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Standardization of *Dooshivishahari Agada* through HPTLC

Research Article

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Abstract

Ayurveda medicines are a time-tested and valuable resource for healing, even today, globally. Hence the quality control standards for the Ayurveda formulations are the need of time because of commercialization. Despite the availability of modern equipments and techniques, not all *Ayurvedic* formulations have been standardized as per modern-day protocol. *Dooshivishari agada* (DVA), a herbo-mineral preparation; is mentioned in the text of *Asthangha hrudaya* in the context of treatment of poisoning, specifically for *dooshivisha* (cumulative poison). High-performance thin-layer chromatography (HPTLC) is a validated analytical tool for qualitative and quantitative analysis of chromatographic information. In this present study, an effort has been made to standardize DVA through HPTLC. HPTLC photo documentation of DVA showed 8, 11, and 10 spots under short UV, long UV, and under white light after derivatization respectively. A densitometric scan at 254 nm revealed 3 high peaks corresponding to 3 different compounds in the ethanol extract, and at 366 nm and 620 nm there were three high peaks. These Physico-chemical constants, TLC photo documentation, the unique Rf values, and densitogram obtained at different wavelengths can be used as a fingerprint to identify DVA.

Keywords: *Ayurveda, Dooshivishari agada,* HPTLC, Herbo-mineral formulation, Pharmaceutical study, Standardization.

Introduction

There has been exponential growth in the field of *Ayurveda* medicine in the last few decades. Due to its natural origin and lesser side effects, *Ayurveda* medicine is getting popularized in developed as well as developing countries. From the global perspective, there is a shift toward the use of *Ayurveda* formulations, as the dangers and the shortcoming of modern medicine have started getting more apparent. (1) *Ayurveda* formulations comprise multiple herbal or herbo mineral ingredients. Each of these ingredients will have various components and their action differs based on the components present in it. (2) It is the prime responsibility of the regulatory authorities to ensure that the health seekers get the medication, which guarantees safety, quality, and efficacy. These regulatory authorities have prescribed standards for raw materials and finished products. HPTLC is one among these to ensure the identity, safety, and quality of the herbs. (3)

Dooshivishari agada is a herbo mineral formulation, mentioned for the management of *dooshivisha* (cumulative poison) condition. (4) To maintain the quality, standardization is needed as it has been said that the indigenous system of medicine has been commercialized in the present scenario leading to the use of quality control standards of formulations. (5)

Dooshivishari agada is a herbo mineral formulation mentioned in the context of treatment of various kinds of poisoning, comprising twelve ingredients. (4)

High-performance thin-layer chromatography (HPTLC) is a sophisticated instrumental technique and is a powerful analytical tool for chromatographic information for various samples. (6) So a formulation like *Dooshivishari agada*, comprising multiple ingredients, was subjected to phytochemical standardization for quality control and authentication of preparation to ensure therapeutic efficacy.

Aims and Objectives

To evaluate the phytochemical standards of *Dooshivishari agada* using the chromatography technique i.e., HPTLC.

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Materials and methods

Plant material

The ingredients of *Dooshivishari agada* (Table 1) were collected from the local market of Mysore. The collected drugs were identified and authenticated at the teaching pharmacy of the Department of Dravyaguna, SDM College of Ayurveda and Hospital, Hassan, Karnataka state, India.

Phytochemical standardization

Phytochemical studies through HPTLC were carried out as per the WHO guidelines. (7) The analytical study was carried out at SDM Centre for Research in Ayurveda and Allied Sciences (AYUSH

Centre for Excellence and Recognized SIROs by DSIR), Laxminarayana Nagar, P.O. Kuthpady - 574 118, Udupi, Karnataka state, India as per the standard procedure.

A pharmaceutical preparation of *Dooshivishari agada*

Table No. 1: Ingredients of *Dooshivishari agada*[4]

Sl. No.	*Dravya*	Botanical name	Part used	Quantity
1	*Pippali*	*Piper longum* Linn.	Fruit	12 gm
2	*Dhyamaka*	*Cymbopogon martini* (Roxb.) W. Watson.	Whole plant	12 gm
3	*Jatamansi*	*Nardostachys jatamansi* (D.Don) DC.	Root	12 gm
4	*Lodra*	*Symplocos racemosa* Roxb.	Bark	12 gm
5	*Ela*	*Elettaria cardamomum* (L.) Maton.	Seeds	12 gm
6	*Suvarchika*	*Tribulus terrestris* Linn.	Fruit	12 gm
7	*Kutannata*	*Oroxylum indicum* (L.) Kurz	Root	12 gm
8	*Natha*	*Valeriana wallichi* DC.	Root	12 gm
9	*Kushta*	*Saussurea lappa* Clarke.	Root	12 gm
10	*Yashtimadhu*	*Glycyrrhiza glabra* Linn.	Root	12 gm
11	*Chandana*	*Santalum album* Linn.	Heart wood	12 gm
12	*Gairika*	Red ochre	-	10 gm

Method of preparation

All 11 herbal ingredients were taken in dry form in equal quantity (12gm each). They were pounded well separately to a coarse form using *khalva yantra* (mortar and pestle). *Gairika* (10gm) was pounded to a coarse powder form and *shodhana* (purification) was done by frying with *ghrita* (ghee) in iron vessel. Then all the pounded ingredients were mixed well into a homogenous mixture and stored in an airtight container.

The total quantity obtained

The total quantity of formulation obtained was 120gm.

The physical characteristic of formulation

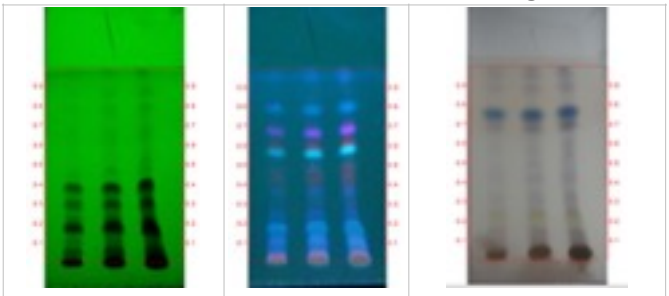
- Appearance: Powder form
- Colour: Reddish-brown
- Odour: Astringent smell
- Touch: Coarse
- Taste: *Kashaya* (Astringent)
- Solubility: Insoluble in water

HPTLC

1gm of the sample of *Dooshivishari agada* was dissolved in 10 ml of alcohol kept overnight and filtered. 3, 6, and 9µl of each of the above extracts were applied on a pre-coated silica gel F254 on aluminum plates to a bandwidth of 7 mm using a Linomat 5 TLC applicator. The plate was developed Toluene: Ethyl acetate (9.0: 1.0). The developed plates were visualized in short UV, long UV, and then derivatized with vanillin sulphuric acid and scanned under UV 254nm, 366nm, and 620nm (following derivatization). Rf, the color of the spots, densitometric scan, and 3-D chromatograms were recorded.

Results

Figure 1: HPTLC Photo documentation of sample of Alcoholic extract of *Dooshivishari agada*



Short UV | **Long UV** | **After derivatization**

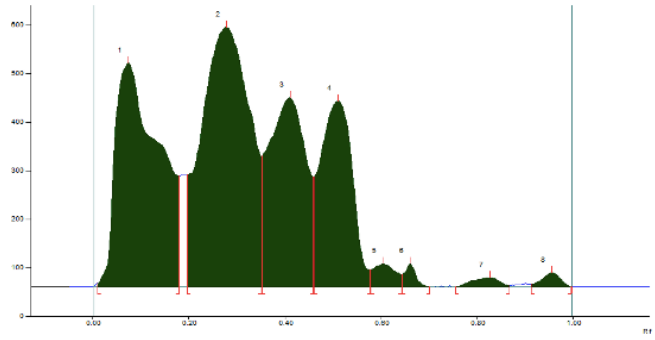
Track 1: Alcoholic extract of *Dooshivishari agada* -3µl
Track 2: Alcoholic extract of *Dooshivishari agada* -6µl
Track 3: Alcoholic extract of *Dooshivishari agada* -9µl

Table 2: Rf values of *Dooshivishari agada*

Short UV	Long UV	After derivatization
0.05 (D. green)	0.05 (F. pink)	-
-	0.12 (F. blue)	-
-	-	0.16 (Purple)
-	0.19 (F. blue)	-
0.21 (D. green)	-	0.21 (Yellow)
-	0.26 (F. blue)	0.25 (Purple)
0.31 (D. green)	-	0.32 (Purple)
-	0.36 (F. blue)	-
0.39 (D. green)	-	-
-	0.44 (F. blue)	0.43 (Purple)
0.47 (Green)	0.47 (F. blue)	-
-	-	0.50 (Purple)
-	0.56 (F aqua. blue)	0.55 (Purple)
-	0.62 (F. red)	-
0.68 (Green)	0.67 (F. purple)	0.68 (Purple)
-	-	0.75 (Copper sulphate blue)
0.78 (Green)	0.79 (F. blue)	-
0.88 (Green)	-	0.89 (Purple)

F – fluorescent

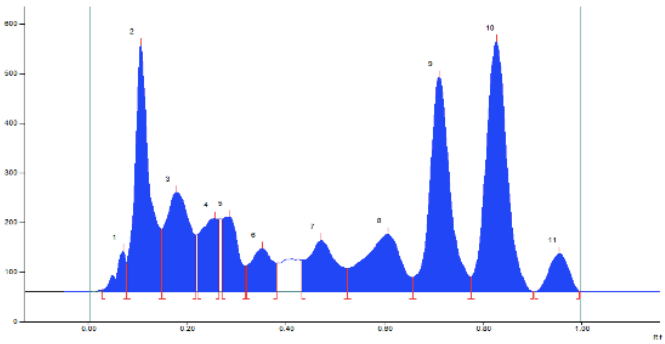
Figure 2: Densitometric scan of the sample of *Dooshivishari agada*



Track 3, ID: Dooshivishari agada

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	7.3 AU	0.07 Rf	460.3 AU	24.12 %	0.18 Rf	27.9 AU	29851.5 AU	26.59 %
2	0.20 Rf	231.2 AU	0.28 Rf	533.5 AU	27.95 %	0.35 Rf	68.1 AU	38691.4 AU	34.46 %
3	0.35 Rf	268.8 AU	0.41 Rf	389.1 AU	20.38 %	0.46 Rf	25.6 AU	21472.5 AU	19.13 %
4	0.46 Rf	226.0 AU	0.51 Rf	381.8 AU	20.00 %	0.58 Rf	34.6 AU	18303.8 AU	16.30 %
5	0.58 Rf	34.9 AU	0.60 Rf	47.5 AU	2.49 %	0.64 Rf	26.1 AU	1580.3 AU	1.41 %
6	0.65 Rf	26.5 AU	0.66 Rf	47.5 AU	2.49 %	0.70 Rf	0.6 AU	772.0 AU	0.69 %
7	0.76 Rf	0.6 AU	0.83 Rf	19.5 AU	1.02 %	0.87 Rf	3.3 AU	785.7 AU	0.70 %
8	0.91 Rf	5.4 AU	0.96 Rf	29.5 AU	1.55 %	1.00 Rf	0.2 AU	815.7 AU	0.73 %

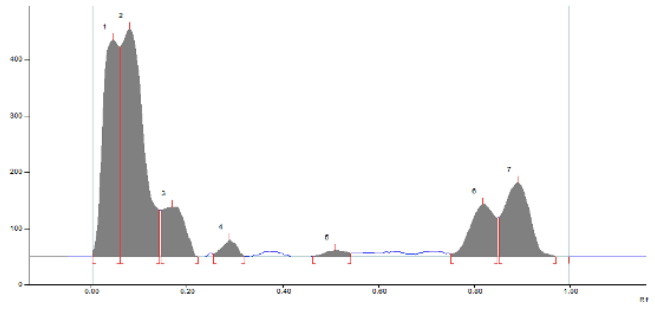
Fig. 2a. At 254nm



Track 3, ID: Dooshivishari agada

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	3.6 AU	0.07 Rf	82.1 AU	3.43 %	0.08 Rf	57.1 AU	1184.8 AU	1.72 %
2	0.08 Rf	57.4 AU	0.11 Rf	495.6 AU	20.71 %	0.15 Rf	25.8 AU	10412.8 AU	15.13 %
3	0.15 Rf	126.1 AU	0.18 Rf	199.9 AU	8.36 %	0.22 Rf	14.5 AU	7024.0 AU	10.21 %
4	0.22 Rf	114.7 AU	0.26 Rf	146.9 AU	6.14 %	0.27 Rf	46.6 AU	3933.1 AU	5.71 %
5	0.27 Rf	146.1 AU	0.29 Rf	151.1 AU	6.32 %	0.32 Rf	51.5 AU	3524.2 AU	5.12 %
6	0.32 Rf	52.2 AU	0.35 Rf	87.2 AU	3.64 %	0.38 Rf	57.4 AU	2740.9 AU	3.98 %
7	0.43 Rf	62.9 AU	0.47 Rf	103.7 AU	4.33 %	0.53 Rf	47.8 AU	4257.4 AU	6.19 %
8	0.53 Rf	47.9 AU	0.61 Rf	115.7 AU	4.84 %	0.66 Rf	28.8 AU	6088.3 AU	8.85 %
9	0.66 Rf	29.1 AU	0.71 Rf	431.2 AU	18.02 %	0.78 Rf	29.8 AU	12423.5 AU	18.05 %
10	0.78 Rf	30.2 AU	0.83 Rf	502.8 AU	21.01 %	0.90 Rf	0.1 AU	15109.9 AU	21.95 %
11	0.91 Rf	0.2 AU	0.96 Rf	76.5 AU	3.20 %	1.00 Rf	1.6 AU	2129.5 AU	3.09 %

Fig. 2b. At 366nm



Track 3, ID: Dooshivishari agada

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	4.7 AU	0.05 Rf	384.1 AU	33.65 %	0.06 Rf	71.0 AU	9217.6 AU	26.31 %
2	0.06 Rf	371.6 AU	0.08 Rf	403.5 AU	35.35 %	0.14 Rf	82.5 AU	13384.9 AU	38.21 %
3	0.15 Rf	82.5 AU	0.17 Rf	88.7 AU	7.77 %	0.22 Rf	0.2 AU	3029.6 AU	8.65 %
4	0.25 Rf	5.0 AU	0.29 Rf	29.3 AU	2.57 %	0.32 Rf	1.1 AU	661.2 AU	1.89 %
5	0.46 Rf	0.8 AU	0.51 Rf	11.5 AU	1.01 %	0.54 Rf	6.6 AU	375.9 AU	1.07 %
6	0.75 Rf	4.7 AU	0.82 Rf	93.1 AU	8.16 %	0.85 Rf	68.6 AU	3395.5 AU	9.69 %
7	0.85 Rf	69.3 AU	0.89 Rf	131.2 AU	11.50 %	0.97 Rf	0.1 AU	4968.6 AU	14.18 %

Fig. 2c. At 620nm

Discussion

All ingredients of DVA are made into coarse powder to rupture its tissue and cell structure, so that the components are easily extracted. As *gairika* is a mineral ingredient, it must be done *shodhana* (purification) before using it in the formulation. Because, *gairika* contains free ferrous ions which is toxic to the body. TLC photo documentation of *Dooshivishari agada* showed 8, 11, and 10 spots under short UV, long UV, and white light after derivatization respectively. Spot with Rf 0.68 was commonly detected in all three detection methods. All three methods gave optimum separation of different bands and hence all of them may be used as TLC fingerprint patterns to identify the composition of *Dooshivishari agada* (Table 2). A densitometric scan at 254 nm revealed four high peaks corresponding to 4 different compounds in the ethanol extract, compounds with Rf 0.51 (16.30%), 0.41 (19.13%), 0.28(34.46%), and 0.07 (26.59%) were the peaks (Figure 2A). At 366 nm there were three high peaks, with Rf 0.83 (21.95%), 0.11 (15.13%), and 0.71 (18.05%) being the major peaks detected (Figure 2B). At 620 nm there were three high peaks, with Rf 0.08 (38.21%), 0.05 (26.31%), and 0.89 (14.18%) being the major peaks detected (Figure 2C). The results obtained through this analytical study can be taken as preliminary standards for further studies.

Conclusion

Standardization aims to maintain the quality and standard of the drug, by which the therapeutic potential can be maintained. Different ingredients of *Dooshivishari agada* have varied chemical constituents that add up to the

therapeutic effect of the drug. It is not a single entity but a combination of all the constituents that give the desired result. *Dooshivishari agada* was prepared from these ingredients will have combined effects of all the individual herbs. The physicochemical standardization of *Dooshivishari agada* was carried out using the HPTLC fingerprint profile for the quality control of the processed pill. But for standardization, it must be tested on 2-3 samples of the formulation.

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