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Research Article

Marketed Formulations Standardization Containing Alkushi viz. Microbial Counts, Heavy Metal Analysis, TLC and Quantification of Levodopa

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Abstract

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Herbal medicine has a strong potential in the treatment of different diseases, however quantum of doubt about its efficacy. Hence, standardization becomes most important with the herbal medicine which assures their quality and efficacy. In this research work an attempt was made to establish system of standardization of formulations containing Alkushi in order to assess the quality.

Key Words: *Rubia cordifolia* Linn, Standardization, HPTLC, Heavy metal analysis, HPLC.

Introduction

Standardization means adjusting the herbal drug preparation to a defined content of a constituent or group of substances with known therapeutic activity so it is the process of evaluating the quality and purity of crude drugs by means of various parameters like microscopically and heavy metal analysis.[1-2] Traditional herbal medicines are used by about 60 per cent of the world's population for primary health care.[3] It is very important to establish a system of standardization for every marketed formulation present in the market, in order to assess the quality of the drugs.[4]

Material and Method

Collection of Marketed Formulations

The marketed formulations containing Alkushi were collected from Local market, Faridabad (Haryana) of India in the month of July named as MF-1, MF-2 and MF-3 respectively. The composition of all formulations is mentioned in Table No. 1

Thin Layer Chromatography [10]

Preparation of test solution

Tablet or Capsule: Around 1 g of the tablet or capsule powder was defatted with petroleum ether (60-80 ^{o}C) and refluxed three times. Petroleum ether extract was discarded. Marc was air dried and refluxed with 20 ml of 0.01M methanolic hydrochloric acid. Cooled and filtered the extract through Whatmann filter paper (No. 41), concentrated the filtrate up to 10 ml in rotary evaporator and used as test solution.

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Procedure

The pre coated aluminium plates with Silica Gel 60 F_{254} (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness, were used as stationary phase. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. n-butanol: glacial acetic acid: water (4:1:1) was used as mobile phase and anisaldehyde sulphuric acid reagent as detecting agent. About (10 µl) of the sample solution was applied with the help of Linomat applicator as 10 mm bands on the TLC plate. The plate was developed with mobile phase upto about 80 mm. The developed chromo-plate was dried by hot air. The plate was sprayed with the spraying reagent, and dried in hot air oven at 105 ºC for 5 to 10 min. The plate was then photo documented with the help of HPTLC visualizer. The corresponding results are showed in Figure No.1

Microbiological Analysis

Microbiological study included Total bacterial counts, Total yeast and mould counts, test for pathogens as per Indian pharmacopoeia (2010) and Ayurvedic Pharmacopoeia (2001).[5, 10] The corresponding results are mentioned in Table No. 3

Heavy metal analysis

About 10 g of samples were weighed and charred on the hot plate at 100 ^{0}C for 1-2 hours. Then samples were kept in the muffle furnace at 600 ^{0}C for 6 hours for complete ashing so that no traces of carbonaceous substances were left. Then crucibles were taken out from the muffle furnace and these were cooled down in a dessicator. Then 5 ml HNO_3 and 5 ml of Millipore water were added and swirled to dissolve the content, cooled and filtered through Whatmann filter paper (No. 42) and the volume was made upto 10 ml in the volumetric flasks. The prepared solutions were used as test solution. Blank solution was prepared by adding 4.0 ml of conc. nitric acid and 1.0 ml of perchloric acid and digested in the same condition. Then, adjust volume to 25.0 ml with distilled water. The corresponding results are mentioned in Table No. 4

Quantification of Levodopa in Marketed formulations by HPLC

Preparation of standard solution

A standard solution of Levodopa (purity 98% w/w) was prepared by dissolving 5.81 mg in 100 ml of phosphate buffer (pH 3) to get stock solution.

Preparation of test solution

Tablet or Capsule: 500 mg powdered tablets and capsules was weighed and transferred in 250 ml round bottom flask containing 30 ml diluents and refluxed on water bath for 30 min. The mixture was cooled and filtered. This process repeated twice and all the filtrates were collected. The combined filtrate was centrifuged for 10 min at 2000 rpm. Supernatant liquid was collected and concentrated up to 50 ml on rotary evaporator and used as test solution for analysis. The corresponding results are shown in Table No. 5.

Conditions for HPLC

Chromatographic system: Waters HPLC; Column: Inertsil C18, 250 mm x 4.8 mm; Detector: UV detector; Flow rate: 1 ml/min; Wavelength: 198 nm; Injection volume: 10 µl; Mobile phase: phosphate Buffer (dissolves 1.36 gm potassium hydrogen phosphate in 1000 ml of distilled water and adjusts the pH 3 with O- phosphoric acid)

Table No. 1: Composition of Marketed formulations containing Alkushi

Formulation A [MF-1] (Each Capsule contains)	Formulation B [MF-2] (Each Tablet contains)	Formulation C [MF-3] (Each Capsule contains)
Asparagus racemous 10 mg, Tribulus terrestris 10 mg, Mineral pitch 10 mg, Terminalia arjuna 65 mg, Embelica officinalis 75 mg, **Alkushi** 10 mg, Astercantha logifolia 10 mg, Withania somnifera 250 mg, Lead, tin and zinc reduced 10 mg, Copper pyrite 10 mg	Powders (**Alkushi** 32 mg , Orchismasula 130 mg, Asteracantha longifolia 64 mg, Lactuca scariola 32 mg, , Suvarnavang 32 mg); Extracts (Argyriaspeciosa 64 mg, Tribulus terrestis 64 mg, Leptadeniareticulata 32 mg)	**Alkushi** 30 mg, Bhringraj 40 mg, yashimadhu 30 mg, Arjuna 40 mg, Lavanga 10 mg, Pippali 30 mg, Shati 10 mg, Shilajeet 20 mg, Citraka 10 mg, Jiraka 20 mg, Jatiphala 30 mg, Kutaja 10 mg, Jhabuk 20 mg, Musli safed 30 mg Satavari 50 mg, Lohabhasma 04 mg, Kumkuma 10 mg, Asvgandha 50 mg, Amalaki 10 mg Haritaki 30 mg.

Table No. 2: List of Instruments used

Instrument	Company Name
Autoclave	Yarko, India
BOD incubator	Narag scientific Industry, India
Electronic balance	Mettler Toledo, India
Horizontal shaker	Bliss scientific Indujstry, India
Hot plate	Ambassadar, India
HPTLC	Camag, Switzerland
HPLC	Waters, USA
Bio safety cabinet	Esco, India
Micro balance	Mettler Toledo, India
Microwave oven	Ambassadar, India
Muffale furnace	Ambassadar, India
pH meter	Utech Instruments, Thailand
Sonicator	Bandelin Sonorex, India
Water bath	Equitron, India
Microscope	Zeiss, India
Millipore Water	Millipore, USA
Atomic absorption spectrometer	Perkin, USA

Results and Discussion

From the microbiological study (Table No.3) it was revealed that in Formulation-1 and Formulation-3, all microbials are in prescribed limits with respect Ayurvedic Pharmacopoeia (2001) but formulation-2 showed the presence of *E. Coli* and *Pseudomonas aeruginosa.*

By the heavy metal analysis (Table No.4) it was concluded that, in case of mercury marketed formulation-2 show little deviation but in formulation-1 and formulation-3 mercury is in prescribed limits. In case of arsenic, all marketed formulations showed the presence of arsenic. The cadmium and lead were in prescribed limits in all samples of formulations.

By TLC profiling for all samples of Marketed formulations at 254 nm, 366 nm and after derivitzation, well resolved spots were obtained as mentioned in quality standard of medicinal plants.

By HPLC analysis (Table No. 5) the content of Levodopa in marketed formulation-1 and formulation-2 was found to be **0.005% w/w** and **0.007% w/w** respectively but did not detect in formulation-3 because of low quantity.

Table No. 3: Microbial counts in Marketed formulations containing Alkushi

Sample name	**Total bacterial count (cfu/g)**	**Total yeast and mould count (cfu/g)**	***E. Coli* (cfu/g)**	***Salmonella typhi* (cfu/g)**	***Pseudomonas aeruginosa* (cfu/g)**	***Staphylococc-us aureus* (cfu/g)**
Limits for Formulations	**1×10^{7}**	**1×10^{5}**	**1×10^{3}**	**Absent**	**Absent**	**Absent**
MF-1	1.9×10^{4}	287	Absent	Absent	Absent	Absent
MF-2	5.3×10^{5}	3.0×10^{4}	**Present**	Absent	**Present**	Absent
MF-3	6.3×10^{3}	<10	Absent	Absent	Absent	Absent

Heavy Metal Analysis

Table No. 4: Heavy metals analysis of Marketed formulations containing Alkushi

Sample name	**Arsenic (ppm)**	**Mercury (ppm)**	**Lead (ppm)**	**Cadmium (ppm)**
Limits for Formulations	Not more than 0.1	Not more than 0.1	Not more than 1.0	Not more than 0.1
MF-1	**0.56**	<0.1	<1.0	0.1
MF-2	**0.35**	**0.1**	<1.0	<0.1
MF-3	**0.8**	<0.1	<1.0	<0.1

Quantification of Levodopa in Marketed formulations containing Alkushi

Table No. 5: Quantification of Levodopa in marketed formulations containing Alkushi by HPLC

***Mucuna pruriens* Linn formulation -1 (MF-1)**					
	Weight (g)	**Dilution**	**Quantity Applied**	**Average Peak Area**	**Purity**
Sample	0.5074	in 50 ml x 1/50 ml	20 µl	50.6	_
Standard	0.0058	in 100 ml x 5/50 ml	20 µl	408.2	98% w/w
% Levodopa					**0.005% w/w**
***Mucuna pruriens* Linn formulation -2 (MF-2)**					
	Weight (g)	**Dilution**	**Quantity Applied**	**Average Peak Area**	**Purity**
Sample	0.5160	in 50 ml x 1/50 ml	20 µl	96.6	_
Standard	0.0058	in 100 ml x 5/50 ml	20 µl	408.2	98% w/w
% Levodopa					**0.007% w/w**

TLC profile of Marketed formulations containing Alkushi

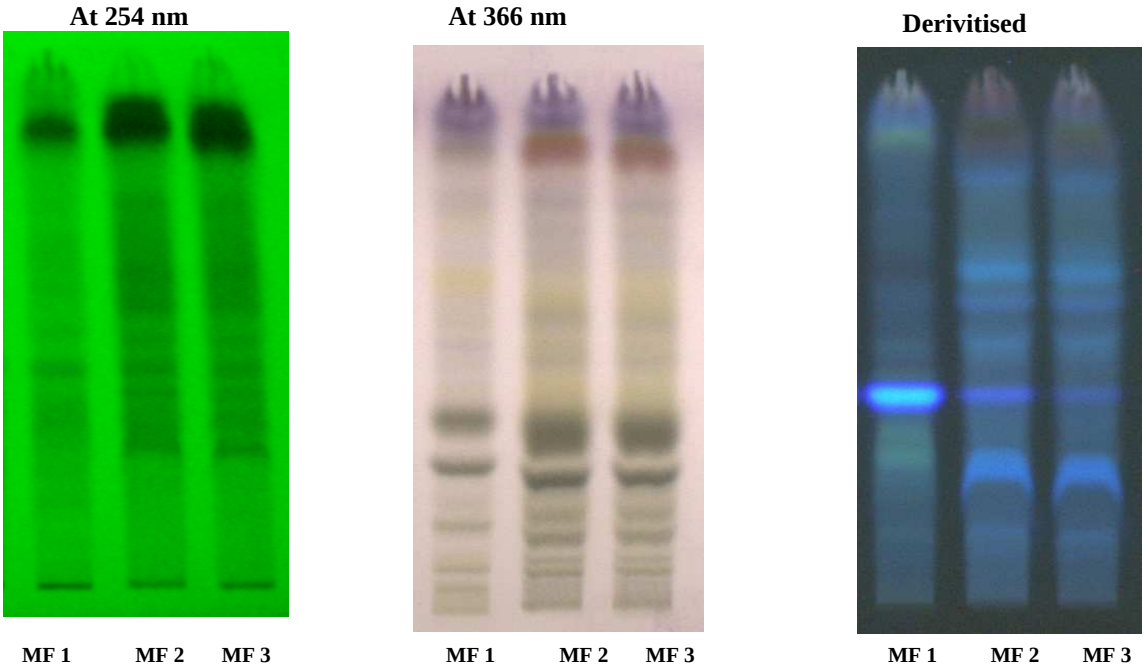

Figure No. 1: TLC profile of Marketed formulations containing Alkushi using HPTLC

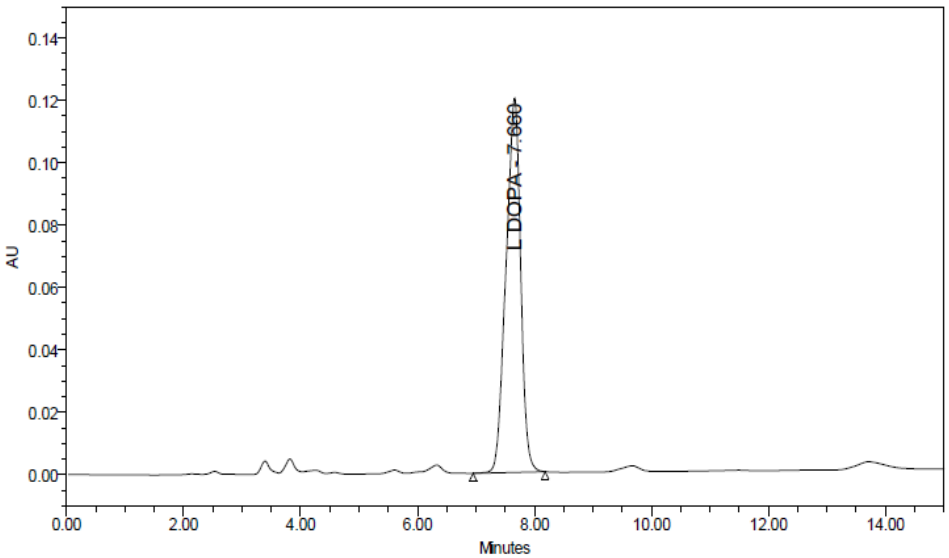

Figure No. 2: Peak of standard Levodopa

Conclusion

In light of results, present study strongly indicates that standardization and quantification of bioactive principle through modern analytical tools is essential for establishing the authenticity, creditability and usage of Ayurvedic medicines or herbal formulations.

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