Research Article

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CHEMICAL ANALYSIS OF DUSHIVISHARI AGADA: AN AYURVEDIC HERBO-MINERAL FORMULATION TO COMBAT RESIDUAL TOXICITY

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Received on: 04/09/15 Revised on: 13/10/15 Accepted on: 22/11/15

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DOI: 10.7897/2277-4343.07133

ABSTRACT

Quality assessment of herbo-mineral formulations is having huge importance in order to justify their acceptability in modern system of medicine. In the present context, herbo-mineral preparations are having a vital role in health care systems, because these drugs are easily available at low cost, safe and people have faith in them. Dushivishari Agada is an Ayurvedic herbo-mineral formulation comprising of Pippali (*Piper longum* Linn.), Dhyamaka (*Cymbopogon martinii* (Roxb.) Wats.), Jatamamsi (*Nardostachys jatamamsi* (D.Don) DC.), Lodhra (*Symplococus racemosa* Roxb.), Ela (*Elettaria cardamomum* Maton.), Suvarchika (*Salt petre*), Kutannatum (*Oroxylum indicum* (L.) Benth. ex Kurz), Natam (*Valeriana wallichii*), Kushta (*Saussurea lappa* DC.), Yastimadhu (*Glycyrrhiza glabra* L.), Chandana (*Santalum album* L.) and Gairika (Red Ochre Fe_2O_3) and is used to combat residual toxicity. Keeping above facts in mind it is aimed to standardize Dushivishari Agada employing standard testing protocol for AYUSH drugs. Physico-chemical studies like loss on drying at 105ºC, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive; total fat, crude fibre, total carbohydrate, total protein and HPTLC were carried out as per the WHO guidelines, Ayurvedic Pharmacopoeia and Indian Pharmacopoeia. Quality indicating physical and chemical tests was done and standard values for Dushivishari Agada were recorded. Standardization tests done on Dushivishari Agada helped in authenticating the polyherbal preparation and also in ensuring the quality of the same.

Keywords: Dushivishari Agada, Pippali, Dhyamaka, Jatamamsi, Lodhra.

INTRODUCTION

The subject matter of herbal and herbo-mineral drug standardization is very wide and deep.[1] For the purpose of research work on standardization of herbal and herbo-mineral formulations, a deep knowledge of the important herbs and minerals found in India and widely used in Ayurvedic formulation is very much essential.[2] India can surface as the major country and play the lead role in production of standardized, therapeutically effective Ayurvedic formulations if we can explore the medicinally important plants and minerals employing modern techniques of standardization.[3] World Health Organisation's specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines are of extreme importance.[4] In spite of these various efforts by WHO, there are not many studies supporting their scientific assessment. The present study is aimed to lay down pharmacopoeial standard for Dushivishari Agada[5] (DA) extremely useful in latent poisoning and its complications, insect poisoning and generalized itching.

DA is an Ayurvedic herbo-mineral formulation comprising of Pippali (*Piper longum* Linn.), Dhyamaka (*Cymbopogon martinii* (Roxb.) Wats.), Jatamamsi (*Nardostachys jatamamsi* (D.Don) DC.), Lodhra (*Symplococus racemosa* Roxb.), Ela (*Elettaria cardamomum* Maton.), Suvarchika (Salt petre), Kutannatum (*Oroxylum indicum* (L.) Benth. ex Kurz), Natam (*Valeriana wallichii*), Kushta (*Saussurea lappa* DC.), Yastimadhu (*Glycyrrhiza glabra* L.), Chandana (*Santalum album* L.) and Gairika (Red Ochre).[6] Keeping these views in mind, DA was subjected for standardization to ensure quality and also to authenticate the preparation.

MATERIALS AND METHODS

Plant material

The ingredients of DA (Figure 1, Table 1) were collected from the local market of Thrissur district, Kerala state, India. The collected drugs were identified and authenticated at the teaching pharmacy of Department of Dravyaguna, SDM College of Ayurveda and Hospital, Hassan, Karnataka state, India.

Physico-chemical standardization

Physico-chemical studies like uniformity of weight, hardness test, diameter, disintegration time and HPTLC were carried out as per the WHO guidelines,[7] Ayurvedic Pharmacopoeia[8] and Indian Pharmacopoeia.[9]

High Performance Thin Layer Chromatography

Two tablets of DA were crushed and extracted with 10 ml of alcohol by cold percolation method. 4, 8 and 12 µl of the extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in toluene: ethyl acetate: acetic acid (8: 2:

0.1). The developed plates were visualized in UV 254, 366 nm and after derivatisation with vanillin-sulphuric acid and scanned under UV 254, 366 nm and 620 nm. R_f, color of the spots and densitometric scan were recorded.[10]

The studies were done 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 standard procedure (Sample code: 13070101).

RESULTS AND DISCUSSION

Standardization tests performed for DA were as per AYUSH testing protocol. DA is found to be light reddish brown in color with no characteristic odor and has pungent taste (Table 2). The %w/w for physio-chemical parameters uniformity of weight, hardness, diameter, disintegration time was 460-570 mg, 4, 10 and more than 1 h respectively (Table 2). TLC photo-documentation of DA showed 14, 8 and 10 spots under 254 nm, 366 nm and under white light after derivatisation respectively. Spot with R_f 0.09, 0.29, 0.76 and 0.81 were commonly detected in all the three detection methods. All the three methods gave optimum separation of different bands and hence all of them may be used as TLC fingerprint pattern to identify the composition of DA (Table 3). Densitometric scan at 254 nm revealed 3 high peaks corresponding to 3 different compounds in the ethanol extract, compounds with R_f 0.02 (13.78%), 0.38 (15.58%) and 0.54 (23.46%) were the peaks (Table 3, Figure 3). At 366 nm there were three high peaks, with R_f 0.11 (13.75%), 0.78 (28.42%) and 0.38 (9.50%) being the major peaks detected (Figure 3 and 4). These physico-chemical constants, TLC photo documentation, the unique R_f values and densitogram obtained at different wavelengths can be used as fingerprint to identify DA. HPTLC is an important tool in standardisation and quality control of polyherbal formulations. As there is more than one ingredient qualitative HPTLC fingerprinting can be used for development of quality standards for polyherbal formulations.[11,12]

Table 1: Ingredients of Dushivishari Agada

Ingredients	Botanical source
Pippali	*Piper longum* Linn.
Dhyamaka	*Cymbopogon martinii* (Roxb.) Wats.
Jatamamsi	*Nardostachys jatamamsi* (D.Don) DC.
Lodhra	*Symplococus racemosa* Roxb.
Ela	*Elettaria cardamomum* Maton.
Suvarchika	Salt petre
Kutannatum	*Oroxylum indicum* (L.) Benth. ex Kurz
Natam	*Valeriana wallichii*
Kushta	*Saussurea lappa* DC.
Yastimadhu	*Glycyrrhiza glabra* L.
Chandana	*Santalum album* L.
Gairika	Red ochre (Fe_2O_3)

Table 2: Pharmacopoeial parameters for Dushivishari Agada

Parameters	Result n = 3 (% w/w)
Colour	Light reddish brown
Odour	Not characteristic
Average weight (10 tablets) mg	508.1
Uniformity of weight	(460 – 570 mg)
Hardness test (kg/cm)	4
Disintegration time (min)	More than 1 hr
Diameter (mm)	10

Figure 1: Dushivishari Agada

Table 3: R_f values at different wavelength

At 254 nm	At 366 nm	Post derivatisation
		0.03 L pink
0.05 Green		
0.09 Green	0.09 F L blue	0.09 L pink
0.11 Green		0.11 L pink
	0.15 F L blue	0.15 L pink
0.17 Green		
	0.20 F red	
		0.22 Yellow
0.24 D green	0.24 F L blue	
0.29 Green	0.29 F L blue	0.29 L pink
0.33 D green		
0.42 Green		
0.47 D green		
		0.53 Violet
0.56 Green		
		0.60 Violet
0.62 Green		
0.70 Green	0.70 F L blue	
0.76 Green	0.76 F red	0.76 Violet
0.81 Green	0.81 F red	0.81 Violet

L: light; F: fluorescent; D: dark

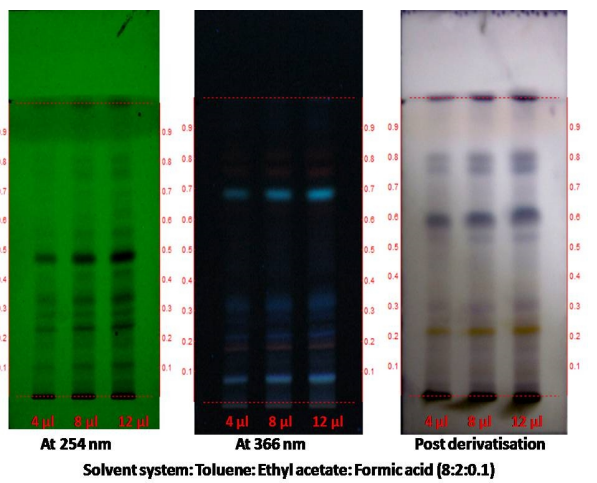
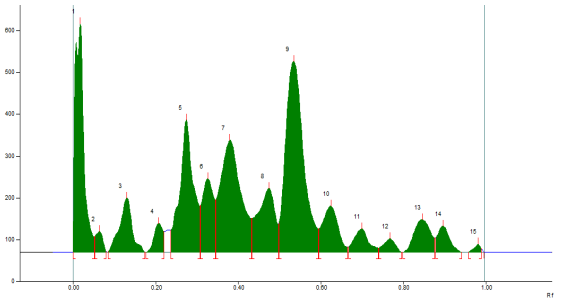


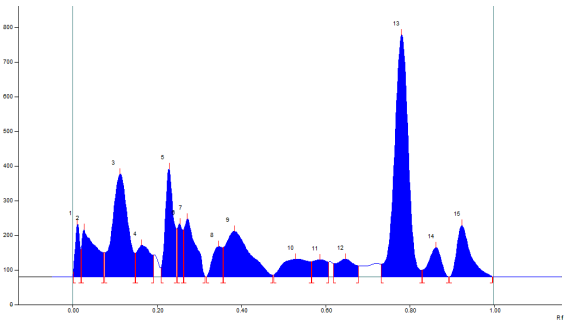
Figure 2: TLC photo documentation of Dushivishari Agada



Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	547.1 AU	21.70 %	0.05 Rf	36.8 AU	8345.0 AU	13.78 %
2	0.05 Rf	37.5 AU	0.06 Rf	49.3 AU	1.95 %	0.08 Rf	1.5 AU	610.7 AU	1.01 %
3	0.09 Rf	1.0 AU	0.13 Rf	129.9 AU	5.15 %	0.17 Rf	0.3 AU	2795.3 AU	4.62 %
4	0.18 Rf	0.2 AU	0.21 Rf	69.1 AU	2.74 %	0.22 Rf	49.1 AU	1063.2 AU	1.76 %
5	0.24 Rf	52.2 AU	0.28 Rf	316.6 AU	12.56 %	0.31 Rf	10.1 AU	7478.5 AU	12.35 %
6	0.31 Rf	112.2 AU	0.33 Rf	176.4 AU	7.00 %	0.35 Rf	25.0 AU	3423.9 AU	5.65 %
7	0.35 Rf	125.3 AU	0.38 Rf	267.3 AU	10.60 %	0.43 Rf	81.0 AU	9431.1 AU	15.58 %
8	0.43 Rf	81.1 AU	0.48 Rf	152.4 AU	6.04 %	0.50 Rf	66.5 AU	4485.8 AU	7.41 %
9	0.50 Rf	70.6 AU	0.54 Rf	456.0 AU	18.09 %	0.60 Rf	55.3 AU	14202.9 AU	23.46 %
10	0.60 Rf	56.1 AU	0.63 Rf	110.0 AU	4.36 %	0.67 Rf	12.7 AU	2961.1 AU	4.89 %
11	0.67 Rf	12.9 AU	0.70 Rf	55.3 AU	2.19 %	0.74 Rf	9.8 AU	1386.1 AU	2.29 %
12	0.74 Rf	9.8 AU	0.77 Rf	32.2 AU	1.28 %	0.80 Rf	0.0 AU	643.0 AU	1.06 %
13	0.80 Rf	0.3 AU	0.85 Rf	78.0 AU	3.09 %	0.88 Rf	33.7 AU	2225.9 AU	3.68 %
14	0.88 Rf	34.3 AU	0.90 Rf	61.9 AU	2.46 %	0.94 Rf	0.0 AU	1289.1 AU	2.13 %
15	0.96 Rf	0.3 AU	0.98 Rf	19.5 AU	0.77 %	0.99 Rf	9.3 AU	205.9 AU	0.34 %

Figure 3: HPTLC Densitometric scan at 254 nm



Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	13.5 AU	0.01 Rf	150.4 AU	5.78 %	0.02 Rf	77.0 AU	1164.6 AU	2.08 %
2	0.02 Rf	80.7 AU	0.03 Rf	137.4 AU	5.28 %	0.07 Rf	69.6 AU	3290.8 AU	5.88 %
3	0.08 Rf	69.8 AU	0.11 Rf	296.7 AU	11.39 %	0.15 Rf	67.9 AU	7700.5 AU	13.75 %
4	0.15 Rf	68.7 AU	0.16 Rf	91.2 AU	3.50 %	0.19 Rf	62.4 AU	2109.8 AU	3.77 %
5	0.21 Rf	26.8 AU	0.23 Rf	311.8 AU	11.97 %	0.25 Rf	37.7 AU	4286.1 AU	7.65 %
6	0.25 Rf	137.7 AU	0.25 Rf	152.1 AU	5.84 %	0.26 Rf	33.7 AU	1425.3 AU	2.55 %
7	0.26 Rf	136.1 AU	0.27 Rf	166.3 AU	6.39 %	0.31 Rf	1.3 AU	3118.1 AU	5.57 %
8	0.32 Rf	0.3 AU	0.35 Rf	86.9 AU	3.34 %	0.36 Rf	84.3 AU	1452.3 AU	2.59 %
9	0.36 Rf	84.9 AU	0.38 Rf	131.2 AU	5.04 %	0.48 Rf	5.4 AU	5321.9 AU	9.50 %
10	0.48 Rf	5.8 AU	0.53 Rf	50.8 AU	1.95 %	0.57 Rf	44.0 AU	2252.1 AU	4.02 %
11	0.57 Rf	44.1 AU	0.59 Rf	49.5 AU	1.90 %	0.61 Rf	42.6 AU	1215.5 AU	2.17 %
12	0.62 Rf	38.0 AU	0.65 Rf	51.3 AU	1.97 %	0.68 Rf	32.2 AU	1600.0 AU	2.86 %
13	0.73 Rf	37.1 AU	0.78 Rf	696.3 AU	26.74 %	0.83 Rf	19.4 AU	15913.5 AU	28.42 %
14	0.83 Rf	19.7 AU	0.86 Rf	84.2 AU	3.23 %	0.89 Rf	0.7 AU	1746.4 AU	3.12 %
15	0.90 Rf	0.2 AU	0.92 Rf	148.0 AU	5.68 %	1.00 Rf	0.7 AU	3402.9 AU	6.08 %

Figure 4: HPTLC Densitometric scan at 366 nm

CONCLUSION

The constituents of DA such as Pippali (*Piper longum* Linn.), Dhyamaka (*Cymbopogon martinii* (Roxb.) Wats.), Jatamamsi (*Nardostachys jatamamsi* (D.Don) DC.), Lodhra (*Symplococus racemosa* Roxb.), Ela (*Elettaria cardamomum* Maton.), Suvarchika (Salt petre), Kutannatum (*Oroxylum indicum* (L.) Benth. ex Kurz), Natam (*Valeriana wallichii*), Kushta (*Saussurea lappa* DC.), Yastimadhu (*Glycyrrhiza glabra* L.), Chandana (*Santalum album* L.) and Gairika (Red Ochre) are endowed with various biological properties and hence this herbo-mineral formulation prepared from these ingredients will have combined integrity of all the individual constituents. In spite of the advent of modern technology in standardization of Ayurvedic formulations, only a few are standardized so far. In the present work, standardization of Dushivishari Agada carried out using physicochemical, phytochemical studies and HPTLC finger print profiles for the quality control of raw material, processed vati. The purpose of standardization of medicinal plants is to ensure therapeutic efficacy and quality thereby ensuring batch to batch consistency of Ayurvedic medicines. The results obtained through this study were quick, reproducible and could be used for routine monitoring of raw material.

ACKNOWLEDGEMENT

Authors are highly grateful to the constant support of Dr. Girish K J, Research Co-ordinator, SDM College of Ayurveda and Hospital, Hassan, Karnataka, India. The Authors thank Dr. B. Ravishankar, Director, SDM Centre for Research in Ayurveda and Allied Sciences for providing the facilities for their guidance. The authors also thank all the faculty members of Department of Agada Tantra, SDM College of Ayurveda and Hospital, Hassan, Karnataka, India for their continuous support.

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Cite this article as:

Sreejith GS, Ittoop J Ancheril, Sunil Kumar KN, Anitha MG, Arun Raj GR. Chemical analysis of Dushivishari agada: An Ayurvedic herbo-mineral formulation to combat residual toxicity. Int. J. Res. Ayurveda Pharm. Jan – Feb 2016;7(Suppl 1):80-83 http://dx.doi.org/10.7897/2277-4343.07133

Source of support: Nil, Conflict of interest: None Declared