Asian Journal of Chemistry Vol. 16, Nos. 3–4 (2004), 1742–1748

Studies on the Standardization of Avalehas; Pugakhanda and Saubhagyasunthi

M.K. SANTOSH*, D. SHAILA and I. SANJEEVA RAO
Varun Herbals Pvt. Ltd., 5-8-293/A, Mahesh Nagar
Chirag Ali Lane, Hyderabad-500 001, India
E-mail: santosh_mkb76@yahoo.com

The present paper deals with the standardization of avalehas such as pugakhanda and saubhagyasunthi which was 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 study. Qualitative organic analysis showed the presence of alkaloids, steroids, phenols, tannins, glycosides, resins, saponins and flavonoids in both pugakhanda and saubhagyasunthi. Qualitative inorganic analysis of pugakhanda revealed the presence of calcium, magnesium and iron whereas saubhagyasunthi showed the presence of aluminum in addition to calcium, magnesium and iron. TLC study of alcoholic extracts of pugakhanda and saubhagyasunthi was carried out in toulene : ethyl acetate solvent system. Alcoholic extracts of both the avalehas were used for UV-Visible spectrophotometry and HPLC fingerprint study. The quantification of gallic acid in pugakhanda and saubhagyasunthi was evaluated using standard gallic acid by HPLC fingerprint study.

Key Words: Standardization, Avalehas, Pugakhanda, Saubhagyasunthi, HPLC.

INTRODUCTION

Avalehas or lehyas are semi-solid or granular medicinal preparations made by the addition of sugar or jaggery to the prescribed drugs, juices or decoctions[1]. Avalehas are confections or thickened and sweetened extract, equivalent to confections, electuaries and conserves of the British Pharmacopoeia[2]. The present communication deals with the standardization of avalehas such as pugakhanda and saubhagyasunthi, which are used for the perinatal care of mother and child health. In Ayurveda, pugakhanda is therapeutically indicated for shula, amlapitta, rajayaksama, ksina, chardi, murccha, pandu and bandhyaroga and saubhagyasunthi for agnimandya, sutikaroga, atisara and grahani[3]. Studies on the standardization of avalehas such as narikelakhanda, kantakaryavaleha, agasthya haritaki rasayana and vasavaleha have been reported[1,4,5]. A few analytical standard values have been prescribed for saubhagyasunthi[3]. Hence, pugakhanda and saubhagyasunthi were taken for the standardization study.

EXPERIMENTAL

The authentic ingredients were procured from the local market of Hyderabad (A.P.), India and were botanically identified. Pugakhanda and saubhagyasunthi were prepared as per the procedure described in Ayurvedic Formulary of India[6, 7]. Standard gallic acid was procured from M/s S.D. Fine Chem. Ltd., Mumbai, India.

The prepared samples were analyzed for the parameters such as organoleptic study, pH (1% aqueous), moisture content, total ash, acid insoluble ash, total sugars, fat content, water soluble extractive, alcohol soluble extractive, successive hexane, benzene and chloroform extractives, qualitative organic and inorganic analysis[3, 8, 9].

The standard gallic acid solution was prepared by dissolving 5 mg of gallic acid in 5 mL of absolute alcohol. The 4% alcoholic extracts of the samples 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. The samples were used for UV-Vis spectrophotometric and HPLC fingerprint study.

TLC plates were prepared as per the procedure described by Stahl[10]. The 4% alcoholic extracts of the samples were prepared by soaking them for 18 h in absolute alcohol. Alcoholic extracts were filtered and concentrated. Respective concentrated alcoholic extracts were redissolved in toluene : methanol (9 : 1) and about 100 µL was loaded on the TLC plate and eluted in toluene : ethyl acetate (93 : 7) solvent system[11]. The plates were sprayed with vanillin-sulphuric acid reagent and the spots were detected after heating at 11°C for 30 min. 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-10 AT VP pumps (Shimadzu), variable wave length 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 5µC_{18}(2) Phenomenex column (250 mm × 4.6 mm) was used. The HPLC system was equipped with software Class VP series version 6.1 (Shimadzu). The mobile phase components water : acetonitrile and methanol were filtered through 0.2 µ membrane filter before use and pumped from the solvent reservoir to the column at a flow rate of 1 mL/min which yielded a column back pressure of 180 kgf/cm^2. The initial concentration of solvent in pump-B (acetonitrile and methanol) was 50% and run time was set at 30 min. 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 organoleptic study, physico-chemical analysis of pugakhanda and saubhagyasunthi is summarized in Table-1. The data of qualitative organic and inorganic analysis of pugakhanda and saubhagyasunthi is summarized in Table-2.

TABLE-1
ANALYTICAL DATA OF PUGAKHANDA AND SAUBHAGYASUNTHI

S.No.	Analytical parameters	Pugakhanda	Saubhagyasunthi
1.	Organoleptic studies		
	Colour	Reddish brown	Brown
	Odour	Fragrant	Aromatic
	Taste	Sweet	Sweet
2.	pH (1% aqueous)	8.16	7.84
3.	Moisture content (%)	2.17	1.17
4.	Total ash (%)	1.27	5.60
5.	Acid insoluble ash (%)	0.36	2.29
6.	Total sugar (%)	40.95	43.70
7.	Fat content (%)	5.10	15.16
8.	Water soluble extractive (%)	45.40	21.55
9.	Alcohol soluble extractive (%)	19.25	23.70
10.	Successive extractives:		
	Hexane soluble (%)	9.31	13.70
	Benzene soluble (%)	0.36	0.68
	Chloroform soluble (%)	0.52	0.62

The organoleptic study of pugakhanda revealed reddish brown powder with fragrant odour and sweet taste. The analytical study of pugakhanda showed pH (1% aqueous solution) 8.16, moisture content 2.17%, total ash content 1.27%, acid insoluble ash 0.36%, total sugar 40.95%, fat content 5.1%, water soluble extractive 45.4%, alcohol soluble extractive 19.25% and successive extractives such as hexane soluble extractive 9.31%, benzene soluble extractive 0.36% and chloroform soluble extractive 0.52%. The alcoholic extract of pugakhanda showed the presence of alkaloids, steroids, phenols, tannins, glycosides, resins, saponins and flavonoids. The qualitative inorganic analysis revealed the presence of calcium, magnesium and iron.

The organoleptic study of saubhagyasunthi revealed brown powder with aromatic odour and sweet taste. The analytical study of saubhagyasunthi showed pH (1% aqueous solution) 7.84, moisture content 1.17%, total ash content 5.6%, acid insoluble ash 2.29%, total sugar 43.7%, fat content 15.16%, water soluble extractive 21.55%, alcohol soluble extractive 23.7% and successive extractives such as hexane soluble extractive 13.7%, benzene soluble extractive 0.68% and chloroform soluble extractive 0.62%. The alcoholic extract of saubhagyasunthi showed the presence of alkaloids, phenols, tannins, glycosides, steroids, resins, saponins and flavonoids. The qualitative inorganic analysis revealed the presence of aluminum, calcium, magnesium and iron. In saubhagyasunthi, standard values for moisture content 6–10%, total sugar 35–40% and fat content 6–15% have been reported[3]. The variation in moisture content may be due to the method of preparation (semisolid or granular form).

TABLE-2
QUALITATIVE ORGANIC AND INORGANIC ANALYTICAL DATA OF PUGAKHANDA AND SAUBHAGYASUNTHI

S. No.	Analytical parameters	Pugakhanda	Saubhagyasunthi
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.	Maganese	–ve	–ve
15.	Iron	+ve	+ve
16.	Mercury	–ve	–ve

The separation of alcoholic extract of pugakhanda and saubhagyasunthi was carried out in the toluene : ethyl acetate (93 : 7) solvent system. The data of TLC study of pugakhanda and saubhagyasunthi is summarized in Table–3.

The UV-Vis spectrophotometric data of pugakhanda and saubhagyasunthi is tabulated in Table-4. Three peaks were detected in alcoholic extract of pugakhanda at wavelengths of 208 nm, 254 nm and 337 nm with optical densities of 0.198, 0.473 and 2.535 respectively. The alcoholic extract of saubhagyasunthi also showed three peaks at wavelengths of 254 nm, 334 nm and 662 nm with optical densities of 0.739, 2.909 and 0.267 respectively.

Gallic acid is a cheap and easily available phenolic standard, which can be used as a marker compound for the qualitative and quantitative evaluation of most of the herbal formulations due to its presence in many flowering plants[12]. 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. 1). The HPLC chromatogram of pugakhanda corresponding to standard gallic acid showed at a retention time of 1.760 min with an area percentage of 69.43 at a wavelength of 272 nm (Fig. 2). Quantitative evaluation of gallic acid in pugakhanda at 272 nm showed 1.739%. The HPLC chromatogram of

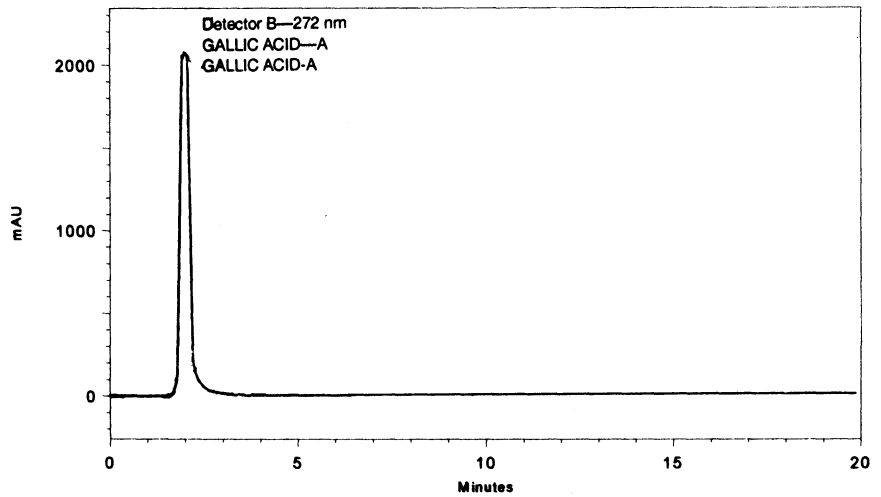

Fig. 1 HPLC chromatogram of gallic acid

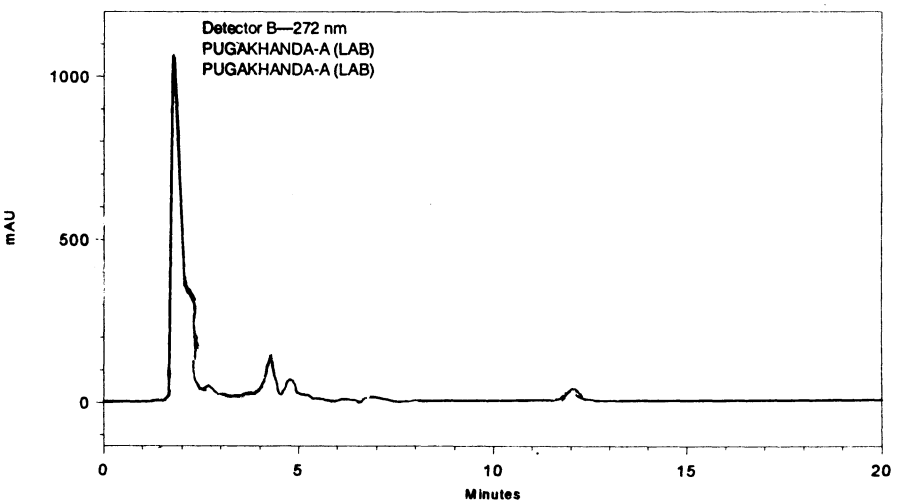

Fig. 2 HPLC chromatogram of pugakhanda

saubhagyasunthi corresponding to standard gallic acid showed at a retention time of 1.909 min with an area percentage of 20.02 at a wavelength of 272 nm (Fig. 3). Quantitative evaluation of gallic acid in saubhagaysunthi at 272 nm showed 0.501%. The variation in retention time of gallic acid in the formulations may be due to the presence of other chemical constituents. The peak corresponding to gallic acid in pugakhanda and saubhagyasunthi at retention times of 1.760 min and 1.909 min respectively has been confirmed by their overlaid spectra.

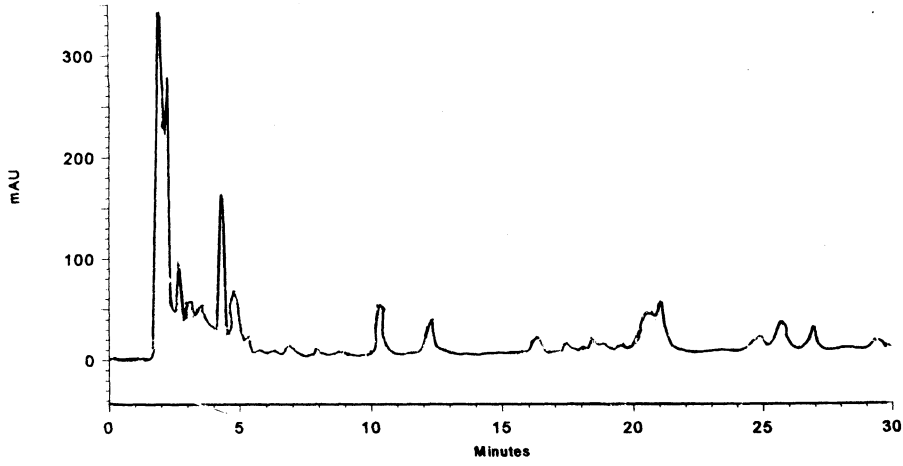

Fig. 3 HPLC chromatogram of saubhagyasunthi

TABLE-3
DATA OF TLC STUDY OF PUGAKHANDA AND SAUBHAGYASUNTHI

S. No.	Pugakhanda		Saubhagyasunthi	
	Colour of spot	R_f value	Colour of spot	R_f value
1.	Violet	0.08	Violet	0.04
2.	Violet	0.14	Violet	0.23
3.	Light violet	0.22	Violet	0.30
4.	Light violet	0.35	Brown	0.36
5.	Light violet	0.51	Light violet	0.41
6.	Violet	0.98	Light violet	0.45
7.	—	—	Light violet	0.50
8.	—	—	Light violet	0.63
9.	—	—	Violet	0.98

TABLE-4
DATA OF UV-VIS SPECTROPHOTOMETRIC STUDY OF PUGAKHANDA AND SAUBHAGYASUNTHI

S. No.	Pugakhanda		Saubhagyasunthi	
	Wavelength (nm)	O.D. value	Wavelength (nm)	O.D. value
1.	208	0.198	254	0.739
2.	254	0.473	334	2.909
3.	337	2.535	662	0.267

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.

ACKNOWLEDGEMENT

The authors are thankful to the Secretary, Department of RCH, Ministry of Health and Family Welfare, New Delhi, for financial support.

REFERENCES

1. M. Alam, K.K. Shanmughadasan, K. Sathiavasan, M. Paranthaman and K.K. Purushothaman, *Bull. Medico-Ethno Botanical Res.*, **3**, 97 (1982).
2. Anonymous, Vaidya Yoga Ratnavali, IMPCOPS, Madras (1982).
3. Anonymous, Pharmacopoeial Standards for Ayurvedic Formulations, Central Council for Research in Ayurveda and Siddha, New Delhi (1982).
4. M. Alam, K.K. Shanmughadasan, K. Sathiavasan, M. Paranthaman and K.K. Purushothaman, *J. Res. Ayurveda Siddha*, **3**, 69 (1982).
5. M. Alam, K. Sathiavasan, K.K.S. Dasan, M. Paranthaman and K.K. Purushothaman, *J. Res. Ayurveda Siddha*, **5**, 33 (1984).
6. Anonymous, Ayurvedic Formulary of India, Part-I, Ministry of Health and Family Welfare, New Delhi (1978).
7. Anonymous, Ayurvedic Formulary of India, Part-II, 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 & Unwin Ltd., London (1969).
11. H. Wagner and S. Bladt, Plant Drug Analysis, Springer-Verlag, Berlin (Germany) (1996).
12. J.B. Harborne, Phytochemical Methods, 3rd Edn., Chapman & Hall, London, p. 42 (1998).

(*Received*: 25 February 2004; *Accepted*: 10 June 2004) AJC-3436