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ORA- Analytical Study

Pharmacognostic and Pharmaceutical Study of *Panchashirisha Agada*: A Novel Ayurveda Formulation

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ABSTRACT:

Introduction: In today's era, assessing the quality of herbal formulations is crucial and rationale for confirming authenticity before its market release. The World Health Organization has established guidelines for evaluating the safety, efficacy, and quality of herbal medicines. *Panchashirisha Agada* is a herbal formulation which comprises of *Shirisha Phala, Shirisha Moola, Shirisha Twak, Shirisha Pushpa, Shirisha Patra* (*Albizzia lebbeck* Benth.)**Aim:** To prepare *Panchashirisha Agada Vati* and authenticate by carrying out physico-chemical assessment. **Materials and Methods:** Pharmacognostical evaluation was carried out at Pharmacognosy department and Pharmaceutical study was completed in the Pharmaceutical chemistry department of ITRA, Jamnagar respectively. **Results:** Pharmacognostical findings confirmed the authenticity and genuine ingredients of *Panchashirisha Agada.* The average values of physicochemical parameters of *Panchashirisha Agada Vati* were found as follows: pH value:8, loss on drying:12.8%, total ash value:30%, water soluble extractive value:11.4%, alcohol soluble extractive value:15.9%. The hardness of the *Vati* 5.02 kg/Cm^2. Qualitatively TLC study showed 7 major spots at 256 nm,13 spots at 366 nm. Further HPTLC study showed 6 major spots at 254 nm, 7 spots at 366 nm, 12 spots at 540 nm. **Conclusion:** The trial drug is genuine and devoid of any adulteration and substitution. Data generated can be used to develop a preliminary standard profile criterion for the formulation *Panchashirisha Agada Vati.*

KEYWORDS: Agad Tantra*, Panchashirisha Agada Vati,* Pharmacognostic study, Pharmaceutical study, HPTLC

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1. INTRODUCTION

Agad Tantra (ayurveda toxicology) deals with the symptoms and treatment related to the poisonous effect of both *Sthavara* and *Jangama Visha*. [1] The anti-poisonous drugs used to counter the action of the poisons are known as *Agada* (antidote). Diseases are caused due to exposure to cumulative, concocted poisons and incompatible dietary habits which signifies the need of detoxification at all levels. The *Agada yogas* (anti-poisonous formulations) mentioned in *Samhitas* (treatises) possess anti-toxic, anti-oxidant and radical scavenging activities. [2, 3, 4] The judicial use of *Agada yogas* (anti-poisonous formulations) in certain diseases is found to be effective as per research.

The formulations in Agad Tantra include drugs of herbal, mineral and animal origin which possess properties opposite to the *Visha* i.e., anti-poisonous properties. These are capable of managing the complications produced by various kinds of poisoning. So here the study has been taken on *Panchashirisha Agada,* which is mentioned in the *Charaka Samhita*. [5]

Currently, the demand for herbal medications is growing rapidly, therefore maintaining their quality and productivity is crucial. [6] However, regulations for herbal medicines are less stringent than for synthetic drugs. Quality can be influenced by various means via: physical, chemical, and geographical factors. [7] Deceptive practices like adulteration or substitution not only harms the herbal quality but also diminish the cost reasonably. This leads to varied health hazards for consumers. [8] Validating herbal materials before market entry is vital. Standardization and phytochemical analysis are done, along with quality control parameters to ensure utmost quality standards. [9] This study aims to develop and authenticate *Panchashirisha Agada's* analytical profile.TLC study is used for qualitative and semi quantitative analysis of natural products. HPTLC study is useful for the qualitative and quantitative analysis, and the detection of adulterants or any impurities.

Aim and Objectives

The study is aimed to prepare *Panchashirisha Agada Vati* and authenticate by evaluating through physico-chemical analysis.

2. MATERIALS AND METHODS

Collection of raw drugs

Raw drugs of *Panchashirisha Agada* like *Shirisha Twak* was collected from the Pharmacy ITRA, Jamnagar, Gujarat. Other parts like *Shirisha Phala, Moola, Pushpa and Patra* were collected from the Dhanvantari pharmacy, Junagadh, Gujarat after proper identification and assessment.

Method of Preparation

All the 5 parts of *Shirisha* (Mentioned in Table 1) were dried and made into fine powder separately with the help of grinder mixer. These individual fine powders obtained were sieved through 100-number mesh. Fine powders of all drugs were taken in the big steel container and mixed uniformly. This mixed Powder was taken in *kharal* (Mortar) followed by mixing of *Kwatha* prepared out of coarse powder of *shirisha panchanga* obtained after the sieving process. Vigorous trituration was continued till the consistency of the mixture achieved *susukshma pishtam* (fine powder paste). Once

the paste consistency appeared, uniform sized tablets of 500 mg were prepared manually. These tablets were dried in the shade for 15 days and preserved in an airtight bag thereafter. (Figure 1)

Table 1: Contents of *Panchashirisha Agada Vati*

Sr.no	Drug	Latin name	Family	Part used	Quantity
1)	*Shirisha*	*Albizzia lebbeck* Benth.	Fabaceae	*Moola*	1 part
2)				*Twak*	1 part
3)				*Pushpa*	1 part
4)				*Patra*	1 part
5)				*Phala*	1 Part

Fig1(a) *Shirisha Moola*

Fig1(b) *Shirisha Patra*

Fig1(c) *Shirisha Phala*

Fig1(d) *Shirisha Pushpa*

Fig1(e) *Shirisha Twak*

Fig1(f)Fine powder of *Shirisha Panchanga*

Fig1(g)Kwatha of *Shirisha Panchanga*

Fig1(h)*Kwatha* poured into fine powder

Fig1(i)Kalka formation of *Panchashirisha*

		
Fig1(j)*Vati* making machine	Fig1(k)*Vati* dried under Sunlight	Fig1(l)Airtight bag packaging of *Vati*

Figure 1: Photographs of Preparation

Ayurvedic Parameters[*Rasapanchaka*]:

Panchashirisha Agada Vati having *Shirisha Twak, Phala, Moola, Pushpa and Patra. Rasapanchaka of shirisha* as per following. [10]

Rasa- Madhura, Katu, Tikta, Kashaya

Guna- Laghu

Virya- Anusna

Vipaka- Katu

Karma- Sothahara, Tridoshahara, Vishaghna, Varnya

Pharmacognostical evaluation:

As per the API standards the drugs which were used in the formulation and prepared as *Panchashirisha Agada Vati* were identified and authenticated by the Pharmacognosy Laboratory, ITRA, Jamnagar. [11, 12]

Powder microscopy

Powdered drug was studied microscopically and microscopic characters of individual drugs were noted. The powder of the drug was dissolved with water followed by microscopy of the sample without stain and after staining with Phloroglucinol + HCl. Microphotographs of the sample were also taken under Carl – zeiss trinocular microscope. [13]

Pharmaceutical Analysis of *Panchashirisha Agada Vati*

Physical properties of the *Panchashirisha Agada Vati* like

1. Hardness
2. Uniformity of weight was performed at pharmaceutical laboratory, ITRA, Jamnagar. [14]

Qualitative Analysis consideration:

Physicochemical analysis was conducted at pharmaceutical laboratory, ITRA, Jamnagar to find out the following parameters.

1. **pH value:** pH value was determined using pH paper. A strip of pH paper was placed on a white tile surface. A drop of *Panchashirisha Agada* sample was poured on pH paper with the help of a dropper. The colour obtained on pH paper was compared with different shades of colour pH chart, and the pH value was noted down, which was 8 (mildly alkaline).
2. **Loss on drying:** Accurately weighed about 1 gm. of sample was transferred to a cleaned, dried and previously weighed petri dish; it was spread evenly and dried in an oven at 105°C till constant weight. From the amount of weight loss the loss on drying was calculated on the basis of air-dried sample.
3. **Total Ash value:** Incinerate about 2 to 3 gm accurately weighed powdered sample in a tarred porcelain crucible at a temperature not exceeding

450^{0}C until free from carbon. The sample was cooled and weighed and the percentage of ash is calculated with reference to the air dried drug.

4. **Water Soluble Extract:** 5 gms of coarsely powdered sample was macerated with 100 ml of water in a closed flask for twenty four hours. The flask was shaken intermittently during first six hours and allowed to stand for eighteen hours. The extract was filtered rapidly taking precaution against loss of solvent. 25 ml of filtrate was evaporated to dryness in a dried, previously weighed, evaporating dish and dried at 110^{0}C in hot air oven. From the weight of the residue, the percentage of water soluble extractive was calculated with reference to the air-dried drug.
5. **Alcohol soluble Extract:** 5 gms of coarsely powdered sample was macerated with 100 ml of alcohol in a closed flask for twenty-four hours. The flask was shaken intermittently during first six hours and allowed to stand for eighteen hours. The extract was filtered rapidly taking precaution against loss of solvent. 25 ml of filtrate was evaporated to dryness in a dried, previously weighed, evaporating dish and dried at 110^{0}C in hot air oven. From the weight of the residue, the percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug. [15, 16]

Thin Layer Chromatography (TLC)

Materials & Methods:

The TLC method is at present an important analytical tool for qualitative and semi quantitative analysis of a number of natural products. The adsorbent, such as Silica Gel G, is coated to a thickness at 0.3 mm on clean TLC plates using commercial spreader, the plate are activated at 105^0 C for 30 minutes and used. The selection of mobile phase depends upon type of constituents to be analysed. After the development of chromatogram by ascending technique, the resolved spots are revealed by spraying with suitable detecting agents. [17]

TLC conditions:

Sample preparation: Drug is powdered and is extracted with Methanol for 1 hour and then filter and filtrate is use for TLC.

Stationary Phase:Merck pre-coated silica gel 60 F254 plate

Solvent system-

Toluene: Ethyl Acetate: Formic acid: Methanol (6:3:0.1:1)

Solvent front: 8.6 cm

Spray reagent: Anisaldehyde Sulfuric Acid Spray

High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC study was carried out at VASU Research Centre, Vadodara, Gujarat. [18]

Sample Preparation for HPTLC:

1 g of sample was weighed accurately in a conical flask. To it 10 mL methanol was added, reflux for 30 minutes on water bath. Then, allowed to cool and filtered with the help of Whatman filter paper No. 1. The filtrate thus obtained was used for HPTLC fingerprinting.

Method for developing HPTLC

HPTLC analysis was performed on a CAMAG TLC system and vision CATS software. HPTLC Silica gel 60 F254 aluminium plate 10 cm X 10 cm from Merck was used as a stationary phase. The plate was activated at 60^{o}C for one hour before use. Before HPTLC analysis, extract was put at room temperature for 2 h. Using the CAMAG Linomat 5 semi-automatic sampler, 5 µl samples were applied using a 100 µl syringe. Samples were applied with 8 mm bandwidth and 8 mm from the bottom of the plate. The CAMAG ADC2 automatic developing chamber was saturated with the mobile phase ethyl acetate: water: formic acid 80:10:10 (v/v/v) for 30 min and then developed until 8 cm height.

3. RESULTS AND OBSERVATIONS

Organoleptic Parameters of Prepared Drug: It refers to analysis methods like *Rupa, Rasa, Gandha, Sparsha* etc. Five tablets were randomly selected from the prepared batch of *Panchashirisha Agada* and was powdered using a mortar and pestle for the study. The identification was carried out on the basis of organoleptic features and microscopy of the prepared drug as mentioned in (Figure 2).

Characters - Results

Rupa (colour) - Greyish - Black

Rasa (taste) - Characteristic

Gandha (odour) - Characteristic

Sparsha (consistency)-Smooth

Finished product microscopy:

Microscopic evaluation of finished product (*Panchashirisha Agada)* was conducted, Characters were noted down and microphotographs were taken. Tannin content, Stone cells, Starch grains, Scleroids, Rhomboidal crystals, Prismatic crystals, Pollen grains, Pitted vessels, Fibres, Epidermal cells, Crystal Fibre of *Shirisha* were seen in Photo Microscopy in 10X.

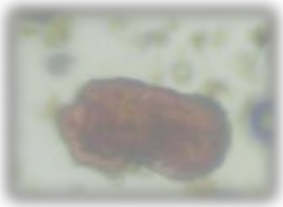
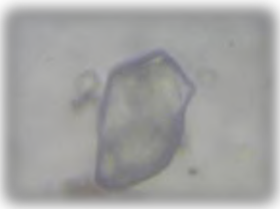


Fig2(a)Tannin content of *Shirisha*	Fig2(b) Scleroids of *Shirisha*	Fig2(c) Prismatic crystal of *Shirisha*	Fig2(d)Pitted vessel of *Shirisha*
			
Fig2(e)Fiber of *Shirisha*	Fig2(f) Crystal of *Shirisha*	Fig2(g)Crystal fibre of *Shirisha*	Fig2(h)Trichome of *Shirisha*

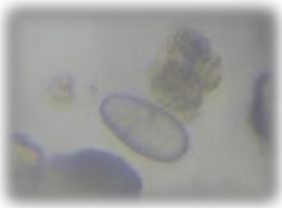
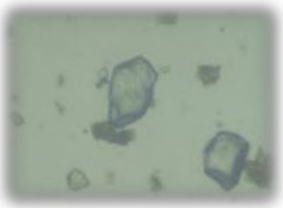
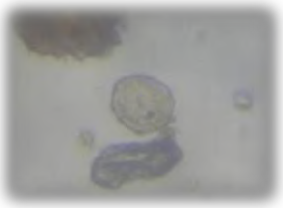
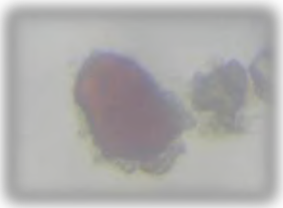
			
Fig2(i)Stone cell of *Shirisha*	Fig2(j) Starch grains of *Shirisha*	Fig2(k)Rhomboidal crystal of *Shirisha*	Fig2(l)Pollen grain of *Shirisha*
			
Fig2(m)Lignified pitted vessels of *Shirisha*	Fig2(n)Epidermal cells of *Shirisha*	Fig2(o) Brown content of *Shirisha*	Fig2(p) Stone cell with brown content of *Shirisha*

Figure 2: Microphotographs of Prepared drug

Pharmaceutical scrutiny

1) Hardness of *Panchashirisha Agada Vati*

The hardness of five *vati* of sample *Panchashirisha Agada* was tested by Monsanto hardness tester and noted & shown in Table 2. The mean of five values was calculated and considered as the hardness of sample *Panchashirisha Agada Vati*. The mean hardness of the sample *Panchashirisha Agada Vati* was 5.02 Kg/cm^2.

Table 2: Hardness of *Panchashirisha Agada Vati* readings

Tablet	Hardness in Kg/cm^2
1	5.0
2	4.5
3	4.2
4	6.0
5	5.4

2) Uniformity of weight :

Table 3: weight of ten *Panchashirisha Agada Vati* individually

Tablet	Weight in mg
1	480
2	499
3	503
4	488
5	501
6	492
7	497
8	504
9	507
10	496

Average weight = Total weight of 10 Vati / 10

= 4967/ 10

= 496.7mg

Average weight = 496.7mg

Highest weight = 507mg

Lowest weight = 480 mg

a) Weight variation of lowest weight :

Average weight- Lowest weight

:496.7-480 = 16.7mg

= 16.7*100/496.7 % = 3.36 %

b) Weight variation of Highest weight : Highest weight - Average weight

: 507–496.7 = 10.3 mg

= 10.3*100/496.7 % = 2.07 %

3) Physicochemical Analysis:

Table 4: Physicochemical analysis of *Panchashirisha Agada Vati*

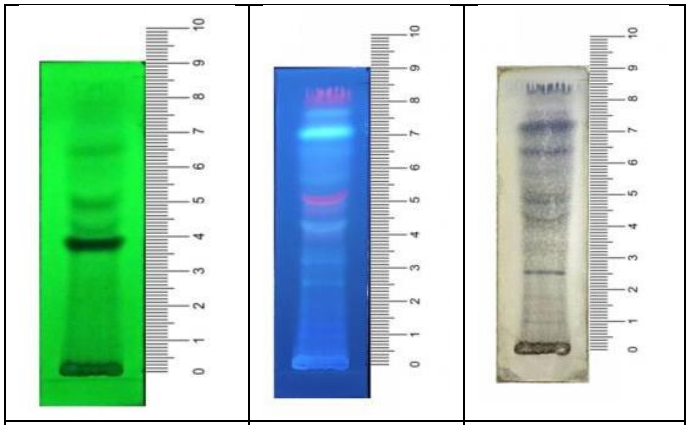
pH	8
Loss on drying	12.8 %
Total Ash	30%
Water soluble extractive	11.4%
Alcohol soluble extractive	15.9%

4) TLC value of *Panchashirisha Agada Vati*:

TLC fingerprinting was one of the fundamental objectives of present study. Below are the images of *Panchashirisha Agada,*TLC showing the separation of components at different level, at different wavelengths and after spray. TLC showed 7 spots under 254 nm, 13 spots under 366 nm and 12 after spraying with Anisaldehyde Sulfuric acid spray. Rf values of all spots and their color are mentioned below in Table 5& Figure 3.

Table 5: TLC rf values of *Panchashirisha Agada Vati*

UV 254 nm		**UV 366 nm**		**After derivatized with Anisaldehyde Sulfuric acid Spray**	
0.26	Grey	0.11	Light Blue	0.11	Black
0.31	Grey	0.15	Light Blue	0.15	Black
0.44	Black	0.29	Sky Blue	0.20	Black
0.50	Grey	0.37	Sky Blue	0.29	Dark Purple
0.58	Grey	0.39	Sky Blue	0.39	Grey
0.69	Grey	0.46	Purple	0.48	Grey
0.75	Grey	0.50	Sky Blue	0.58	Grey
-	-	0.55	Pink	0.66	Grey
-	-	0.60	Red	0.74	Purple
-	-	0.66	Light Blue	0.83	Purple
-	-	0.75	Sky Blue	0.87	Purple
-	-	0.82	Fluorescent Blue	0.93	Purple
-	-	0.89	Sky Blue	-	-



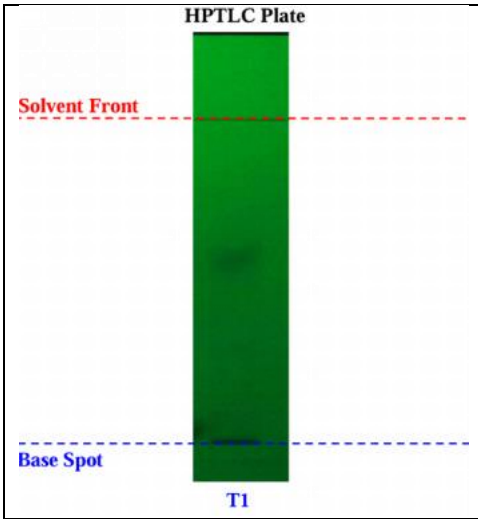
UV 254 nm	UV 366 nm	After derivatized with Anisaldehyde Sulfuric acid Spray

Figure 3: TLC images

5) HPTLC value of *Panchashirisha Agada Vati* & Comparison with reference standard

The HPTLC fingerprinting of *Panchashirisha Agada Vati* was conducted using silica gel 60 F254 aluminum plates, and chromatograms were visualized at 254 nm, 366 nm,

and 540 nm after derivatization. The sample (Track T1) exhibited a complex and well-resolved phytochemical profile across all three wavelengths. At 254 nm, six peaks were observed at Rf values 0.12, 0.32, 0.42, 0.59, 0.70, and 0.83, indicating the presence of UV-absorbing constituents (Figure 4(a)). Visualization under 366 nm revealed seven peaks at 0.32, 0.56, 0.59, 0.65, 0.73, 0.76, and 0.86, (Figure 4(b)) suggestive of fluorescent compounds, possibly flavonoids or alkaloids. After derivatization and visualization at 540 nm, twelve peaks were noted at Rf values 0.18, 0.21, 0.26, 0.29, 0.36, 0.42, 0.56, 0.59, 0.60, 0.70, 0.76, and 0.83, reflecting the presence of chromophoric phytoconstituents such as glycosides, terpenoids, or phenolics. (Figure 4(c)). Catechin was seen as a Dark blue band under 366 nm UV light with a Rf value of 0.38(Figure 4(d)) and as grey colour band under 254 nm UV light (Figure 4(e)). A similar blue band in 366 and grey band in 254 nm with the same Rf value was seen on plate with extract of *Panchashirisha Agada* in triplicates.



UV 254nm

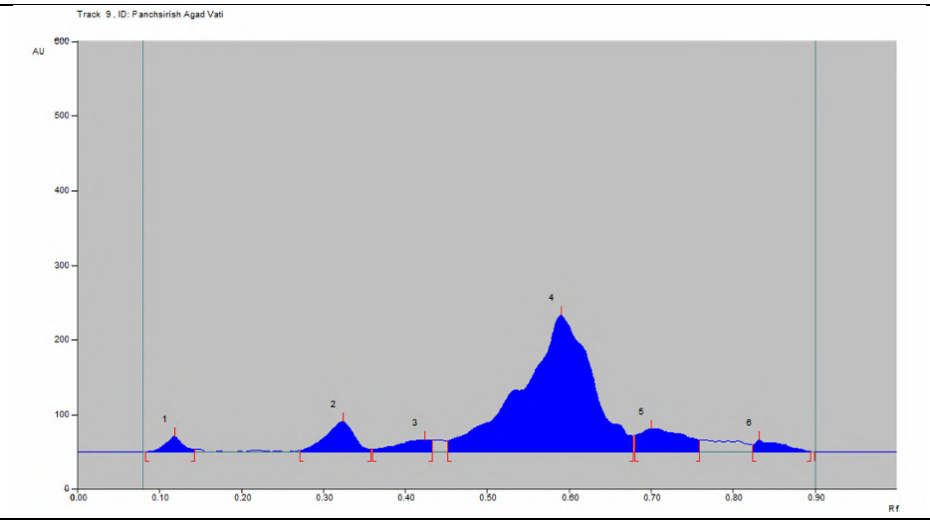
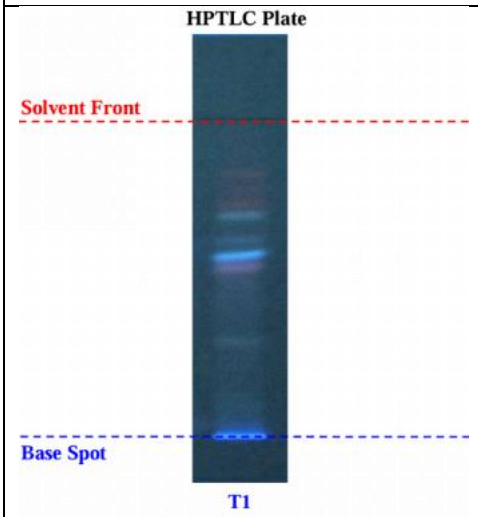


Fig. 4 (a) HPTLC Chromatogram 254nm



UV 366nm

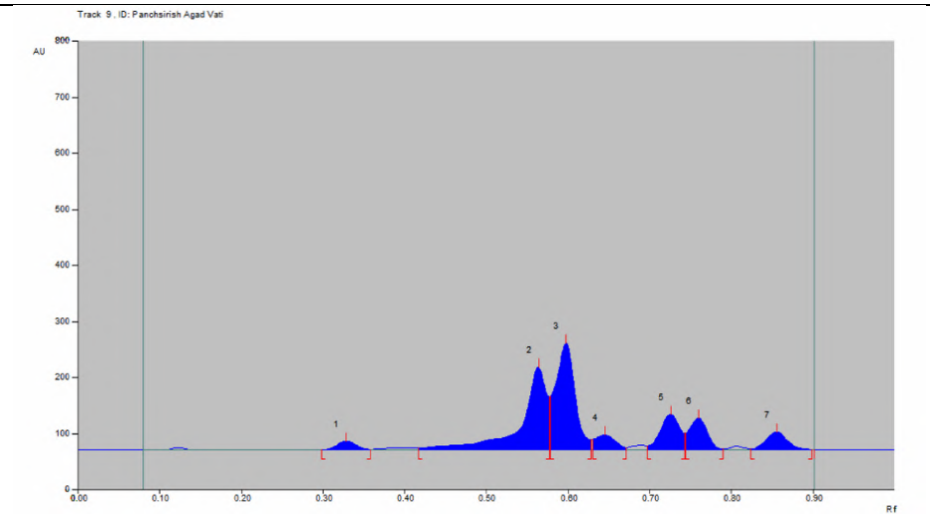
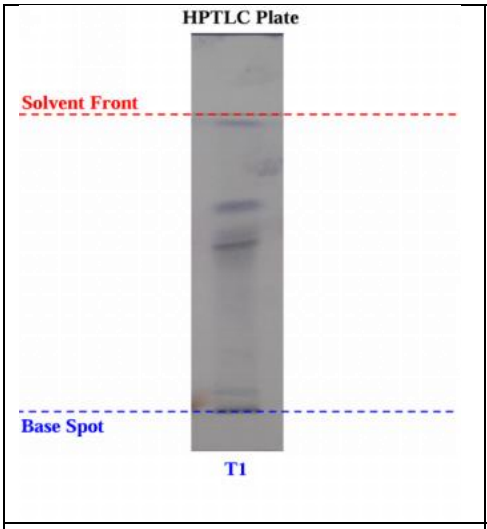


Fig. 4(b) HPTLC Chromatogram 366nm



UV 540nm

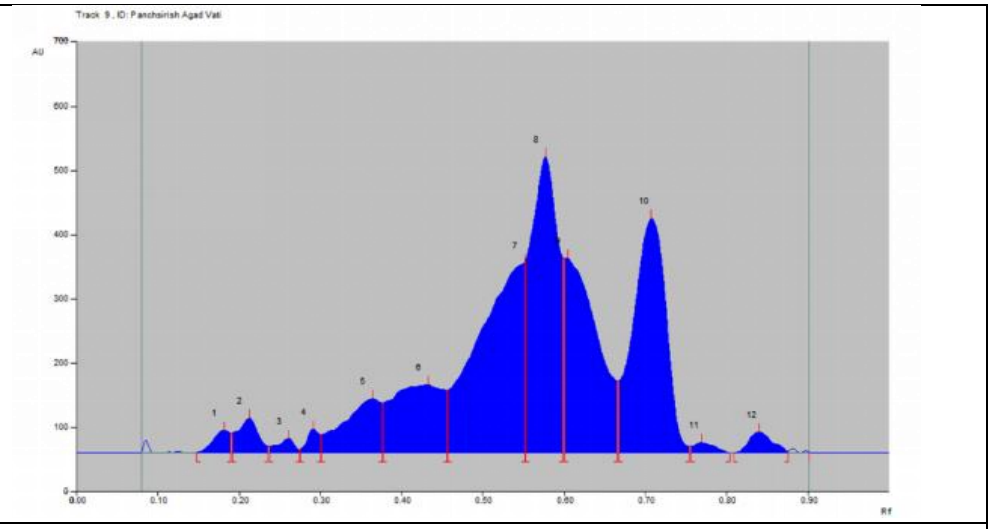


Fig. 4(c) HPTLC Chromatogram 540nm

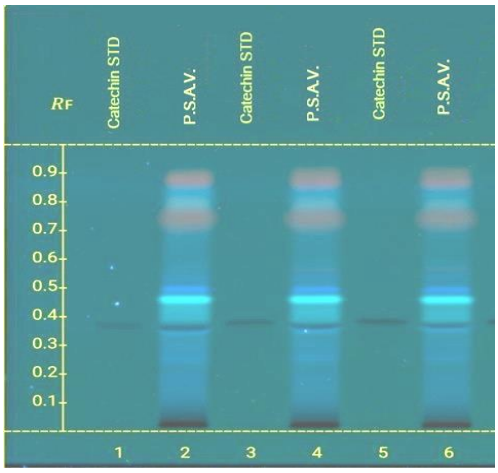


Fig. 4(d) HPTLC Chromatogram with ''Reference Standard'' Catechin at 366nm

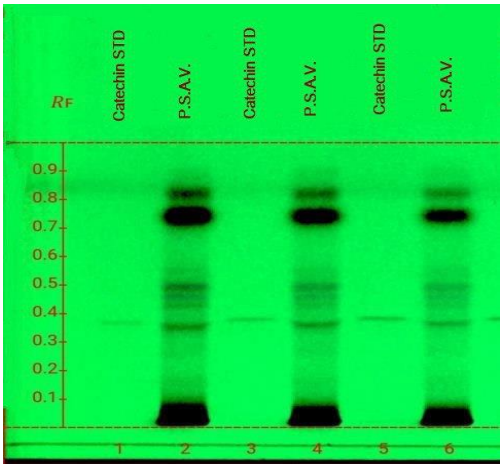


Fig. 4(e) HPTLC Chromatogram with ''Reference Standard Catechin'' at 254 nm

Figure 4 : HPTLC chromatogram

5) Analytical Profile of *Panchashirisha Agada Vati*

Table 6: analytical profile of final drug

Parameter	Observation	Inference
• Organoleptic Evaluation	*Sparsha* (texture): Smooth *Rupa* (colour): Greyish - Black *Rasa* (taste): Characteristic *Gandha*(smell):Characteristic	Passes the test
• Microscopic Characteristics	Tannin content, Stone cells, Starch grains , Prismatic crystals Epidermal cells	Passes the test
• Physicochemical Evaluation		
Ph	8	Mildly Alkaline

Loss on drying	12.8 %	-
Total Ash	30%	-
Water soluble extractive	11.4%	-
Alcohol soluble extractive	15.9%	-
• Pharmaceutical Analysis of *Panchashirisha Agada Vati*		
Hardness	5.02 Kg/cm2	Passes the test
Uniformity of weight	Less than ± 5%	Passes the test
• TLC	7 spots at 254nm, 13 spots at 366nm , 12 spots after derivatized with Anisaldehyde Sulfuric acid Spray	-
• HPTLC	6 spots at 254nm, 7 spots at 366nm, 12 spots at 540 nm	-

4. DISCUSSION

In order to verify *Panchashirisha Agada's* safety, quality & effectiveness, a pharmaceutico-analytical study examines its chemical and physical characteristics. Using the combined effects of its herbal ingredients, *Panchashirisha Agada* is a novel *Ayurvedic* formulation that is frequently used to treat a variety of ailments. In the current study, *Panchashirisha Agada Vati* was prepared which is outlined in Charaka samhita. This traditional formulation comprised *Panchanga* of *Shirisha* that were collected and authenticated. All parts were ground into a fine powder and mixed thoroughly after adding *kwatha* & make uniform sized *vati.*

The final product underwent analysis for organoleptic properties, pharmacognostic characteristics, pharmaceutical parameters and other quality assurance measures in accordance with the Ayurvedic Pharmacopoeia of India. The pharmacognostic assessment revealed the presence of prismatic crystals, lignified stone cells, cork cells, and starch grains from various components. To validate the pharmaceutical process, several physicochemical parameters were assessed. The hardness of *Panchashirisha Agada Vati* was 5.02 kg/cm2, indicating good mechanical resistance for the *vati* while handling or shipping and in weight variation lowest weight *vati* & highest weight *vati* was 3.36% and 2.07% respectively so here deviation of *vati* is less than ± 5% which proves uniformity of weight .The pH indicated the Mild Alkaline nature of the final product. A reduced loss on drying suggested a lower risk of microbial contamination and indicated good stability of the drug. The ash value 30% suggests the amount of inorganic residue left over after burning organic stuff. The water-soluble extractive value, which is 11.4% suggesting a moderate presence of water-soluble compounds in formulation. Thin-Layer Chromatography (TLC) analysis of *Panchashirisha Agada Vati* revealed 7 spots at 254 nm, 13major spots at 366 nm, 12 spots

after derivatized with Anisaldehyde Sulfuric acid Spray indicating the potential compounds within the formulation that may contribute to its therapeutic effects.

In HPTLC Study the recurrence of peaks at 0.42, 0.59, 0.70, 0.76, and 0.83 across two or more wavelengths suggests these as consistent marker compounds, indicative of the formulation's chemical integrity and reproducibility. The rich diversity of Rf values reflects the herbal nature of *Panchashirisha Agada Vati*, supporting its broad therapeutic potential. Catechin, a active chemical constituent of *Shirisha* plant was chosen as reference standard, and its Rf values were measured. Catechin was seen as a Dark blue band under 366 nm UV light with a Rf value of 0.38 and as grey colour band under 254 nm UV light. A similar blue band in 366 and grey band in 254 nm with the same Rf value was seen on plate with extract of *Panchashirisha Agada Vati* in triplicates, suggesting Catechin's presence in all analysed extracts. This HPTLC fingerprint provides us a reliable baseline for future comparative studies and quality control & standardization.

This study will not only help in enhancing the quality control of novel *Ayurvedic* formulations but also increase their acceptance in practices by proving their effectiveness with scientific backing and data preservation.

5. CONCLUSION

Panchashirisha Agada is a novel herbal formulation which comprises of *Shirisha Phala, Shirisha Moola, Shirisha Twak, Shirisha Pushpa, Shirisha Patra* (*Albizzia lebbeck* Benth.) Pharmacognostical findings confirmed the ingredients of *Panchashirisha Agada*. Physico-chemical analysis helps to create a standard analytical profile for *Panchashirisha Agada Vati* as no standard is available in the Ayurvedic Formulary of India. Hence, the data generated by this study can be used for the identification and purity of the formulation i.e *Panchashirisha Agada.*

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