

Standardization Study of Dadimastaka and Pushyanuga Churnas

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The present paper deals with the standardization of dadimastaka and pushyanuga churnas. These are the important Ayurvedic formulations used for perinatal care of mother and child health. Standardization of churnas were achieved by organoleptic study, physico-chemical analysis, qualitative organic and inorganic analysis, thin layer chromatography (TLC), UV-visible spectrophotometry and high performance liquid chromatographic (HPLC) fingerprint studies. Qualitative organic analysis of both the churnas revealed the presence of alkaloids, steroids, phenols, tannins, glycosides, resins, saponins and flavonoids. Qualitative inorganic analysis revealed the presence of calcium, magnesium, iron and silica in dadimastaka churna and aluminium, calcium, magnesium, iron and silica in pushyanuga churna. TLC study of both the churnas was carried out in toluene : ethyl acetate solvent system. Alcoholic extracts of churnas were used for UV-visible spectrophotometry and HPLC fingerprint study. Quantitative evaluation of piperine in dadimastaka churna and gallic acid in pushyanuga churna by HPLC study showed 0.456 and 1.626% respectively.

Key Words: Standardization, Dadimastaka, Pushyanuga Churnas, TLC, HPLC.

INTRODUCTION

Churna is a fine dry powder of a drug or drugs. The term churna may be applied to the powder of a single or a mixture of two or more drugs, which are powdered separately prior to their mixing. Dadimastaka churna and pushyanuga churna are the important Ayurvedic preparations used for the perinatal care of mother and child health. In Ayurveda, dadimastaka churna is prescribed for grahani and pushyanuga churna for asrgdara, raktatisara, yonidosa, rituroga, rajodosa, etc.^{1, 2} Several workers have reported the standardization study on churnas, viz, aswagandha churna, madhusnuhi churna, parangipattai churna, trivrt churna and yasti churna³, attaticcuranam⁴, pancatipakkiniccuranam⁵, talisyadi churna⁶, amukkaraccuranam⁷, trikatukuccuranam⁸, kazharchhi choornam⁹ and navayasa churna¹⁰. Detailed analytical data of dadimastaka and pushyanuga churnas is not available. Hence, these were taken for standardization study.

EXPERIMENTAL

The authentic ingredients were procured from the local market of Hyderabad (A.P.), India and were botanically identified. Dadimastaka and pushyanuga churnas were prepared as per the procedure described in Ayurvedic Formulary of India^{1,2}. The product obtained was a fine powder, which passed through a No. 170 sieve.

Standard piperine was procured from M/s Sigma-Aldrich Chemie GmbH, Germany. Standard gallic acid was procured from M/s s.d. Fine Chem. Ltd., Mumbai, India.

The dadimastaka and pushyanuga churnas were analyzed for the parameters such as organoleptic study, pH (1% aqueous), moisture content, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, successive hexane, benzene and chloroform extractions^{11,12} and qualitative organic and inorganic analysis^{13,14}.

The standard piperine solution was prepared by dissolving 5 mg of piperine in 5 mL of absolute alcohol and standard gallic acid solution was prepared by dissolving 5 mg of gallic acid in 5 mL of absolute alcohol. The 4% alcoholic extracts of dadimastaka and pushyanuga churnas were prepared by soaking them for 18 h in absolute alcohol. The extracts were centrifuged at 3000 rpm and then filtered through Whatman filter paper No. 1 using high-pressure vacuum pump. These samples were used for UV-vis spectrophotometric and HPLC fingerprint study.

TLC plates were prepared as per the procedure described by Stahl¹⁵. The 4% alcoholic extracts of both the churnas were prepared by soaking the respective churna for 18 h in absolute alcohol. Alcoholic extracts were filtered and concentrated. Concentrated alcoholic extracts were redissolved in toluene : methanol (9 : 1) and about 100 μ l was loaded on the TLC plate and eluted in toluene : ethyl acetate (93 : 7) solvent system¹⁶. The plates were sprayed with vanillin-sulphuric acid reagent and heated at 110°C for 30 min. Colours of the spots were noted and R_f value of each spot was calculated.

The samples were scanned over a range of 200–800 nm using Elico (SL-159) UV-Vis spectrophotometer equipped with quartz cuvettes of 10 mm path length and UV-Vis Spectrasoft software. Absolute alcohol was used as a reference.

A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps (Shimadzu), variable wavelength programmable photo diode array detector SPD-M10A VP (Shimadzu), CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and reverse phase Luna 5 μ C₁₈(2) Phenomenex column (250 mm \times 4.6 mm) was used. The HPLC system was equipped with software Class VP series version 6.1 (Shimadzu).

The mobile phase components water : methanol were used for dadimastaka churna and water : acetonitrile and methanol were used for pushyanuga churna. The respective solvents were filtered through 0.2 μ membrane filter before use and pumped from the solvent reservoir at a flow rate of 1 mL/min which yielded a column back pressure of 220 kgf/cm² for dadimastaka churna and 180 kgf/cm² for pushyanuga churna. The initial concentration of solvent in pump-B was 50%

and run time was set at 30 min. The column temperature was maintained at 27°C. 20 μ L of sample was injected by using Rheodyne syringe (Model 7202, Hamilton).

RESULTS AND DISCUSSION

The data of organoleptic study and physico-chemical analysis of churnas is summarized in Table-1. The standard values for pushyanuga churna, moisture content not more than 10%, ash value 12–14%, acid insoluble ash not more than 4%, water soluble extractive 10–20% and alcohol soluble extractive 15–20% have been reported¹⁴.

TABLE-1
ANALYTICAL DATA OF DADIMASTAKA AND PUSHYANUGA CHURNAS

S.No.	Analytical parameters	Dadimastaka churna	Pushyanuga churna
1.	Organoleptic studies:		
	Colour	Greyish brown	Light reddish brown
	Odour	Aromatic	Faint
	Taste	Pungent and sweet	Astringent
2.	pH (1% aqueous)	6.42	5.96
3.	Moisture content (%)	3.15	6.26
4.	Total ash (%)	2.72	12.36
5.	Acid insoluble ash (%)	1.30	2.64
6.	Alcohol soluble extractive (%)	16.50	19.20
7.	Water soluble extractive (%)	37.95	15.15
8.	Successive extractives:		
	Hexane soluble (%)	1.29	2.32
	Benzene soluble (%)	1.08	0.70
	Chloroform soluble (%)	0.98	2.52

The data of qualitative organic and inorganic analysis is summarized in Table-2. The data of TLC study of dadimastaka and pushyanuga churna is summarized in Table-3. The UV-visible spectrophotometric data of dadimastaka and pushyanuga churna is tabulated in Table-4.

The HPLC fingerprints of piperine, gallic acid, dadimastaka churna and pushyanuga churna are shown in Fig. 1–4 respectively. In the present study, standard piperine was used as marker compound for dadimastaka churna due to its presence in three out of fourteen ingredients (*Piper longum* Linn.—fruit and root and *Piper nigrum* Linn.—fruit) of this churna. Standard gallic acid was used as marker compound for pushyanuga churna due to its presence in the maximum ingredients of this churna. The HPLC chromatogram of standard piperine at an optimum wavelength of 245 nm showed an area percentage of 98.53 at a retention time of 23.680 min (Fig. 1). The HPLC chromatogram of standard gallic acid was selected at a retention time of 1.941 min with an area percentage of 99.83 at a wavelength of 272 nm (Fig. 2). The HPLC chromatogram of dadimastaka

TABLE-2
 QUALITATIVE ORGANIC AND INORGANIC ANALYTICAL DATA OF
 DADIMASTAKA AND PUSHYANUGA CHURNAS

S. No.	Analytical parameters	Dadimastaka churna	Pushyanuga churna
1.	Alkaloids	+ve	+ve
2.	Steroids	+ve	+ve
3.	Phenols	+ve	+ve
4.	Tannins	+ve	+ve
5.	Glycosides	+ve	+ve
6.	Resins	+ve	+ve
7.	Saponins	+ve	+ve
8.	Flavonoids	+ve	+ve
9.	Aluminium	-ve	+ve
10.	Arsenic	-ve	-ve
11.	Lead	-ve	-ve
12.	Calcium	+ve	+ve
13.	Magnesium	+ve	+ve
14.	Silica	+ve	+ve
15.	Manganese	-ve	-ve
16.	Iron	+ve	+ve
17.	Mercury	-ve	-ve

TABLE-3
 DATA OF TLC STUDY OF DADIMASTAKA AND PUSHYANUGA CHURNAS

S. No.	Dadimastaka churna		Pushyanuga churna	
	Colour of spot	R _f value	Colour of spot	R _f value
1.	Brownish violet	0.04	Violet	0.04
2.	Brownish violet	0.10	Violet	0.09
3.	Violet	0.21	Light violet	0.17
4.	Light violet	0.29	Light violet	0.22
5.	Brown	0.35	Light violet	0.34
6.	Violet	0.45	Light violet	0.40
7.	Violet	0.51	Light violet	0.56
8.	Light violet	0.65	Light violet	0.64
9.	Light violet	0.76	Light violet	0.77
10.	Violet	0.97	Violet	0.96

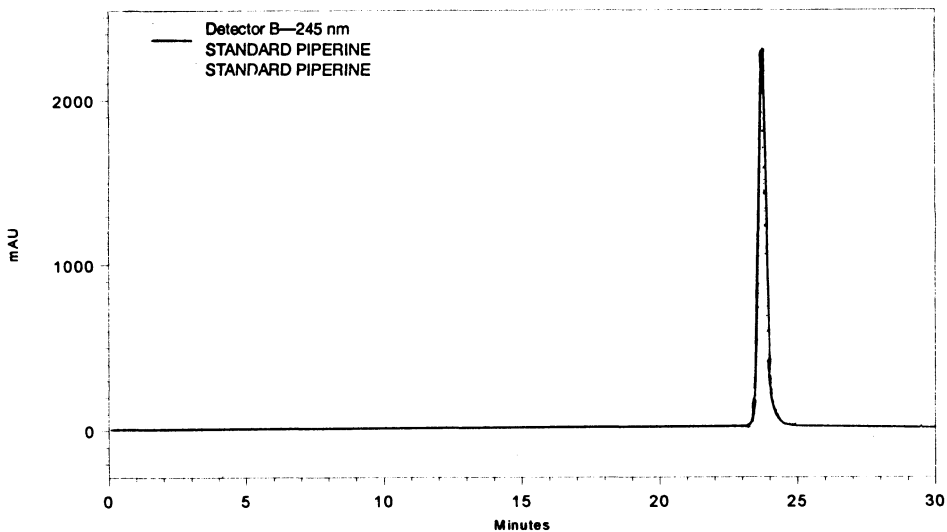


Fig. 1 HPLC chromatogram of standard piperine

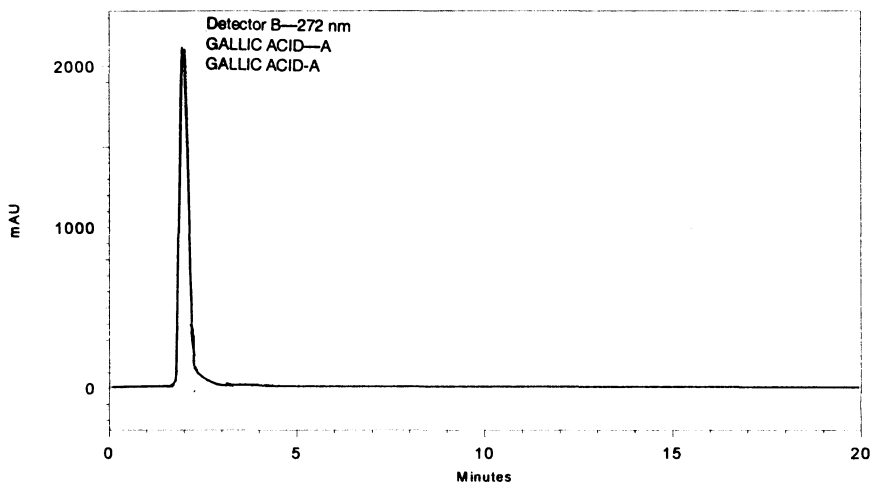


Fig. 2 HPLC chromatogram of standard gallic acid

churna corresponding to standard piperine showed a retention time of 23.765 min with an area percentage of 17.98 at a wavelength of 245 nm (Fig. 3). The HPLC chromatogram of pushyanuga churna corresponding to standard gallic acid showed a retention time of 1.760 min with an area percentage of 64.93 at a wavelength of 272 nm (Fig. 4). The variation in retention time of gallic acid in pushyanuga churna may be due to the presence of other chemical constituents. The peak corresponding to gallic acid in pushyanuga churna at retention time of 1.760 min has been confirmed by its overlaid spectrum. Quantitative evaluation

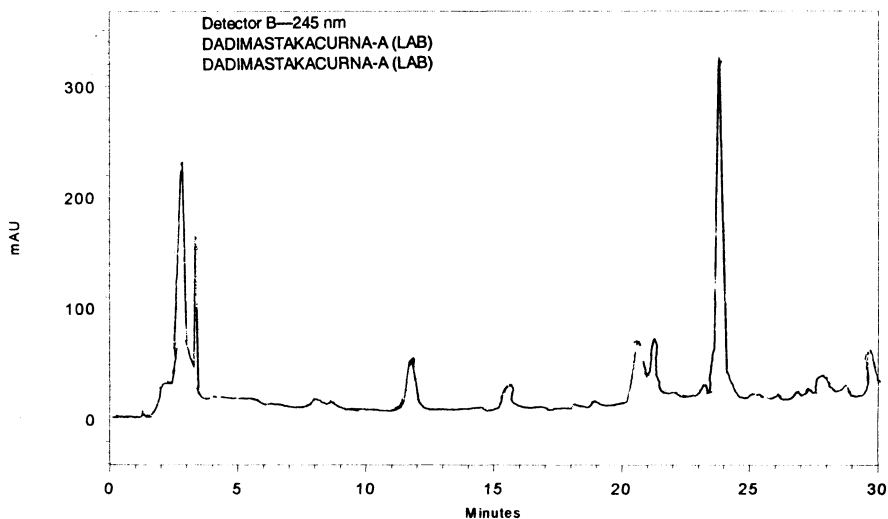


Fig. 3 HPLC chromatogram of dadimastaka churna

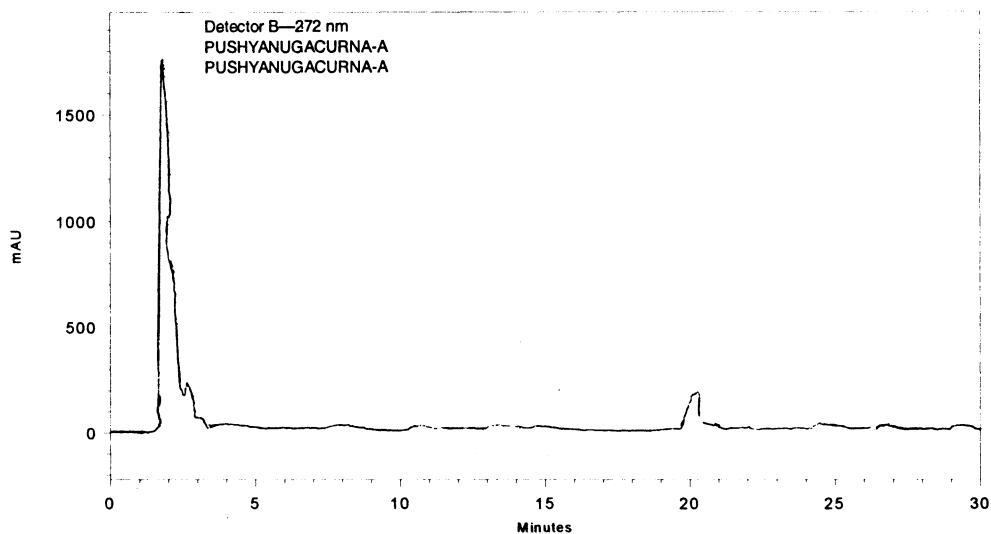


Fig. 4 HPLC chromatogram of pushyanuga churna

of piperine in dadimastaka churna at 245 nm showed 0.456%. Quantitative evaluation of gallic acid in pushyanuga churna at 272 nm showed 1.626%.

The analytical data, TLC, UV-visible spectrophotometric and HPLC fingerprint profiles evolved can be considered as viable parameters which will go a long way for prescribing a dependable standard to these preparations.

TABLE-4
UV-VISIBLE SPECTROPHOTOMETRIC DATA OF DADIMASTAKA
AND PUSHYANUGA CHURNAS

S. No.	Dadimastaka churna		Pushyanuga churna	
	Wavelength (nm)	O.D. value	Wavelength (nm)	O.D. value
1.	208	0.258	272	2.821
2.	253	0.546	296	2.864
3.	341	2.763	480	3.127
4.	396	2.267	504	3.113
5.	606	0.137	664	0.184
6.	664	0.401	—	—

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