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Comparative Physicochemical Analysis of Dushivishari Agada: An Ayurvedic Herbo-minerals Formulation W.S.R to Market Sample

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Abstract

The quality control assessment of herbo-mineral formulations is of great significance in order to justify their acceptability in modern system of medicine though the drug may be therapeutically potent. Ayurveda and its rapidly increasing use by public have given rise to many newer issues and challenges.

Ayurvedic formulations prepared by several manufactures are guaranteed to carry out the quality control test as per preliminary guidelines given by CCRAS (Central Council of Research in Ayurveda and Siddha). Though the standards are followed, still the variability in their results has been observed when compared between same formulations.

Dushivishari Agada is one such herbo-mineral formulation consisting of 13 drugs & used for treatment in Dushivisha janya vikaras.

An attempt is made here to compare Dushivishari agada prepared by GMP certified pharmacy with in-house preparation. Results revealed that the both the samples differ in their organoleptics, pH and Physicochemical properties, Thin layered chromatographic study showed sample (a) and (b) different number of bands at the wavelength of 255nm and 365nm, major difference was seen in disintegration time and hardness of sample (a) i.e., hardness is 10.4 and disintegrated in 1hr, whereas sample (b) hardness is 4.4, but disintegrated in 15min. Statistical analysis shows significant variations in both the samples. The physicochemical data of this comparative study assists in maintaining the standard limits of Dushivishari agada.

Key words: Herbo-Minerals Formulation; Physicochemical Analysis; TLC.

Introduction

In the present day Ayurveda has gained popularity globally due to its effective results witnessed in various diseases and syndromes in the recent epic.

Quality control for Ayurvedic formulations is need of the hour therefore, they are to be viewed and answered looking in the advancement of science and technology in current days. Hence, there is very much need to validate Ayurvedic formulations with the aid of sophisticated instrumental and analytical techniques.

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Dushivishari agada is one such herbo-mineral formulation explained in the Ayurvedic classics which is a combination of 13 drugs [7].

Materials and Methods

Pharmaceutical Part

Raw Material Procurement

Market sample

Two samples of Dushivishari agada, one is in house prepared and another is manufactured by GMP certified pharmacy collected from the Belgaum market and given the code as DVA (a) and DVA (b) respectively.

In-house sample preparation

Dushivishari agada comprising of 13 ingredients of which 12 are herbal and 1 is mineral. All the drugs of Dushivishari agada were collected from market

dealer and were authenticated at AYUSH approved Central Research Laboratory of KLE University's Shri B.M. Kankanwadi Ayurveda Mahavidhyalaya Belgaum, Karnataka, India. Solvents and chemicals of analytical grade were procured from E. Merck and S.D. fine chemicals, Mumbai for analysis of Dushivishari Agada.

Table 1: Ingredients of dushivishari agada

Sl. No.	Dravya	Latin Name [7]	Official part	Quantity
1.	Pippali	Piper longum Linn.	Phala (Fruit)	1part
2.	Pippalimula	Piper longum Linn.	Mula (root)	1part
3.	Dhyamaka	Cymbopogon martinii (Roxb.) Wats.	Patra (Leaves)	1part
4.	Jatamamsi	Nardostachys jatamamsi DC.(N. grandiflora)	Mula (Root)	1part
5.	Lodra	Symplocos racemosa Roxb.	Twak (Stem Bark)	1part
6.	Ela	Elettaria cardamomum Maton	Phala (Fruit)	1part
7.	Suvarchika	Tribulus terrestris Linn.	Phala (Fruit)	1part
8.	Kutanattam	Oroxylum indicum (Linn) Benth.Ex Kurz.	kanda twak (stem bark)	1part
9.	Natam	Valeriana wallichii D.C.	Mula (Root)	1part
10.	Kusta	Saussurea lappa C.B. Clarke.	Mula (Root)	1part
11.	Yastimadhu	Glycyrrhiza glabra Linn.	Mula (Root)	1part
12.	Rakhtachandana	Pterocarpus santalinus Linn. f.	Khandasara (Heartwood)	1part
13.	Gairika	Red ochre		1part

Method of preparation

Instruments and Equipment's

Weighing machine, Analytical balance, Pulverizer, Clean cotton cloth, Steel vessel, Mask, Cap, Apron, Sieve no 85 and 120, Gas and stove.

Preparation of Churna

The *Churna* (powder) was prepared as per the procedure explained in Ayurvedic Formulary of India. All drugs were made into fine powder in a pulverizer. These *churna* are passed first through 85# mesh followed by 120 # sieve individually and then all are mixed together in equal proportions to get uniformly blended homogenous mixture.

Preparation of Dushivishari agada

Step1: Gairika was subjected to shodhana according to Ayurvedic prakasha [1] by ghritha bharjana (ghritha was homemade) at KLEU's Shri B M K Ayurveda Mahavidyalaya and Research centre Rasa Shasthra laboratory.

Step2: All the individual churnas (30gm each) were mixed with gairika (30 gm) and bhavana was given. Bhavana dravya was not mentioned hence the Qwath of the same drugs. (ie the bharad which remained after pulverisation of ingredients of Dooshivishari Agada was taken).

Step3: Qwatha was prepared in classical way with ratio of 1 part drug and 8 parts water reduced to ¼ the quantity and used in QS [5].

Step 4: 5 hours Bhavana was done daily for 7 days at KLEU's Shri B M K Ayurveda Mahavidyalaya and Research centre's Bhaishajya kalpana laboratory.

Step 5: Daily observation of bhavana Dravya pH, odour, colour, taste and consistency of Agada were noted down.

Step 6: On 8th day the vatis were prepared by hand rolling and shade dried in Stainless steel Plates (dhoopana with guggulu was done before drying the rolled pills.)

Step 7: Dried Dooshivishari Agada were then kept in clean and dry sterile glass bottles.

Analytical study

Analytical study was carried out in AYUSH approved Central Research Laboratory of Shri B.M.K. Ayurveda Mahavidyalaya Belgaum. Microbial Limit Test was carried out in Microbiology Laboratory of KLE University's Shri B.M. Kankanwadi Ayurveda Mahavidhyalaya Belgaum, Karnataka, India.

Gairika was sent to Test house centre at Bangalore for quantity estimation of Fe % Dushivishari agada was subjected to following analysis [2].

The samples were analyzed for:

1. Organoleptic Characters
2. Quantitative parameters
3. Microbial limit
4. Physicochemical Properties
5. Qualitative Properties
6. TLC

Results & Discussion

Fe -11. 14 % in quantity, as per the report from Test house centre, Bangalore.

Physicochemical analysis of *Dushivishari agada* of market sample and in house preparation were carried out and the results are shown in tables. Ayurvedic formulations claimed to be made according to CCRAS guidelines are effective but it is very difficult to maintain uniformity in formulations which may be due to natural heterogeneity, the quality of herbal material obtained from wild collection shows more and more fluctuations which can be depicted from our experimental data [3].

Organoleptic study

Organoleptic observation of both samples reveal adequate differences observed in the presentation of the formulation and their taste i.e. sample (a) are handmade pills, they are round and dark brown in color and sample (b) are punched tablets indicates about addition of some binders and are light brown in color.

Physicochemical properties

Moisture content: Moisture content in a drug is an important tool for the stability of any formulation. If moisture is high, it provides healthy environment for microbial growth. Sample (a) is having more moisture content than sample (b) i.e., 12.75%w / w & 9.9% w / w respectively.

Ash value: Ash value represents amount of non-physiological components present in the drug [4]. Lesser the amount of ash, less the impurities. Sample (a) has more ash value when compared to sample (b) i.e. 14.33%w / w and 12.14% w / w.

Extractive values: Extractive value explains the amount of constituents that are extracted from a drug in a given solution. As the *Vati*s are administered along with water as a common *anupana*, maximum extraction must be observed in aqueous extract. Both samples have shown high aqueous extractive value.

pH value: pH determines acidity or alkalinity of a drug. pH for sample (a) is 4.94 which is a bit acidic compared to the sample (b) had 5.06

Physical characteristics for tablets: The physical test for tablets i.e. Weight variation test where 20 tablets are randomly selected and weighed. The mean and standard deviation was calculated. Here in this study sample (a) and (b) have shown significant difference in their weight. This might be due to the sample (a) pills prepared were handmade. The disintegration time and hardness of tablet are important tools for physical stability and absorption rate.

Both procedures must be directly proportional to each other. But in this study the hardness of sample (a) is 10.4 kg / cm3 but disintegration time is 1hr. Disintegration time should not be more than an hour, unless specified otherwise [6]. Whereas hardness of sample (b) is 4.4kg / cm3 and disintegration time is 15min. Disintegration test of both samples were done for 18 tablets. Such change affects the bioavailability of the drug to withstand in the body and show its efficacious results. But in this case as agadas were powdered and administered, therefore there is no much importance of hardness.

Phytochemical analysis

Phytochemical analyses are done to know the presence of functional group, which play a vital role in expression of therapeutic efficacy. Both the samples showed presence of Carbohydrates, reducing sugars, glycosides, steroids, alkaloids and tannins & phenols shown in table 9.

Qualitative analysis

TLC study is carried out on 60F254 pre-coated TLC plates under the solvent system Toulene and Ethyle acetate in the ratio of 7:3 after various trial and errors. Ethanol extracts of all the samples have been taken and visualized under UV light chamber at the range of 255nm and 365nm. This parameter gives idea about qualitative estimation presence of various components of drugs. Results of TLC are shown in table 6. Sample (a) has shown highest number of

Table 2: Organoleptic characters

Samples	DVA(a)	DVA(b)
Colour	Dark brown	Light brown
Odour	Characteristic	Characteristic
Taste	Sweet, Bitter	Bitter
Form	Round pills	Punched tablet

Table 3: Statistical analysis of weight variation test

Samples	DVA(a)	DVA(b)
Number of Samples	20	20
Mean	0.522gms	0.67gms
S.D	0.3908	0.2291
S.E.M	0.0873	0.0512

S.D* – Standard deviation; S.E.M – Standard error mean

Table 4: Determination of tablet disintegration and hardness

Samples	DVA(a)	DVA(b)
Hardness*	10.4kg/cm^2	4.4 kg/cm^2
Disintegration time**	1hr	15min

Monsanto's Hardness Tester
**Tablet Disintegration Apparatus - Solution – Distilled water, Oscillations 30/min, Temperature-39°C.

Table 5: Microbial limit test

Sl.No	Microbial organisms	Limit as per IP	DVA(a)	DVA(b)
1	Escherichia coli	Absent	Absent	Absent
2	Staphylococcus aureus	Absent	Absent	Absent
3	Pseudomonas aeruginosa	Absent	Absent	Absent
4	Salmonella abony	Absent	Absent	Absent

Table 6: Determination of moisture content, ash values and extractive values

Samples	DVA(a)	DVAb)
Moisture content	12.75% W/W	9.9% W/W
Total Ash	14.33%W/W	12.14%W/W
Acid Insoluble Ash	2.45 %W/W	0.975% W/W
Alcoholic Extract	24.56%W/W	11.2%W/W
Aqueous Extract	42.16%W/W	18.4%W/W
pH	4.94	5.06

Table 7: Qualitative parameters of dushivishari agada

Sl.No	Parameters	DVA(a)	DVA(b)
1	Carbonate	–	–
2	Calcium	–	–
3	Magnesium	–	–
4	Potassium	–	–
5	Iron	Present	Present
6	Sulphate	Present	Present
7	Chloride	Present	–
8	Nitrate	Present	–
9	Sodium	Present	Present

Table 8: Preliminary phytochemical screening

Sl.No	Parameters	test	DVA(a)		DVA(b)	
			Aqueous , Alcohol		Aqueous , Alcohol	
1	Carbohydrates	Molish	Present	Present	–	Present
2	Reducing Sugar	Benedicts	Present	Present	Present	Present
3	Monosaccharides	Barfords	Present	–	Present	–
4	Pentose	Bails	–	–	–	–
5	Hexose	Selwinoffs	–	–	–	–
6	Non-reducing sugar	Benedicts	–	–	–	–
7	Polysaccharide	Iodine test	–	–	–	–
8	Proteins	Millons test	–	–	–	–
9	Amino Acids	Ninhydrin test	–	–	–	–
10	Steroids		Present	–	–	Present
11	Glycosides	Cardiac Glycosides	Present	–	–	–
12	Saponins		Present	–	Present	–
13	Flavonoids		Present	Present	–	Present
14	Alkaloids	Dragandroff's	Present	Present	–	Present
15	Tannins & phenolic		Present	Present	Present	Present

Table 9: Determination of TLC study

Samples	Wavelength	RF Value
DVA(a)	254	0.18, 0.0.22, 0.28, 0.32, 0.36, 0.38, 0.42, 0.46, 0.55, 0.63, 0.69
	366	0.14, 0.21, 0.25, 0.28, 0.30, 0.34, 0.39, 048, 0.55, 0.67, 0.76, 0.84, 0.90
DVA(b)	254	0.07, 0.12, 0.16, 0.21, 0.25, 0.29, 0.35, 0.38, 0.43, 0.48, 0.53, 0.58, 0.64
	355	0.07, 0.11, 0.16, 0.20, 0.24, 0.30, 0.38, 0.43, 0.47, 0.73

Samples
Ethanol Extract of Dushivishari agada sample (a) & (b)

Solvent System

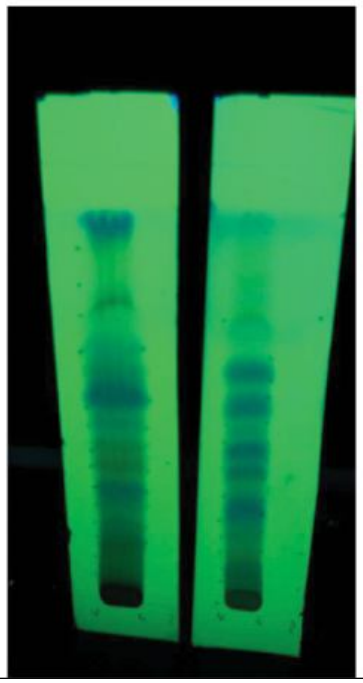
Mobile phase
Toulene: Ethyl Acetate [7 : 3]

Stationary phase
Pre-coated thin films of Silica plates

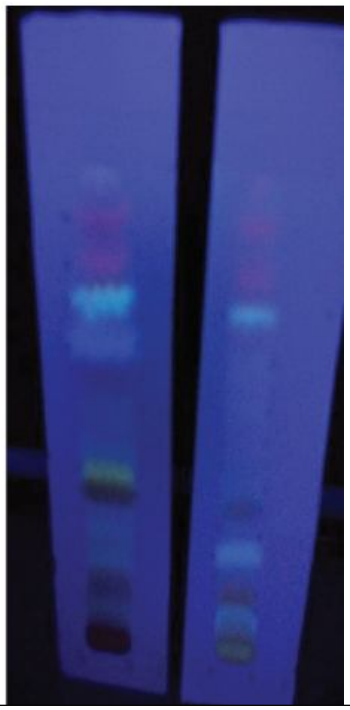
Development
Stepwise in Stahl chamber

Visualization
Under UV chamber
Short wave – 254nm
Long wave – 366nm

Sample (a) & (b)
Short wave 254nm



Sample (a) & (b)
Long wave 356nm



bands i.e. at 255 nm 11 bands and at 365nm 13bands. When compared sample (b).

Microbial limit test

Microbial limit test was carried out for all the samples and the results revealed that all samples were within the limits as per Indian Pharmacopeia Standards.

Conclusion

*Dushiv*ishari agada a herbo-mineral formulation used for various ailments. The ailments like bhinnapurisha varna (loose motins), murcha (unconscious), gadgada vak (stammering), vichitra vikara (various ailments) which are seen in Dushivisha condition**.** Being the same formulation prepared by various manufacturers yet there is difference observed when markets sample and in house preparation are compared through standard quality control parameters as per Ayurvedic Pharmacopeia of India. Hence, it is the need of hour that the Ayurvedic formulations should be standardized in order to make them potent and therapeutically efficient.

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