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STANDARDIZATION STUDY OF A FEW AYURVEDIC FORMULATIONS

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ABSTRACT

The present paper deals with the standardization of a few Ayurvedic formulations such as boladi vati, rajahpravartini vati, dhatri lauha and kumaryasava. These are the important Ayurvedic formulations used for peri–natal care of mother and child health. Standardization of these drugs was achieved by physico–chemical analysis, qualitative inorganic and organic analysis, thin layer chromatography (TLC), UV– visible spectrophotometry and high performance liquid chromatographic (HPLC) fingerprint studies. Alcoholic extracts of the drugs were used for UV– visible spectrophotometry and HPLC fingerprint study. Quantitative evaluation of gallic acid in dhatri lauha and kumaryasava and aloin–A in rajahpravartini vati has been done.

Key words: Standardization, Ayurvedic formulations, TLC and HPLC.

INTRODUCTION

The present communication deals with the standardization of formulations such as boladi vati, rajahpravartini vati, dhatri lauha and kumaryasava, which are used for the peri–natal care of mother and child health. A few analytical standard values have been prescribed for kumaryasava, rajahpravartini vati and dhatri lauha[1]. Bhavsar *et al.*[2] reported studies on kumaryasava. Kumaryasava prepared in the laboratory has higher values of specific gravity, solid content and sugar content as compared to the market sample and have acidic pH. The alcohol content in kumaryasava is inversely proportional to the sugar content[2]. Dash *et al.*[3] reported clinical trial of suthasekhara rasa, dhatri lauha and kamdudharasa in the management of parinaamasula – duodenal ulcer[3]. Santosh *et al.*[4], reported the standardization of selected asavas and arishtas. Aravindasava, asokarista, dasamularista and kumaryasava gave positive results for Borntrager reaction. Analytical study revealed the lowest pH 3.42 in kumaryasava and highest pH 4.8 in amritarista [4].

Kumaryasava, an ayurvedic drug has been used in the Ayurvedic system of medicine for many centuries in various disorders of gastrointestinal, liver, uterus, etc. It is a polyphar-

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maceutical product which contains 48 ingredients of which kumari (*Aloe vera*) is the main constituent. Standardization of kumaryasava was done biologically and chemically[5].

EXPERIMENTAL

The authentic ingredients were procured from the local market of Hyderabad, Andhra Pradesh and were botanically identified. Boladi vati, rajahpravartini vati, dhatri lauha and kumaryasava were prepared as per the procedure described in Ayurvedic Formulary of India[6,7].

Standard aloin–A 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.

Analytical study

The prepared samples were analyzed for the physico–chemical parameters such as pH (1% aqueous), moisture content, total ash, acid insoluble ash, alcohol soluble extractive, water–soluble extractive, total solids, specific gravity, alcohol content, assay for iron, assay for borax, qualitative inorganic and organic analysis[1, 8, 9].

Thin layer chromatography

TLC plates were prepared as per the procedure described by Stahl[10].

The 4% alcoholic extract of boladi vati was redissolved in toluene : methanol (9 : 1) and about 100 µL was used for TLC. The solvent system used for elution was toluene : ethyl acetate (93 : 7). Spots were detected after spraying vanillin–sulphuric acid and heating the plate at 110°C for 30 min.

The 4% alcoholic extract of rajahpravartini vati was redissolved in diethyl ether and about 100 µL was used for TLC. The solvent system used for elution was toluene : ethyl acetate: formic acid (75 : 25 : 1). Spots were detected after spraying with 10% alcoholic KOH.

The 4% alcoholic extract of dhatri lauha was redissolved in dichloroethane : butanol (1 : 1) and about 100 µL was used for TLC. The solvent system used for elution was toluene : ethyl acetate : glacial acetic acid : formic acid (20 : 45 : 20 : 5). Spots were detected after spraying with vanillin–sulphuric acid and heating the plate at 110°C for 30min.

Kumaryasava was mixed with 7.5% HCl (1 : 1) and kept overnight. The mixture was extracted by shaking with diethyl ether. The ether phase was concentrated and about 100 µL was used for TLC. The solvent system toluene : ethyl acetate : formic acid (75 : 25 : 1) was used for the elution. Spots were detected after spraying with 10% alcoholic KOH. R_f value of each spot was calculated and noted[11].

Standard preparation

Standard gallic acid solution was prepared by dissolving 5 mg of gallic acid in 5.0 mL of absolute alcohol. Standard aloin– A was prepared by dissolving 3 mg of aloin–A in 5.0 mL of absolute alcohol. The standard samples were used for the HPLC study.

Sample preparation

The 4% alcoholic extracts of dhatri lauha, rajahpravartini vati and boladi vati were prepared by soaking them for 18 h in absolute alcohol. 10 mL of kumaryasava was evaporated and redissolved in 10 mL of ethanol. The extracts were filtered through Whatman filter paper No. 1 using high–pressure vacuum pump. The samples were used for UV–visible spectrophotometric and HPLC fingerprint study.

UV–visible spectrophotometric analysis

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

HPLC analysis

A gradient and isocratic reverse phase 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 5μ C_{18} (2) Phenomenex column (250 mm x 4.6 mm) was used. The HPLC system was equipped with software Class *VP* series version 6.1 (Shimadzu).

The mobile phase components (acetonitrile– methanol) : water in gradient system was used for gallic acid, boladi vati, dhatri lauha and kumaryasava. Acetonitrile : water in isocratic system was used for aloin–A and rajahpravartini vati. The respective solvents were filtered through 0.2 μ membrane filter before use and pumped from the solvent reservoir at a flow rate of 1mL/min, which yielded a column back pressure of 180 kgf/cm^2 for gallic acid, boladi vati, dhatri lauha and kumaryasava and 140 kgf/cm^2 for aloin–A and rajahpravartini vati. In gradient system, the initial concentration of solvent in pump–B was 50%. The column temperature was maintained at 27°C. 20 μL of sample was injected by using Rheodyne syringe (Model 7202, Hamilton).

RESULTS AND DISCUSSION

The data of physico–chemical analysis of boladi vati, rajahpravartini vati, dhatri lauha and kumaryasava is summarized in Table 1. The standard values for rajahpravartini vati – moisture NMT 9.5%, ash 39–43%, acid insoluble ash NMT 3%, iron 8–10% and borax 20–25%. The

standard values for dhatri lauha – ash 30–35%, acid insoluble ash NMT 4% and iron 17–23%. The standard values for kumaryasava – total solids 7–10%, specific gravity 1.02–1.05, pH 3.5–5.0 and alcohol content 2.8–4.1% w/w have been reported[1].

Table 1. Physico–chemical data of Ayurvedic formulations

Analytical parameters	Boladi vati	Rajahpravartini vati	Dhatri lauha	Kumary–asava
pH(1%)	7.25	7.07	4.68	2.75
Moisture (%)	6.84	5.95	7.01	–
Total ash (%)	35.2	28.46	24.95	–
Acid insoluble ash (%)	3.36	5.84	10.87	–
Alcohol soluble extractive (%)	5.0	6.65	6.85	–
Water soluble extractive (%)	42.05	30.6	18.1	–
Total solids (%)	–	–	–	19.9
Specific gravity	–	–	–	1.067
Alcohol content (%)	–	–	–	6.9
Assay for iron (%)	–	8.58	–	–
Assay for Borax (%)	–	24.72	–	–

The data of qualitative inorganic and organic analysis of the drugs is summarized in Table 2. The presence of saponins, steroids, phenols and glycosides such as O–glycosides and C–glycosides have been reported from kumaryasava[2, 4].

Table 2. Qualitative inorganic and organic analytical data of Ayurvedic formulations

Analytical parameters	Boladi vati	Rajahpravartini vati	Dhatri lauha	Kumary–asava
Calcium	+ve	+ve	+ve	–ve
Magnesium	+ve	+ve	+ve	–ve
Iron	+ve	+ve	+ve	–ve
Aluminum	+ve	–ve	–ve	–ve
Lead	–ve	–ve	–ve	–ve
Arsenic	–ve	–ve	–ve	–ve
Mercury	–ve	–ve	–ve	–ve
Sodium	–ve	+ve	–ve	–ve

Table 2 Continued.,...

Table 2 Continued.,...

Borate	–ve	+ve	–ve	–ve
Silica	–ve	–ve	+ve	–ve
Alkaloids	–ve	+ve	+ve	–ve
Steroids	–ve	+ve	+ve	+ve
Phenols	+ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve
Glycosides	–ve	+ve	+ve	+ve
Resins	+ve	+ve	+ve	–ve
Saponins	+ve	–ve	+ve	+ve
Flavonoids	–ve	+ve	+ve	–ve

The data of TLC study of the drugs is summarized in Table 3. The UV–visible spectrophotometric data of the drugs is tabulated in Table 4.

Table 3. Data of tlc study of ayurvedic formulations

R_f value (color)			
Boladi vati	**Rajahpravartini vati**	**Dhatri lauha**	**Kumary–asava**
0.03 (V)	0.29 (G)	0.21 (BG)	0.12 (R)
0.06 (V)	0.34 (Y)	0.41 (BG)	0.21 (LY)
0.1 (LV)	0.51 (Y)	0.60 (LB)	0.28 (LP)
0.17 (LV)	0.59 (PR)	0.75 (LB)	0.32 (R)
0.24 (LV)	0.66 (Y)	0.89 (LB)	0.42 (LY)
0.79 (LP)	0.74 (LPY)	0.97 (V)	0.58 (LP)
0.82 (LP)	0.81 (Y)	–	–
0.92 (P)	–	–	–

Where,
V– Violet; LV – Light Violet; PR – Pinkish Red; LP – Light Pink; LY – Light Yellow; R – Red; LPY – Light Pinkish Yellow; Y – Yellow; G– Green, P– Pink, BG– Bluish green and LB – Light blue.

The HPLC fingerprint of standard gallic acid, dhatri lauha, kumaryasava, standard aloin–A, rajahpravartini vati and boladi vati are shown in Figure – 1 to 6, respectively. In the present study, standard gallic acid was used as a marker compound for dhatri lauha and kumaryasava and standard aloin–A for rajahpravartini vati. In rajahpravartini vati, exudate of *Aloe vera* contains aloin–A and in dhatri lauha, *Emblica officanalis* is rich in gallic

acid[12]. Gallic acid and tannins are reported from most of the asavas and arishtas. *Aspergillus niger* has converted gallotannin from *Woodfordia fruticosa* flower, an important ingredient of all the asavas and arishtas, into gallic acid[13&14].

Table 4. UV–visible spectrophotmetric data of ayurvedic formulations

Boladi vati		Rajahpravartini vati		Dhatri lauha		Kumaryasava	
W.L.	O.D.	W.L.	O.D.	W.L.	O.D.	W.L.	O.D.
296	2.709	286	2.755	201	0.153	235	2.849
		395	2.161	208	0.170	258	3.009
				254	0.436		
				340	2.606		

Where,
W.L.– Wavelength (nm); O. D.– Optical density

The HPLC chromatogram of standard gallic acid was selected at a retention time of 1.941min with an area percentage of 99.83 at a wavelength of 272 nm (Figure 1). The HPLC chromatogram of dhatri lauha corresponding to standard gallic acid showed at a retention time of 1.963 min with an area percentage of 89.04 at a wavelength of 272 nm (Figure 2). The HPLC chromatogram of kumaryasava corresponding to standard gallic acid

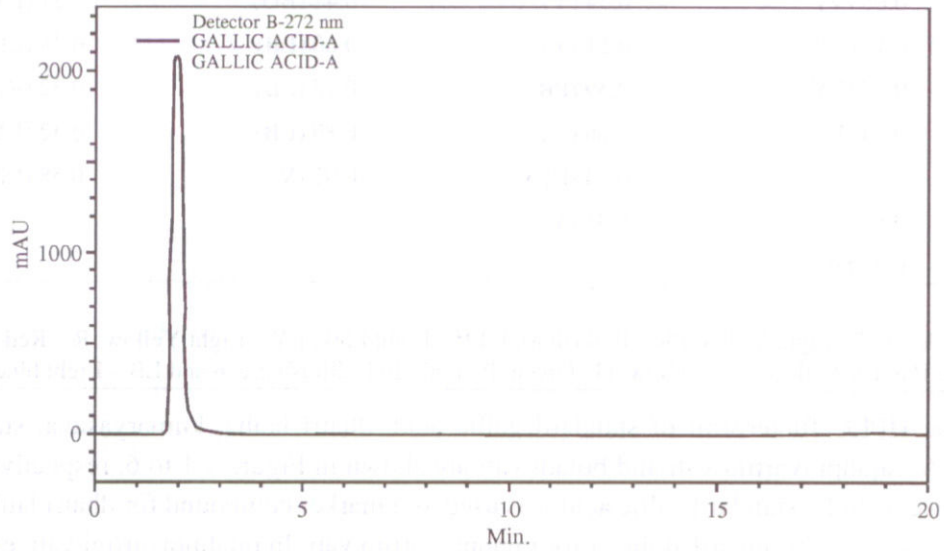

Figure 1. HPLC Chromatogram of standard gallic acid

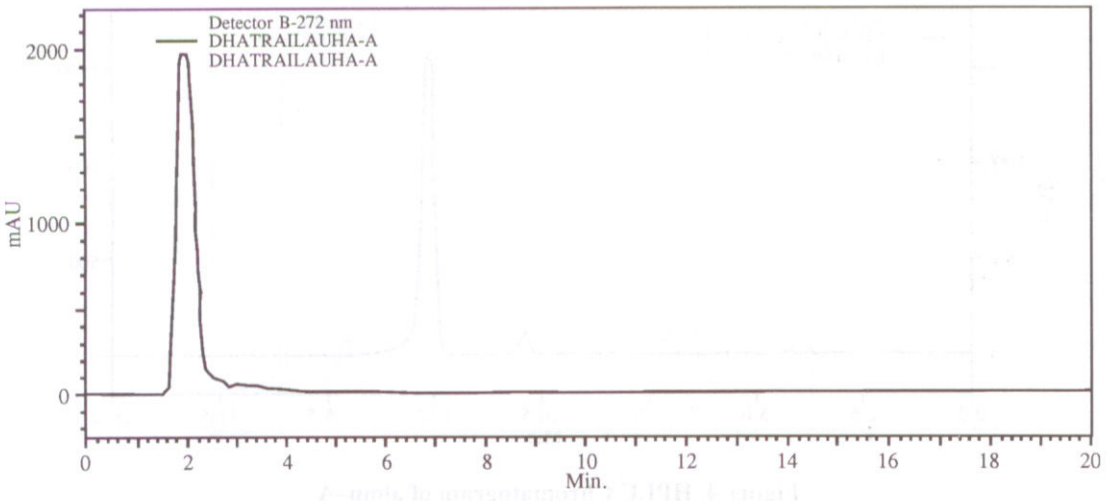

Figure 2. HPLC Chromatogram of dhatri lauha

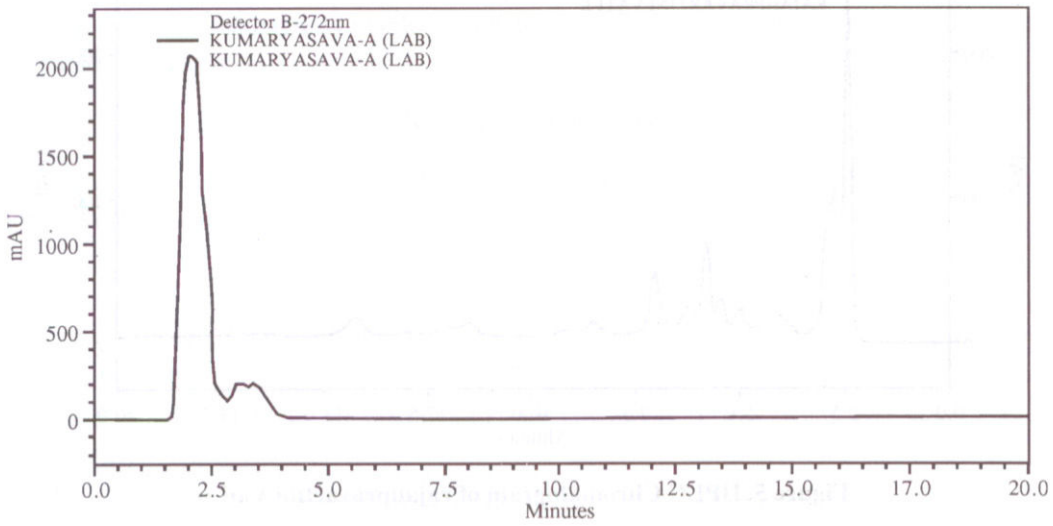

Figure 3. HPLC Chromatogram of kumaryasava

showed at a retention time of 2.080 min with an area percentage of 86.82 at a wavelength of 272 nm (Figure 3). The variation in retention time of gallic acid in dhatri lauha and kumaryasava may be due to the presence of other chemical constituents. The HPLC chromatogram of standard aloin–A at an optimum wavelength of 220 nm showed an area percentage of 91.94 at a retention time of 12.640 min (Figure 4). The HPLC chromatogram of rajahpravartini vati corresponding to standard aloin–A showed at a retention time of 12.096 min with an area percentage of 2.33 at a wavelength of 220 nm (Figure 5). The peak

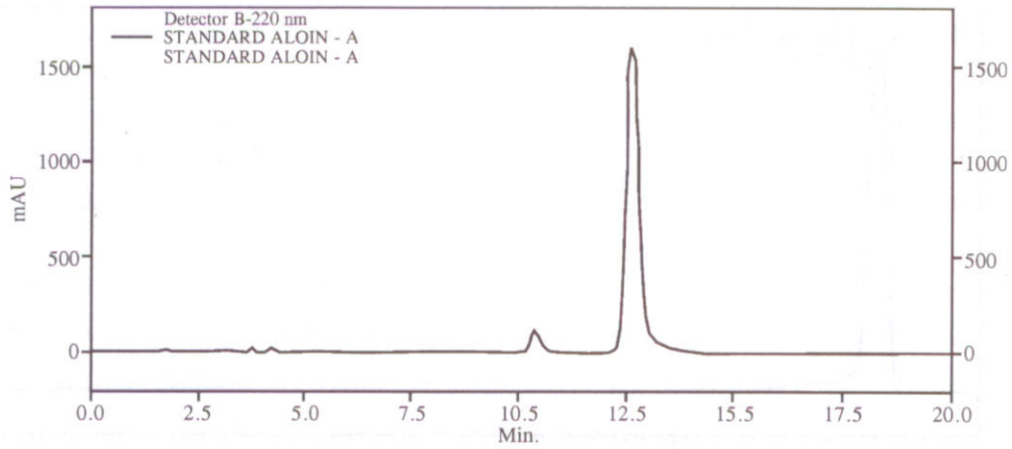

Figure 4. HPLC Chromatogram of aloin–A

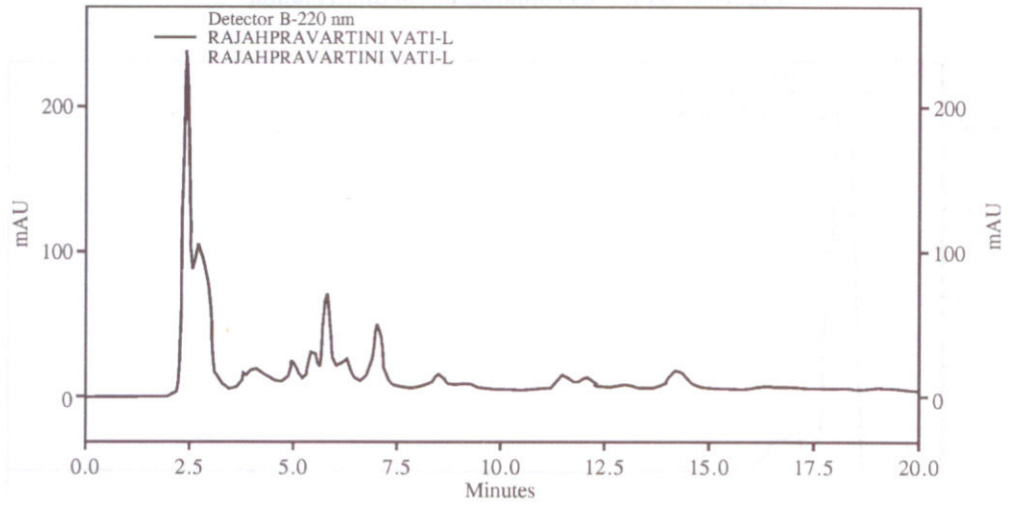

Figure 5. HPLC Chromatogram of rajahpravartini Vati

corresponding to standard marker compounds in dhatri lauha, kumaryasava and rajahpravartini vati has been confirmed by their overlaid spectrum. Quantitative evaluation of gallic acid in dhatri lauha and kumaryasava at 272 nm showed 3.232% w/w and 1.852% w/w. Quantitative evaluation of aloin–A in rajahpravartini vati at 220 nm showed 0.014% w/v. The qualitative HPLC fingerprint of boladi vati was shown in Figure 6. The HPLC chromatogram of boladi vati showed ten peaks at a retention time of 2.613 min, 4.992 min, 14.571 min, 17.259 min, 17.824 min, 18.229 min, 20.256 min, 23.019 min, 26.304 min and 27.883 min with an area percentage of 12.39, 6.97, 4.26, 4.12, 2.52, 5.09, 1.16, 18.17, 2.93 and 15.28 at a wavelength of 318 nm.

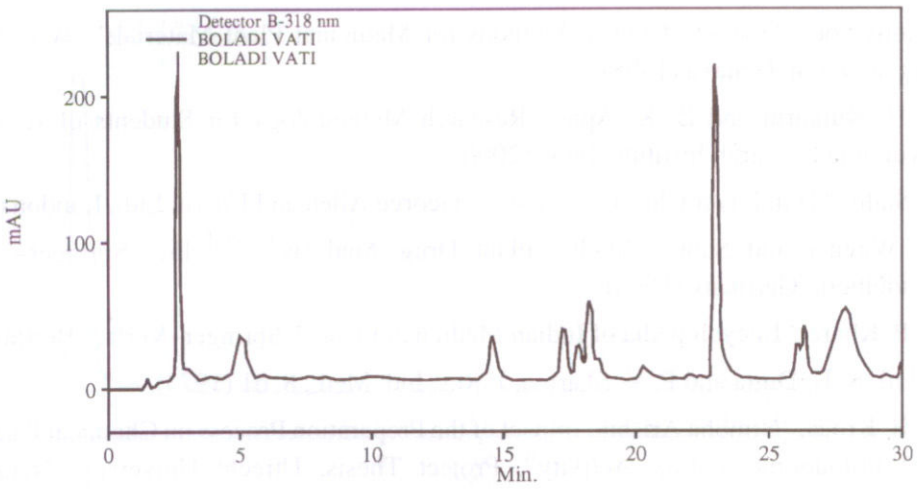

Figure 6. HPLC Chromatogram of boladi vati

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 standards to these preparations.

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REFERENCES

1. Anonymous, "Pharmacopoeial Standards for Ayurvedic Formulations", Central Council for Research in Ayurveda and Siddha, New Delhi (1987).
2. G. C. Bhavasar, M. G. Chauhan and U. M. Upadhyay, J. Res. Ayurveda Siddha, **1–4**, 50 (1990).
3. S. Dash, N. S. Tewari and Prem Kishore, J. Res. Ayurveda Siddha, **10(1–2)**, 41 (1989).
4. M. K. Santosh, D. Shaila, and I. Sanjeeva Rao, Asian J. Chem., **15(2)**, 884 (2003).
5. R. B. Arora, J. N. Sharma, Lalit Gupta and S. S. Agarwal, Jour. Res. Ind. Med., **8(2)**, 37 (1973).
6. Anonymous, "Ayurvedic Formulary of India", Part–I, Department of ISM & H, Ministry of Health and Family Welfare, New Delhi (1978).
7. Anonymous, "Ayurvedic Formulary of India", Part–II, Department of ISM & H, Ministry of Health and Family Welfare, New Delhi (2000).

8. Anonymous, "Quality Control Methods for Medicinal Plant Materials", World Health Organization, Geneva (1998).

9. P. H. Kulkarni and B. K. Apte, "Research Methodology for Students of Ayurveda", Ayurveda Research Institute, Pune (2000).

10. E. Stahl, "Thin Layer Chromatography", George Allen and Unwin Ltd., London (1969).

11. H. Wagner and Sabine Bladt, "Plant Drug Analysis", 2nd Ed., Springer– Verlag, Heidelberg, Germany (1996).

12. C. P. Khare, "Encyclopedia of Indian Medicinal Plants", Springer–Verlag, Berlin (2004).

13. J. Lal, S. K. Dutta and P. V. Sharma, J. Res. Ind. Med., **8**, 61 (1973).

14. B. H. Kroes, "Nimbha Arishta: Impact of the Preparation Process on Chemical Parameters and Immunomodulatory Activity", Project Thesis, Utrecht University, Netherlands (1990).

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