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Standardization and TLC Fingerprint Analysis of Ashtanga Hridayokta Dushivishari Agad

Rashi Negi[1], Bhawana Mittal[2], Ramesh Chandra Tiwari[3]
PG Department of Agadtantra Evum Vidhi Vaidyaka, Uttarakhand Ayurved University
Rishikul Campus, Haridwar, 249401, Uttarakhand[1,2,3]
PG scholar[1], Assistant Professor[2], Professor & HOD[3]
Email id- rashinegi12345@gmail.com

ABSTRACT- *Dushivishari Agad, a classical polyherbal formulation indicated in the management of Dushivisha (cumulative or latent toxicity), has been widely described in Ayurvedic literature. The present study was undertaken to establish quality control parameters and generate standard analytical data for Ashtanga Hrudayokta Dushivishari Agad. Analytical study was accessed by organoleptic parameters including colour, odour, taste, and texture were recorded. Physicochemical parameters such as loss on drying, ash values, extractive values, and pH were assessed using standard laboratory procedures. Chromatographic profiling was carried out by Thin Layer Chromatography (TLC) to identify characteristic phytochemical markers and evaluate the chemical consistency of the formulation. pH value, loss on drying, ash value, acid insoluble ash, water soluble ash, water soluble extractive value, and alcohol soluble extractive value were 5.30, 6.882%, 16.529%, 7.005%, 3.314%, 15.596%, and 5.825%. All parametric tests were done on Dushivishari Agad, these values can be used as reference values for further research.*

Keywords: *Dushivishari Agad: Dushi visha: Agadtantra: Herbal standardization.*

I. INTRODUCTION

Ayurveda, the traditional system of medicine of India, is broadly divided into eight clinical branches collectively known as *Ashtanga Ayurveda*. Among these branches, *Agadtantra* deals with the science of toxicology and focuses on the study of different types of poisons, their origin, properties, toxic manifestations, and therapeutic management. Poisons may arise from plant, mineral, animal, or artificial sources, and their effects on the human body may vary depending on their potency and duration of exposure. *Agadtantra* provides detailed descriptions of several *Vishaghna Yogas* (antitoxic formulations) that help in neutralizing toxins and managing various toxins as well as disease conditions[1,2].

Dushivisha is a unique concept described in classical texts of *Ayurveda.* It refers to low-grade or cumulative toxins that remain in the body in a dormant state for a prolonged period. These toxins may gradually disturb normal physiological functions and lead to the development of different pathological conditions. The principle of *Ayurveda* focuses holistically on the equilibrium of *Dosha, Dhatu, Mala* and *Agni* in order to have a healthy and positive mental health. Formulation of *Agad Yogas* is used to counteract the negative effects of toxic substances in which *Dushivishari Agad* is mentioned in *Sushrut, Ashtanga Hrudayam,* and *Bhavprakash Samhita* with different ingredients under the treatment of *Dushivisha*. For the present study, *Ashtanga Hrudayokta Dushivishari Agad* is described in *Uttarsthan*, 35th chapter, 39th *Shlok*. It is a poly-herbal formulation with 12 herbs such as *Pippali* (*Piper longum)*, *Dhyamak* (*Cymbopogon martini*), *Jatamansi* (*Nardostachys jatamansi*), *Lodhra* (*Symplocus racemosa*), *Ela* (*Ellataria cardamomum*), *Suvarchika* (*Cleome viscosa*), *Kuttannata* (*Oroxylum indicum*), *Nata* (*Valleriana jatamansi*), *Kustha* (*Sassurea lappa*), *Yashti* (*Glycyrrhiza glabra*), *Chandana* (*Pterocarpus santalinus*), *Gairika* (Red Ochre)[3]. Ingredients of DVA (*Dushivishari Agad)* having anti-inflammatory, anti-oxidant, and neuroprotective, properties[4-8].

The aim of the analytical study of *Dushivishari Agad* is to confirm the identity, purity, and quality of the formulation by using organoleptic, physicochemical and TLC (Thin Layer Chromatography) analysis. Analytical study was done at an AYUSH approved ASU drug testing Laboratory; Central Research Facility; KLE, Belagavi, Karnataka; (Reference No: CRF/FG/345/2025-2026). The World Health Organization (WHO) has also emphasized the importance of establishing proper standardization of herbal medicines. Therefore, ensuring all the guidelines, standardization tests were done on DVA in authenticating the poly-herbal preparation and ensuring its quality, safety, and effectiveness.

II. MATERIALS AND METHODS

Dushivishari Agad is described in *Ashtanga Hrudaya, Uttarsthan adhyaya* 35, verse 39. Following under is the preparation of the formulation.

A. Collection of raw drugs

The ingredients of DVA (Table1) were collected from various sources

- Patra of *Dhyamaka,* Bark of *Kutannata* were collected from Haridwar
- Root of *Kushta* was collected from Uttarkashi, Uttarakhand
- *Pippali, Jatamamsi, Lodhra, Ela, Suvarchika, Natam, Yashti, Rakta Chandana* and *Gairika* all dried herbs were purchased from Herbal Automation, Anamika Pharmacy, Haridwar, Uttarakhand.

B. Authentication of raw drugs

The collected drugs were identified and authenticated at *Dravyaguna* Department of Uttarakhand Ayurveda University, Rishikul Campus, Haridwar, Uttarakhand with reference no-DG/RC/UAU-266

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C. Drying of raw drugs

All the raw herbal *drugs* were cleaned and properly sun dried except the aromatic ingredients were shadow dried.

TABLE 1: Ingredients of DVA

S. No.	Ingredients	Botanical Name	Part Used	Quantity
1.	***Pippali***	*Piper longum Linn.*	*Phala* (fruit)	**1 part**
2.	***Dhyamaka***	*Cymbopogon martini Roxb.*	*Patra* (leaves)	**1 part**
3.	***Jatamansi***	*Nardostachys jatamansi DC*	*Moola* (root)	**1 part**
4.	***Lodhra***	*Symplocus racemosa Roxb.*	***Twak* (bark)**	**1 part**
5.	***Ela***	*Elettaria cardamomum Maton.*	***Phala* (fruit)**	**1 part**
6.	***Suvarchika***	*Cleome viscosa Linn.*	***Beeja* (seed)**	**1 part**
7.	***Kuttannata***	*Oroxylum indicum Linn.*	***Twak* (bark)**	**1 part**
8.	***Natam***	*Valleriana jatamansi Jones*	*Moola* (root)	**1 part**
9.	***Kushta***	*Sassurea lappa C.B.*	*Moola* (root)	**1 part**
10.	***Yashti***	*Glycyrrhiza glabra* Linn.	*Moola* (root)	**1 part**
11.	***Chandan***	*Pterocarpus santalinus* l.f.	*Kandasara* (heartwood)	**1 part**
12.	***Gairika***	Red ochre		**1 part**

*Lemon grass, *Hurhur* were used as a substitute ingredient for *Dhyamaka* & *Suvarchika*

D. Instruments Used

Digital balance, Magnifying lens, Hot air oven, Silica crucible, Hot plate, Grinder, Water bath, Muffle furnace, Common glassware, Desiccator, Rotary shaker, and TLC chamber.

E. Method for preparation of Dushivishari Agad

- DVA was prepared using the method described in the classical text of *Sharangdhar Samhita* at GMP approved Anamika Pharmacy, Sidcul, Haridwar, Uttarakhand.
- All 12 herbal ingredients were cleaned, dried and taken in equal quantity.
- They were initially coarsely grounded separately with *khalva yantra* (iron mortar-pestle) and then grinded into fine powder.
- Then the mineral drug- *gairika* underwent *shodhan* (purification) by roasting with *goghrita* (ghee) in an iron vessel[9].
- Thereafter, the coarse powder was passed through Sifter machine sieve number 80 to form fine powder.
- The powdered ingredients were thoroughly mixed to form the final formulation.
- This homogenous mixture was stored in an air tight container as *Dushivishari Agad.* The final product was in the form of *churna* (fine powder)

III. Analytical Study of *Dushivishari Agad*

The analytical study was conducted at an AYUSH approved ASU drug testing Laboratory; Central Research Facility; KLE, Belagavi, Karnataka;(Reference No: CRF/FG/345/2025-2026) The following parameters were analyzed:

1. Organoleptic study
2. Physicochemical study
3. TLC (Thin layer chromatography)

A. Organoleptic parameters[10]

Organoleptic evaluation serves as a simple and rapid method for preliminary assessment in the standardization and quality control of Ayurvedic formulations. DVA was evaluated based on these sensory characteristics: Appearance, Colour, Odour, Taste, Texture. This sensory assessment of pharmaceutical formulations represents a critical yet often underappreciated dimension in ensuring the efficacy of the formulation. (Table 2)

B. Physicochemical parameters

In pharmacognosy and pharmaceutico-analytical studies, physicochemical parameters are used to evaluate the purity, quality, and identity of crude drugs or formulations. These tests help detect adulteration, moisture content, inorganic impurities, and extractable active constituents. It is like a report-card of the formulation. (Table 3)

Dushivishari Agad powder was used for analysis of different physicochemical parameters as mentioned in the Ayurveda Pharmacopoeia of India (API). DVA was subjected to various assessment such as loss on drying, ash value, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, pH Value.

C. Chromatographical assessment

The assessment was done using Thin Layer Chromatography (TLC). Table 4, (Fig. 1)

Thin Layer Chromatography (TLC) is a simple, rapid, and inexpensive analytical technique used to separate, identify, and

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analyze the purity of a substance. Ayurvedic Pharmacopoeia of India (API) guidelines were followed. the alcoholic extract of DVA powder was formed using precoated silica gel TLC plates as the stationary phase with Toluene: Ethyl acetate (7:3) as the mobile phase for chromatographic separation. After development, the plate was dried and visualized under UV light at 254 nm (short wave) and 366 nm (long wave) for accurate identification and purity of the compounds in *Dushivishari Agad.*

IV. Observations and Results

TABLE 2: Organoleptic parameters

Sr. No.	**Organoleptic parameters**	**Results**
1.	Appearance	*Churna* (powder)
2.	Colour	Brownish
3.	Odour	Aromatic
4.	Taste	Sweet, Bitter
5.	Texture	Smooth, grainy

TABLE 3: Shows the Physicochemical analysis of *Dushivishari Agad* [11]

Sr. No.	**Physicochemical parameters**	**Results**
1.	Loss on drying	6.882%
2.	Ash value	16.529%
3.	Acid Insoluble Ash	7.005%
4.	Water soluble Ash	3.314%
5.	Water soluble extractive	15.596%
6.	Alcohol soluble extractive	5.825%
7.	pH	5.30

A. TLC of DVA

TABLE 4: Thin Layer Chromatography

	***Dushivishari Agad churna* (powder)**	Alcohol extract
	Mobile phase	Toluene: Ethyl acetate
	Stationary phase	Silica gel
	Ratio	7:3
	Rf values	**Short Wave:** 0.06, 0.08, 0.13, 0.18, 0.22, 0.27, 0.31, 0.37, 0.43, 0.50, 0.54, 0.69, 0.75, 0.83, 0.90, 0.95
		Long Wave: 0.06, 0.13, 0.51, 0.62, 0.70

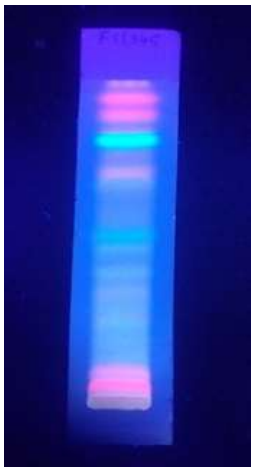

Fig 1: TLC plate at wavelength 366nm

V. Discussion

Analytical evaluation is essential for establishing quality standards and ensuring the identity, purity, and consistency of herbal formulations.

A. The organoleptic evaluation of the formulation showed brown colour, bitter and sweet taste, aromatic odour, and smooth, grainy texture which are mainly attributed to the presence of various herbal ingredients in the formulation. These sensory parameters serve as preliminary indicators for identification and help detect any possible adulteration or deterioration of the drug.

B. Physicochemical parameters such as moisture content, ash value, acid-insoluble ash, water-soluble ash, extractive values, and pH provide important information regarding the purity and quality of the formulation. The loss on drying at 105°C (6.882%) indicated that the formulation contains an acceptable level of moisture which is almost within limit of 6.8%. Lower the value of LOD indicates drug is properly dried, stable, less susceptible to microbial growth, and indicates the shelf-life stability of the drug.

C. The Total Ash Value (TAV) (16.529%) reflects the total inorganic content present in the formulation. High TAV indicates presence of possible contamination with soil, sand, or adulterant. The acid-insoluble ash (7.005%) represents the amount of silica and earthy matter present. Low value indicates minimal contamination. The water-soluble ash (3.314%) indicates the proportion of soluble salts and minerals present in the formulation. For *Dushivishari Agad,* the lower value reflects low levels of inorganic matter indicating purity and cleanliness of raw materials.

D. The water-soluble extractive value (15.596%) and alcohol-soluble extractive value (5.825%) suggest the presence of polar and moderately polar constituents in the formulation. Extractive values are useful indicators for determining the active constituents extracted with particular solvents. High alcohol-soluble extractive value shows the presence of polar ingredients such as steroids, phenolics, flavonoids, and glycosides, whereas water-soluble extractive value shows the presence of acids, sugars, and inorganic compounds[12].

E. The pH value (5.30) indicates the weakly acidic nature of the formulation which indicate the presence of tannins and phenols which make their aqueous extracts. Weakly acidic drugs are generally better absorbed in acidic environments whereas weakly basic drugs may require more alkaline conditions for optimal absorption. Monitoring the pH of samples is important for a wide range of applications, including drug absorption, stability, and taste[13].

F. The TLC fingerprint profile of the alcoholic extract of *Dushivishari Agada* using

Toluene: Ethyl acetate (7:3) as the mobile phase showed multiple spots under both short (254nm) and long (366nm) wavelength UV light. The presence of several Rf values indicates rich phytochemical profile in *Dushivishari Agad*, reflecting its complex herbal composition. Total 16 spots for short wave and 5 spots for long wave was detected which meant the presence of aromatic compounds. The TLC fingerprint obtained is consistent with the multiple-ingredient herbal formulation which can further serve as a reference in chromatographic profile for the identification and quality control of the formulation.

VI. Conclusion

This study provides a preliminary analytical evaluation of *Dushivishari Agad* through organoleptic, physicochemical, and TLC analysis.

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The obtained results establish the characteristic parameters and a chromatographic fingerprint profile for the formulation, which serve as reference data for its identification and quality control.Chromatographical data of *Dushivishari Agad* shows rich phytoconstituents of raw drugs and their Rf value proves the standardisation of DVA. These parameters can be used as a reference value for further research on *Dushivishari Agad.* The findings provide a comprehensive analytical profile *of Dushivishari Agada* and can serve as reference data for its quality control and standardization.

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