Standardization of 'Dashamularishta': A Polyherbal Formulation

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ABSTRACT

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. The present work is an attempt to standardize Dashamularishta, a traditional formulation, used in the normalization of physiological processes after child birth. Four marketed preparations and three in-house preparations were used for the study. The various parameters performed included organoleptic characteristics, physicochemical and toxicological parameters. HPTLC was carried out for quantitative analysis of piperine in all the formulations. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organizations and manufacturers for developing standards.

Keywords: Dashamularishta, standardization, botanical parameters, physico-chemical parameters, toxicological parameters

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INTRODUCTION

Standardization is an essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principles (1). It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical location. The increasing demand of the population and the chronic shortage of authentic raw materials have made it incumbent, so there should be some sort of uniformity in the manufacture of Ayurvedic medicines so as to ensure quality control and quality assurance. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.

The present study was aimed at standardizing Dashamularishta, a traditional polyherbal formulation, which is used for the normalization of physiological processes after child birth. It is also used in treating fatigue, removing dead cells from the body, in regeneration of the cells, sedative and tonic (2). It consists of ten main herbs, collectively called as Dashamula class and thirty five other herbs, collectively called as general class of herbs (Tables 1A and B).

MATERIALS AND METHODS

Drug Samples and Method of Preparation (3, 4)

The crude drugs used in preparation of Dashamularishta were collected from local market, Indore and identified in Department of Botany, Government Agriculture College, Indore. Three in house formulations were prepared, with varying concentrations of one of the ingredient, Dhataki, as per the procedure mentioned in Ayurvedic text "Ayurveda Sar Sangrah" and four marketed formulations of different manufacturers were procured. The Dashamula class of herbs were taken in equal proportions (18.30 gm each) and powdered coarsely, then to it the general class herbs (7.32 gm each) were added in powdered form, and prescribed amount (5.5 Ltr) of water was added i.e. 8 times the weight of the herbs taken and kept for boiling until one-fourth (1.5 Ltr) of the decoction was left. Simultaneously jaggery was prepared by taking the prescribed quantity (0.885 gm) of munnaka dissolved in water 4 times (3.5 Ltr) of its weight and was kept on boiling until three-fourth (2.6 Ltr) of its decoction was left. When both decoctions were prepared, they were transferred to

Table I-A:. Dashamula class herbs

S.No	Botanical Name	Common Name
1	*Aegle marmelous*	Bael
2	*Oryxlum indicum*	Sonpataha
3	*Premna mucronata*	Arani
4	*Sterospermum suaveolens*	Patala
5	*Tribulus terristris*	Gokhru
6	*Desmodium gangeticum*	Shalparni
7	*Solanum xanthocarpum*	Choti kateli
8	*Uracaria picta*	Prishparni
9	*Solanum indicum*	Badi kateli
10	*Gmelina arborea*	Gambhir

Table I-B:. General Class of herbs

1	*Plumbago zeylanica*	Chitrak
2	*Inula racemosa*	Puskar moola
3	*Sympiocos racemosa*	Sodhra
4	*Tinospora cordifolia*	Giloy
5	*Acacia catechu*	Khadir
6	*Pterocarpus marsupium*	Vijayasar
7	*Terminalia chebula*	Harad
8	*Terminalia belerica*	Bahera
9	*Sauassurea costus*	Kuth
19	*Cedrus deodara*	Deodar
11	*Embelia ribe*	Vidanga
12	*Piper cubeba*	Kebab chini
13	*Santalum album*	Chandan
14	*Myristica fragrans*	Jaiphal
15	*Asparagus racemosus*	Shatavari
16	*Pueraria tuberose*	Vidarikand
17	*Withania somnifera*	Ashwagandha
18	*Woodfordia fruticosa*	Dhakati
19	*Cinnamomum zeylanicum*	Dalchini
20	*Cinnamomum tamala*	Tejpatra
21	*Eletlaria cardamom*	Badi elachi
22	*Piper longum*	Pipali
23	*Hemidesmus indicus*	Sariva
24	*Mesua ferrea*	Nageskeshwar
25	*Callicarpa marcophyll*	Priyangu
26	*Carum carvi*	Kalazeera
27	*Ipomoea turpethum*	Nisoth
28	*Vitex negundo*	Renuka
29	*Pluchea Lanceolata*	Rasna
30	*Areca catechu*	Supari
31	*Cyperus rotundus*	Nagar motha
32	*Cyperus seariosus*	Jeevak
33	*Pistacia Iintegerrima*	Kakadsingi
34	*Jaggery*	Guda
35	*Vitis vinifera*	Munnaka

three earthen pots in equal amount. To each earthen pot Dhataki was added in different concentration, then other supportive ingredients such as Guda (1400 gm), Pipali, Badi elachi etc were added to it and then it was packed tightly and kept beneath the earth for the period of one month.

The coding of the marketed and in-house formulations was done as follows:

Marketed Preparations

Code	Manufacturer
SD1	Sandu Pharmaceuticals
SD2	Baidyanath Pvt. Ltd.
SD3	Dabur India Ltd.
SD4	Vyas Pharmaceuticals

In-house Preparations

SD5	containing Dhataki + 10% of the prescribed quantity
SD6	containing prescribed amount of Dhataki
SD7	containing Dhataki – 10% of the prescribed quantity

Botanical Parameters (5)

Organoleptic evaluation was carried out to assess the color, odor and taste of the marketed and in-house formulations.

Determination of total solid content(6)

10 ml of the samples were taken in tared dish and evaporated at low temperature until the liquid was removed and then heated until the residue was apparently dried. Thereafter, it was transferred to an oven and dried to constant weight at 105°C.

Determination of specific gravity

The specific gravity was measured using the standard procedure (7) using a pycknometer.

Determination of viscosity (8)

Viscosity was determined with the help of Ostwald's viscometer.

Determination of alcohol content

25ml of the sample was taken in a distillation flask. Then it was diluted with 150 ml of water and little pumice powder was added to it, distillation head and condenser were attached. 90 ml of distillate was collected in a 100 ml volumetric flask and cooled to 25°C. The volume was adjusted to 100 ml. Then the specific gravity of the sample

was measured and then alcohol content was determined as per the table given in I .P. (9)

Determination of sugar content (10)

Sucrose (0.475g) was dissolved in 250ml of distilled water. It was converted into invert sugar, by adding conc. HCl (2ml) to it and boiling gently for 30min. The solution was kept on boiling water-bath for about 2h. and neutralized with sodium carbonate. The neutralized solution was diluted up to 500 ml. 5ml of each sample i.e. SD1, SD2, SD3, SD4, SD5, SD6 and SD7 were taken and to each 25 ml of water was added, followed by 2 ml HCl and boiled for 2 hrs. Then it was filtered and the filtrate was collected and neutralized with sodium bicarbonate and the volume was made up to 250 ml. Fehling's solution was prepared freshly every time, by mixing equal volumes of Fehling's A and B. 10ml of Fehling's solution was taken in porcelain evaporating basin and diluted with equal volume of distilled water. The solution was allowed to boil, and titrated against standard invert sugar solution until the blue color entirely disappeared. Then the solution was allowed to cool till the precipitate of cuprous oxide was settled and the solution was boiled again until the end-point was approached. 5ml of sample was dissolved in water and diluted upto 250 ml, and titrated against 25ml. of the standard Fehling's solution.

Determination of pH (11)

The pH of the all the seven formulation SD1, SD2, SD3, SD4, SD5, SD6 and SD7 was determined with the help of pH meter.

Determination of refractive index (12)

It was determined with the help of Abbes Refractometer.

Determination of acid value (13)

10 ml of sample was taken and dissolved in 50 ml of equal mixture of solvent ether and alcohol. This solution was titrated with 0.1 N NaOH, 1 ml Phenolphthalein was added as indicator and was titrated until the solution remained faintly pink after shaking for 30 sec.

The acid-value of sample was calculated by following formula

$$\text{Acid value} = \frac{n \times 5.61}{w}$$

n = the number of ml of 0.1 N sodium hydroxide required

w = the weight in g of the substance

Quantitative determination of piperine by HPTLC (14)

Chromatographic Condition

- Test plate: HPTLC pre-coated plates (25 TLC Aluminum sheets), silica gel 60 F_{254} (Merck KgaA, 64271 Darmstadt, Germany)
- Format: 10 × 20 cm
- Thickness: 200µm
- Application mode: CAMAG Linomat IV applicator
- Development chamber: CAMAG Twin Trough Chamber
- Densitometric scanning: CAMAG TLC Evaluation software CATS 3.20.
- Detection: Deuterium lamp
- Measurement mode: Absorption/Reflection
- Wavelength : 254 nm
- Solvent System: Acetone: n Hexane 3: 7

Sample and standard (piperine) were prepared as per the standard procedure.

Procedure

The plates were pre-washed with methanol before spotting. Standard and sample solutions were applied to the plates as sharp bands by means of Linomat IV sample applicator. The spots were dried in a current of air. 20 ml of mobile phase was poured into one trough of the Camag twin trough glass chamber and the plate was placed in another trough of the chamber. The whole assembly was left to equilibrate for 30 min. The plates was then developed until the solvent front had traveled a distance of 80 mm above the base of plates. The plate was then removed from the chamber and dried in current of air. Detection and quantification was performed with Camag TLC scanner II at wavelength of 254 nm for evaluation of data.

Quantification of active ingredient

Quantification of active ingredient was done by comparing the peak areas and R_f values of standard piperine with the sample and the % was calculated accordingly.

Microbial contamination (15)

The samples were tested for the presence of microorganisms like *E.coli, Salmonella, Pseudomonas aeruginosa and Staphylococcus aureus*

Heavy metal analysis (16)

To 3 ml of the sample, 10 ml water, 2 ml Hydrochloric acid and 2 ml Nitric acid was added and boiled for 10 minutes

The mixture was cooled down and volume made up to 100 ml with water. 0.1N Nitric acid was used as blank. The samples were detected for presence of heavy metals like lead, copper, arsenic and mercury.

RESULTS AND DISCUSSION

All the formulations of Dashamularishta were evaluated as per WHO guidelines. Botanical parameters revealed that the formulations were red to reddish brown in color, with pleasant odor and sour taste (table II). The values for percentage of total solid content, specific gravity, viscosity, refractive index, acid value, alcohol content, sugar content and pH in all formulations of Dashamularishta are presented in table III. Alcohol content in the in-house formulation having prescribed amount of Dhataki was found to be 11.0 %. Formulation containing more than prescribed amount of Dhataki contained 22.0 % of alcohol and formulation containing less amount of Dhataki showed no alcohol production. Dhataki is the component responsible for causing fermentation and generation of alcohol in any arishtha preparation (15). The concentrations of Dhataki were varied in the three in-house formulations to observe its effect in generation of alcohol. The results obtained revealed that the amount prescribed in Ayurveda Sar Sangrah is optimum for generation of alcohol. In HPTLC study, the R_f value of standard Piperine was found to be 0.37 where as R_f values of piperine and its quantity in all the formulations were found to be in the range of 0.36–0.38 and 0.11–0.12% respectively (Table IV). The peaks obtained for the formulations matched with that of the standard piperine thereby confirming the presence of piperine in all the samples of Dashamularishta (fig. 1–8).

Various microorganisms like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* contaminate herbal drugs and cause serious health hazards (16). For detection of such microorganisms, colonies obtained on specific media were subjected to suitable microbial tests along with pure strains to detect their presence or absence. The results obtained (Table V) revealed the absence of these microorganisms thereby confirming the non toxic nature of Dashamularishta. Heavy metals may be present in crude drugs through atmospheric pollution and through the soil. Moreover minerals and metals are also used in preparing Ayurvedic formulations. However, heavy metals have been associated with various adverse effects (17) including status epilepticus, fatal infant encephalopathy, hepatotoxicity, congenital paralysis and deafness, and developmental delay. Many case studies have reported serious adverse conditions due to heavy metals in Ayurvedic and other herbal drugs (18). Hence, heavy metals need to be detected in such preparations. In this study, all the samples tested negative for the presence of heavy metals (Table VI), thereby further confirming the non toxic nature of the preparation. Hence, Dashamularishta is a safe polyherbal formulation and is free from any toxic materials,

Table II: Botanical evaluation of marketed and in-house formulations of Dashamularishta

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Color	Dark brown	Dark reddish brown	Dark reddish brown	Dark brown	Dark brown	Dark brown	Dark brown
Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Taste	Sour	Sour	Sour	Sour	Sour	Sour	Sour

Table III: Physico-chemical evaluation marketed and in-house formulations of Dashamularishta

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Total solid content	11.42 ± 0.03	16.61 ± 0.02	11.96 ± 0.01	11.78 ± 0.01	12.15 ± 0.00	12.15 ± 0.00	12.63 ± 0.02
Specific gravity	1.07 ± 0.01	1.08 ± 0.00	1.04 ± 0.29	1.11 ± 0.001	1.02 ± 0.02	1.07 ±.006	1.01 ± 0.01
Viscosity	2.90 ± 0.02	2.76 ± 0.02	3.87 ± 0.01	4.18 ± 0.00	2.74 ± 0.01	2.80 ± 0.04	2.83 ± 0.03
Refractive index	1.410 ± 0.001	1.424 ± 0.004	1.413 ± 0.00	1.413 ± 0.01	1.443 ± 0.003	1.433 ± 0.02	1.393 ± 0.003
Alcohol content	12 ± 0.00	10.00 ± 0.33	11.00 ± 0.33	11.00 ± 0.00	22.00 ± 0.33	12.00 ± 0.00	0.00
Sugar content	20.91 ± 0.05	23.48 ± 0.14	19.53 ± 0.13	26.30 ± 0.29	19.17 ± 0.08	20.83 ± 0.01	21.40 ± 0.05
Acid value	3.03 ± 0.00	3.12 ± 0.05	2.96 ± 0.17	2.91 ± 0.25	2.94 ± 0.00	2.80 ± 0.07	2.01 ± 0.18
pH	4.16 ± 0.03	3.91 ± 0.03	4.26 ± 0.00	4.18 ± 0.01	4.83 ± 0.09	4.11 ± 0.04	3.73 ± 0.05

Values are means ± SEM of three experiments.

Table IV: Screening for micro-organisms in marketed and in-house formulations of Dashamularishta

Formulation Code → Name of Microbes↓	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Escherichia coli	–	–	–	–	–	–	–
Salmonella typhi	–	–	–	–	–	–	–
Pseudomonas aeruginosa	–	–	–	–	–	–	–
Staphylococcus aureus	–	–	–	–	–	–	–

'–' indicates absence.

Table V: Heavy metal analysis of marketed and in-house formulations of Dashamularishta

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Arsenic	–	–	–	–	–	–	–
Lead	–	–	–	–	–	–	–
Mercury	–	–	–	–	–	–	–
Copper	–	–	–	–	–	–	–

'–' indicates absence.

Table VI: Quantitative estimation of piperine in marketed and in-house formulations of Dashamularishta by HPTLC

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
R_f Values	0.36	0.37	0.37	0.37	0.36	0.38	0.39
Amount of Piperine	0.11%	0.11%	0.11%	0.11%	0.12%	0.12%	0.12%

generally associated with polyherbal preparations. The results obtained in this study may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standards for the preparation.

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