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Quality Standardization of *Dooshivishari Agada*

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ABSTRACT

Dooshivishari Agada (DVA) one of formulation described in Ayurvedic classics, commonly used for toxic conditions. Standards of DVA are not present in API. In this study DVA is prepared in three Batches and subjected to analysis like organoleptic studies, pH, LOD (Loss on Drying), total ash, acid insoluble ash, hardness, disintegration time, phytochemical screening and thin layer chromatography. The results obtained in this study did not show any variation in phytochemical screening but negligible variation in physico-chemical analysis. Further studies in this line we being carried out.

KEYWORDS

Dooshivishari Agada (DVA), Ayurvedic classics, Quality Analysis

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INTRODUCTION

Some of the well acclaimed regularly prescribed during practice in *Ayurveda*, are combination of herbs and minerals and which not yet standardized and included in the Ayurvedic Pharmacopeia of India. One of such formulation is *Dooshivishari Agada* (DVA), which is present in classics and indicated for allergic conditions as well as toxic presentation [1, 2].

This drug is widely prescribed by Ayurvedic Physicians in Andhra Pradesha, Karnataka, Kerala and Maharashtra. Standard preparation methods and quality assessment report for this formulation is not available. The present Study is aimed to fill these existing lacunae.

MATERIALS AND METHODS

a) Raw drugs required for preparation of DVA were procured from GMP Certified KLE Ayurved Pharmacy, Khasbag, and Belagavi.

b) Procured drugs were authenticated in AYUSH approved Central research Facility, KLE Academy of Higher education & Research, Shri B M K Ayurveda Mahavidyalaya and Research Centre, Belgavi.

Preparation of DVA was done using 13 ingredients[3] as below; ingredients and official parts used are shown in **Table 1**.

a. Drugs were pulverized to powder form and then sieved through sieve No 120 to which *Shoditha Gairika* was added.

Table 1 Ingredients of *Dooshivishari Agada*

Sl. No.	*Dravya*	Botanical Name	Official part
1	*Pippali*	*Piper longum* Linn.	*Phala* (Fruit)
2	*Pippali mula*	*Piper longum* Linn.	*Mula* (root)
3	*Dhyamaka*	*Cymbopogon martinii* (Roxb.) Wats.	*Patra* (Leaves)
4	*Jatamamsi*	*Nardostachys jatamamsi* DC. (N. grandiflora)	*Mula* (Root)
5	*Lodra*	*Symplocos racemosa* Roxb.	*Twak* (Stem Bark)
6	*Ela*	*Elettaria cardamomum* Maton	*Phala* (Fruit)
7	*Suvarchika*	*Tribulus terrestris* Linn.	*Phala* (Fruit), *Mula* (Root)
8	*Katunnatum*	*Oroxylum indicum* (Linn) Benth.Ex Kurz.	*Mulatwak* (Root bark)
9	*Natam*	*Valeriana wallichii* D.C.	*Mula* (Root)
10	*Kusta*	*Saussurea lappa* C.B. Clarke.	*Mula* (Root)
11	*Yastimadhu*	*Glycyrrhiza glabra* Linn.	*Mula* (Root)
12	*Chandana*	*Santalum album* Linn.	*Kandasara* (Heartwood)
13	*Gairika*	*Red ochre*	

b. Individually all *choornas* were weighed (100 gm each) and mixed with *Gairika*. As *Bhavanadravya* was not mentioned in texts we preferred the *kwath* of same drugs (as mentioned in Table 1). *Kwath* was prepared as classical way. *Kwath choorna* which remained after pulverization of ingredients in ratio of 1 part drug and 8 parts water

which reduced to ¼ the quantity and used in QS[4].

c. The process of *Bhavana* was carried out 8 hours a day for 7 days at Bhaishajya Kalpana laboratory, KLE Academy of Higher education & Research, Shri B M K Ayurveda Mahavidyalaya. On 8th day the *vati* were prepared and kept in stainless steel plates and dried under shade.

Dried *Dooshivishari Agada* vati was placed in clean and dry sterilized glass bottles till analysis was done.

During preparation of *Dooshivishari Agada* care was taken to use daily prepared fresh *Bhavana Dravya* which was subjected for *mardana* for seven days, Maintenance of hygiene was taken care throughout preparation by wearing gloves, masks and head caps by all laboratory staff, and equipment used for preparation ware cleaned daily.in this way DVA is prepared in three successive batches.

Quality Analysis of *Dooshivishari Agada*

Analysis of DVA was carried out at AYUSH Approved Drug Testing Laboratory Central research Facility KLE Academy of Higher education & Research, Shri B M K Ayurveda Mahavidyalaya and Research Centre, Belgavi. DVA was subjected to following analysis according to the methods described elsewhere.

DVA underwent following quality tests

1. Prepared Vati were subjected to organoleptic characters like form, odour, colour and taste.
2. Physico-Chemical Analysis
3. Thin Layer Chromatography and Screening of Phytochemicals

Physico-Chemical Analysis

We followed the methods given in API[5]

A. Loss on Drying:

Method: A small amount (2-5) of sample was taken in silica crucible and weighed accurately using fine electronic weighing machine (Shumadzu Japan), then it was transferred and kept in electric hot air oven (Phatak India) at 110^0 for Three hours. After cooling in a desiccator it was weighed, the difference in weight was calculated as the formula given below:

$$\text{Loss on drying (\%)} = \frac{\text{Loss in weight}}{\text{Weight of the sample}} \times 100$$

B. Total ash:

Method: Ground material, was weighed (2-3 gm) in a tarred silica crucible previously ignited, cooled and weighed. Crucible was placed in muffle furnace (Phatak Ltd). After ignition is completed, gradually transfer crucible to the desiccator. Allowed it to cool to room temperature. Weighed to nearest 0.1 mg. Content in mg of ash per g of air-dried material was calculated using following formula:

Weight of ash = Tare weight of the crucible - weight of the crucible + ash

% Total ash = weight of ash x 100 / weight of sample

C. Acid insoluble ash

Method: Previously obtained ash was boiled with 25 ml of 2M hydrochloric acid for 5 min on water bath and filtered through ashless filter paper (Whatsman no1). Collected the insoluble matter on ashless filter paper, washed with hot water. Kept the filter paper in crucible and ignited in a furnace for 1 hour. Cooled in a desiccator and weighed .Calculated the percentage of acid insoluble ash with reference to air dry drug

Calculation

% acid Insoluble ash = weight of Ash x100 / weight of sample

D. *Tablet Hardness Test*

Method: Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the formulation was determined by using Monsanto Hardness tester. It is expressed in kg/cm^2. Six tablets according to guidelines were randomly picked and analyzed for hardness. The mean was calculated.

E. *Tablet Disintegration time*

Method: Introduced one tablet into each tube of Tablet disintegration tube and, added a disc to each tube. Suspended the assembly in the beaker containing the specified liquid and operate the apparatus for the specified time. Removed the assembly from the liquid. The tablets failed the test if all of them for disintegration. If 1 or 2 tablets or capsules fail to disintegrate, repeat the test on 12 additional tablets or capsules; not less than 16 of the total of 18 tablets tested disintegrate.

F. Thin Layer Chromatography

Thin layer chromatography enables qualitative analysis of phytochemical constituents of herbal drugs.

Sample preparation: Test solution: Form of (50gm) powdered drug DVA and 100ml ethanol were taken in 100ml stoppered conical flask and the mixture was kept on mechanical shaker for 6 hrs and kept for overnight. Extract was filtered & kept aside as test solution.

Procedure: Prior to development of TLC of DVA with toluene: ethyl acetate (7:3) Chamber was allowed for 30 min saturation. With capillary tube the extract was applied to silica gel 60F254 TLC plate (E Merck) of uniform thickness (0.2mm).Developed a plate in solvent system to a distance of 3 cm. After drying silica plate it was kept in saturated chamber for development of TLC. Fully developed TLC was dried with help of drier.

Visualization: The plate was observed in UV chamber at 254nm and 366nm. Later to

this Rf values were calculated with following formula;

Rf = Distance travelled by solute / Distance travelled by solvent

G. *Preliminary Phytochemical Screening*[6]**:**

Powdered sample was subjected for Cold maceration method for extraction and tests were carried out for Carbohydrates, Reducing Sugars, Monosaccharaides, pentose sugars, Non-reducing sugars, Polysaccharides, Proteins, Amino acids, steroids, Tannins and Phenolic Compounds and Alkaloids.

RESULTS

All the batches of DVA were shown similar results as colour (Brown), taste (Sweet and bitter), aromatic odour and little hard consistency. Physico-chemical analysis results and TLC profile of DVA is explained in **Table 2** and **Table 3** respectively.

Phytochemical Screening of DVA shown Presence of Reducing sugars, Mono-saccharides, non-reducing sugars, poly saccharides tannins & phenolic compounds and alkaloids were present. Pentose sugar, Protein and Amino acids were absent in all samples.

Table 2 Results of Physico-chemical analysis of DVA

Parameters	**Sample 1**	**Sample 2**	**Sample 3**
pH at 5% aqueous solution	5.20	5.2	5.2
Loss on Drying at 1100C (% w/w)	7.8	7.04	9.01
Total Ash (% w/w)	15.06	13.54	11.37
Acid Insoluble Ash (% w/w)	8.2	6.147	8.20
Water Soluble Extractive (%w/w)	14.31	14.11	14.11
Alcohol Soluble Extractive (%w/w)	11.76	11.92	11.92
Hardness kg/cm^2	11	11	12
Disintegration Time (Minutes)	40-45	40-45	45

DISCUSSION

In Ayurveda classics *Dooshivishari Agada* (DVA) is advised for allergic and subclinical or toxic conditions. This formulation has been in widely prescribed in southern part of India for above mentioned conditions. Ayurvedic Pharmacopeia of India which sets standards for drugs and formulations helps to maintain the quality assessment through various analytical parameters. Some drugs and formulations have not gained entry in pharmacopeia texts. Reasons may be several not practiced widely, different preparation method followed or standards have not been developed. In this study attempt were made to standardize.

DVA was prepared as per classical method adopting standard operating procedures. During preparation the formulation smelt similar odour of few ingredients like *Tagara* and *Jatamamsi*. Prepared

formulation had the same odour but with less intense.in this study DVA was prepared successively for three batches and analysed for Basic analytical parameters.

Table 3 TLC Profile of DVA

	R_f Value at 254 nm	R_f Value at 366 nm
Sample 1	0.087	0.063
	0.125	0.15
	0.26	0.625
	0.35	0.68
	0.4	0.75
	0.563	0.775
	0.713	0.938
	0.913	
	0.938	
Sample 2	0.125	0.15
	0.175	0.25
	0.26	0.350
	0.337	0.412
	0.487	0.562
	0.912	0.750
	0.962	0.950
		0.975
Sample 3	0.60	0.08
	0.10	0.15
	0.26	0.35
	0.62	0.71
	0.68	0.91
	0.75	0.93
	0.77	
	0.93	

Physicochemical analysis carried out in batch did not show much variation batches except in case of total ash and acid insoluble ash. Variation obtained may serve as range value for this formulation.

Phytochemical screening showed presence of carbohydrates, reducing sugars, monosaccharaides, non-reducing sugars, polysaccharides (starch), steroids, tannins and phenolic compounds and alkaloids.

Understanding the presence of drugs Thin Layer Chromatogram was run with different solvent system & the best profile was observed in Toluene and Ethyl acetate (7:3). Nearly couple of bands in TLC had R_f value as per API standards for drugs like *Pippali* (0.15, 26).Though the standard reference was not run laterally but that could be adopted and standardized.

CONCLUSION

As DVA is not popularized in practice, by adopting SOP it can be prepared and practiced. Obtained values can be taken as reference values for Quality control. The results obtained in this study did not show any variation in phytochemical screening but negligible variation in physico-chemical analysis.

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