin G1	Not Detected	0.5 PPB	
4.	Aflatoxin G2	Not Detected	0.1 PPB	

<sup>\*</sup>PPB- Parts Per Billion

#### 7. Honey Analysis

The results of Organoleptic, physicochemical, reducing sugars, and purity test for honey used in the formulation are shown in table 8 and 9 respectively.

Table 8: Organoleptic parameters of Honey.

S. No.	Macroscopic study	Honey
1.	Colour	Light red brown
2.	Odour	Sweet
3.	Taste	Sweet

Table 9: Quality control parameters of Honey.

S. No	Test Parameters	Values	Reference ISI	API Value	FSSAI
1.	Moisture Content (%)	51.23	25	NMT 25 %	NMT25 %
2.	Density (gm/ml)	1.419		NLT 1.35	
3.	Sp. Gravity	1.465	1.37		
4.	Refractive Index	1.498			
5.	Determination of Total Ash (%)	0.098	0.5	NMT 0.5	
6.	Reducing Sugars (%)	2.5-3.5	65	NMT 65	
7.	Fiehe's test	- ve	- ve	- ve	
8.	Acidity	0.15 %	0.2 %	NMT 0.2	

<sup>\*</sup>ISI- Indian Standard Institute, \*API- Ayurvedic Pharmacopeia of India, \*FSSAI- Food safety and Standards Authority of India, \*NMT- Not more Than

#### **DISCUSSION**

The present study aimed to evaluate the pharmacognostic and analytical parameters of Pancha Shirishanama Agad, an Ayurvedic formulation prepared from five parts of Shirish (Albizia lebbeck) along with Trikatu, Pancha Lavana, and honey. The organoleptic evaluation of Pancha Shirishanama Agad indicated characteristic attributes expected of a well-prepared Kwatha formulation: dark brown colour, sweet-bitter taste, and smooth liquid texture, reflecting the amalgamation of diverse herbal and other supportive constituents.

Physicochemical parameters are fundamental in assessing the stability and shelf-life of any herbal formulation. The moisture content, as indicated by loss on drying (6.95%), was slightly higher than the standard limit (6.8%) but within an acceptable range, suggesting minimal risk of microbial growth. The ash values (total ash: 8.65%, acid-insoluble ash: 3.32%, water-soluble ash: 5.69%) reflect low levels of inorganic and extraneous matter, indicating the purity and cleanliness of raw materials. The high extractive values in both water (21.23%) and alcohol (11.36%) indicate a good yield of polar and non-polar constituents, signifying the formulation's rich phytoconstituent profile.

Microscopic analysis revealed essential diagnostic features such as stone cells, pitted cells, starch grains, and fibre fragments, which align with the identity and authenticity of the ingredients used. These microscopic markers are crucial for raw drug standardization and serve as a baseline for ensuring quality control.3

Phytochemical screening confirmed the presence of carbohydrates, steroids, saponins, glycosides, tannins, and phenolic compounds. These bioactive components are known for their anti-inflammatory, antioxidant, immunomodulatory, and detoxifying activities, supporting the therapeutic rationale of *Pancha Shirishanama Agad* in managing inflammatory or allergic conditions. Notably, alkaloids in both the extracts and proteins in aqueous extract were absent, which may suggest a relatively lower risk of adverse immunogenic responses in certain sensitive individuals.

TLC profiling demonstrated the presence of multiple well-resolved spots with distinct Rf values, indicative of significant phytochemical diversity and corroborating the complex, multicomponent composition of *Pancha Shirishanama Agad*. These chromatographic fingerprints can serve as reference standards for future batch-to-batch comparison and quality consistency.

Microbial analysis confirmed that *Pancha Shirishanama Agad* complies with API standards, with bacterial and fungal counts well within permissible limits, ensuring its safety for internal use. Aflatoxins, which pose serious health risks, were not detected, further affirming the safety of the formulation.

The honey used met all macroscopic and physicochemical standards, although the higher-than-permissible moisture content (51.23%) suggests a need for cautious storage to prevent fermentation or microbial contamination.

All These findings endorse *Pancha Shirishanama Agad* potential as a standardized and effective *Ayurvedic* formulation for clinical and therapeutic applications. Further studies can be undertaken to establish standardized parameters for *Pancha Shirishanama Agad*, which would serve as a foundation for future research. The present study provides a preliminary benchmark that can support more in-depth analytical, pharmacognostical, and quality control investigations in the field of Ayurvedic drug testing.

#### **CONCLUSION**

The comprehensive pharmacognostical and analytical evaluation of *Pancha Shirishanama* Agad confirms its identity, purity, and safety as per Ayurvedic Pharmacopeial standards. Microscopic features validated the presence of genuine plant materials, while physicochemical parameters and extractive values indicated good formulation quality. Phytochemical screening revealed the presence of therapeutically relevant constituents like saponins, tannins, and phenolics. TLC profiling provided a distinct chemical fingerprint, and microbial analysis confirmed acceptable safety margins. The absence of aflatoxins and adherence to standard limits further establish the formulation's quality. In conclusion, the study affirms the quality, safety, and therapeutic potential of *Pancha Shirishanama Agad*. The results justify its classical indications, and the presence of active phytochemicals supports its efficacy in inflammatory and allergic conditions, reinforcing Pancha Shirishanama Agad as a potent Ayurvedic formulation worth further pharmacological exploration.

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