



HPTLC and ¹H-NMR as Fingerprints to Spot Ingredients of a Polyherbal Medicine Bhunimbadi Kvatha Churna

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ABSTRACT

Introduction: Bhūnimbadi Kvātha Cūrṇa (BKC) is a polyherbal medicine formulated from eight ingredients viz. plant of *Swertia chirata* Buch. Ham., root of *Aconitum heterophyllum* Wall. ex. Royle, stem bark of *Symplocos racemosa* Roxb., rhizome of *Cyperus rotundus* Linn., seed of *Holarrhena antidysenterica* Wall., root of *Coleus vettiveroides*. K. C. Jacob, fruit of *Coriandrum sativum* Linn. and fruit pulp of *Aegle marmelos* Corr. It is used for the treatment of asthma, cough, fever due to pitta dosa, and bleeding disorders according to Ayurvedic Formulary of India. The current study is focused on identification of ingredients and their formulation BKC using qualitative high performance thin layer chromatography (HPTLC) and ¹H-nuclear magnetic resonance (¹H-NMR) spectra fingerprint. **Methods:** Diagnostic HPTLC method is optimised to spot each ingredient in BKC. ¹H-NMR spectroscopy of total methanolic extract in DMSO was also carried out to detect the presence of corresponding signals from ingredients. **Results:** HPTLC as well as ¹H-NMR was found to be effectual in identifying ingredients of BKC. The results can be employed to identify BKC of different manufacturers of herbal markets. **Conclusion:** The test can also be employed in pharmacopoeias for quality control of multi-herb formulations used in Ayurveda.

KEYWORDS

Ayurvedic Formulary of India, chemical fingerprint, HPTLC, ¹H-NMR, polyherbal, quality control.

Received: 04.10.2016

Accepted: 07.12.2016

DOI: 10.5530/jams.2016.1.10

Poly-herbal medicines (PM) which are combination of several herbs are popular in Indian Systems of Medicines like Ayurveda, Siddha and Unani. The synergistic therapeutic properties of these PMs are dependent on several phyto-constituents contributed from each of the ingredients. Owing to variability and complexity of chemical constituents there is need for development of methods for establishing quality for many of poly-herbal medicines used in traditional systems of medicine^[1]. Chromatographic techniques are widely used for detecting plant drugs as it easy to run, reproduce, and requires a few equipments.^[2,3] ¹H-NMR spectroscopy is another important fingerprint technique employed for analysis of multi-constituent herbal extracts in recent times.^[4] Bhūnimbādi Kvātha Cūrṇa (AFI, 2011)^[5] is a poly-herbal medicine formulated from equal quantities of eight herbs viz. Bhūnimba (*Swertia chirata* Buch.Ham - plant), Ativiṣā (*Aconitum heterophyllum* Wall. ex. Royle - root), Lodhra (*Symplocos racemosa* Roxb. - stem bark), Mustaka (*Cyperus rotundus* Linn. - rhizome), Indrayava (*Holarrhena antidysenterica* Wall. - seed), Bālaka (*Coleus vettiveroides*. K. C. Jacob - root); Dhānyaka (*Coriandrum sativum* Linn. - Fruit) and Bilva (*Aegle marmelos* Corr. – fruit pulp). It is used in the treatment of asthma, cough, fever due to pitta dosa, bleeding disorder and fever.^[5] In the present study an effort has been made to find out the suitability of qualitative HPTLC analysis and ¹H-NMR spectroscopy fingerprinting to identify ingredients in the multi-herb formulation BKC.

MATERIALS AND METHODS

Collection and identification of plant samples

Dry raw samples required for the study were collected from the raw drug section of SDM Ayurveda pharmacy, Udipi and from authentic raw drug suppliers. The samples were authenticated using macro-microscopic examination, and voucher specimens (No. SDM/VGST/SMYSR/BKC/01-08) have been deposited in the crude drug museum of Pharmacognosy department of SDMCRAAS, Udupi, Karnataka.

TLC/HPTLC

Sample preparation

Ingredients- 5 g each of Bhūnimba, Ativiṣā, Lodhra, Musthaka, Indrayava, Bālaka, Dhānyaka and Bilva were extracted exhaustively with methanol using Soxhlet apparatus. The filtrate was concentrated to dryness and 100 mg of dried residue was dissolved in 5 ml of methanol in a standard flask individually.

Formulation- 40 g of BKC was extracted exhaustively methanol using Soxhlet apparatus. The filtrate was concentrated to dryness and 100 mg of dried residue was dissolved in 5 ml of methanol in a standard flask.

Method

Chromatography profile for each ingredient with BKC was developed individually using separate mobile phase as follows: Bhūnimba - toluene: ethyl acetate: formic acid (7: 2: 0.2 v/v), Ativiṣā - toluene: ethyl acetate: formic acid (7: 2: 0.1 v/v), Lodhra - chloroform: methanol (9.6:0.8 v/v), Musthaka - toluene: ethyl acetate: formic acid (9: 1 v/v), Indrayava - *n*-hexane: diethyl ether (5:2 v/v), Bālaka - toluene: ethyl acetate (9: 1 v/v), Dhānyaka - toluene: ethyl acetate (9.3: 0.7 v/v), Bilva - toluene: ethyl acetate (8: 1 v/v).

Sample extracts, at two concentration levels were applied on aluminium plates precoated with silica gel 60 F₂₅₄ of 0.2 mm thickness (Merck, Germany) using CAMAG (Muttentz, Switzerland) Linomat 5 applicator.^[6] The plate was developed in CAMAG glass twin trough chamber previously saturated with respective mobile phase. The developed plate was visualized in CAMAG visualizing chamber under short UV and long UV and then scanned in CAMAG Scanner 4 at 254 and 366 nm. Derivation was done using vanillin- sulphuric acid (VS) spray followed heating at 105°C till the spots appeared and scanned at 620 nm. R_f values and densitograms were recorded with the help of CAMAG WinCATS software.^[7,8]

¹H NMR spectroscopy

Sample preparation

The extraction of samples was as per method reported by Kim & Verpoorte^[9] with slight modification. Approximately 20 mg of all the samples were weighed into a 1.5 ml reaction tube and 1.5 ml of deuterated DMSO-D₆ containing 0.05% TMS was added. One millilitre of the supernatant was added to a 5 mm diameter NMR spectroscopy tube and the samples were submitted for NMR analysis.

Operation parameters

¹H NMR spectra of the eight ingredients and the formulation BKC were obtained using Bruker Avance 500 MHz spectrophotometer (Bruker Analytik, Rheinstetten, Germany) equipped with a 5 mm cryoprobe head and operating at proton frequency of 500.13 MHz. The spectra were acquired with 256 scans, requiring approximately 12 min acquisition time. The acquisition parameters were: ≈0.16 Hz/point, pulse width (PW) 30° and relaxation delay = 1 s. FIDs were Fourier transformed with LB = 0.30 Hz. The spectra were corrected to the internal TMS peak. TOPSPIN version 1.3 software was used for acquisition and processing of the ¹H NMR spectra which were manually corrected for phase and baseline distortions. The residual solvents signals for DMSO (2.65–2.45 ppm) and water (3.60–3.10 ppm) were excluded.

RESULTS AND DISCUSSION

HPTLC (Figure 1) and ¹H-NMR fingerprinting (Figure 2) of the individual and compounded powder was developed in this study.

On densitometric scan at 366 nm, out of 10 peaks seen in BKC, 4 peaks were corresponding to Bhūnimba. After derivatisation followed by scan at 620 nm, out of 10 spots of BKC, 4 spots were from Ativiṣā. At 620 nm post derivatisation, out of 9 peaks observed in BKC, 2 peaks have similar R_f as that of Lodhra. After derivatisation, scan at 620 nm showed 13 peaks in BKC, 4 peaks being at same R_f as that of Musthaka. After derivatisation, scan at 620 nm there were 9 peaks in BKC, in which 2 peaks were from Indrayava. At 620 nm after derivatisation, out of 11 peaks of BKC, 4 peaks obtained from Bālaka. After derivatisation, at 620 nm, 13 peaks in BKC, 5 peaks are detected from Dhānyaka. On densitometric scan at 254 nm out of 11 peaks of BKC, 5 peaks belonged to Bilva.

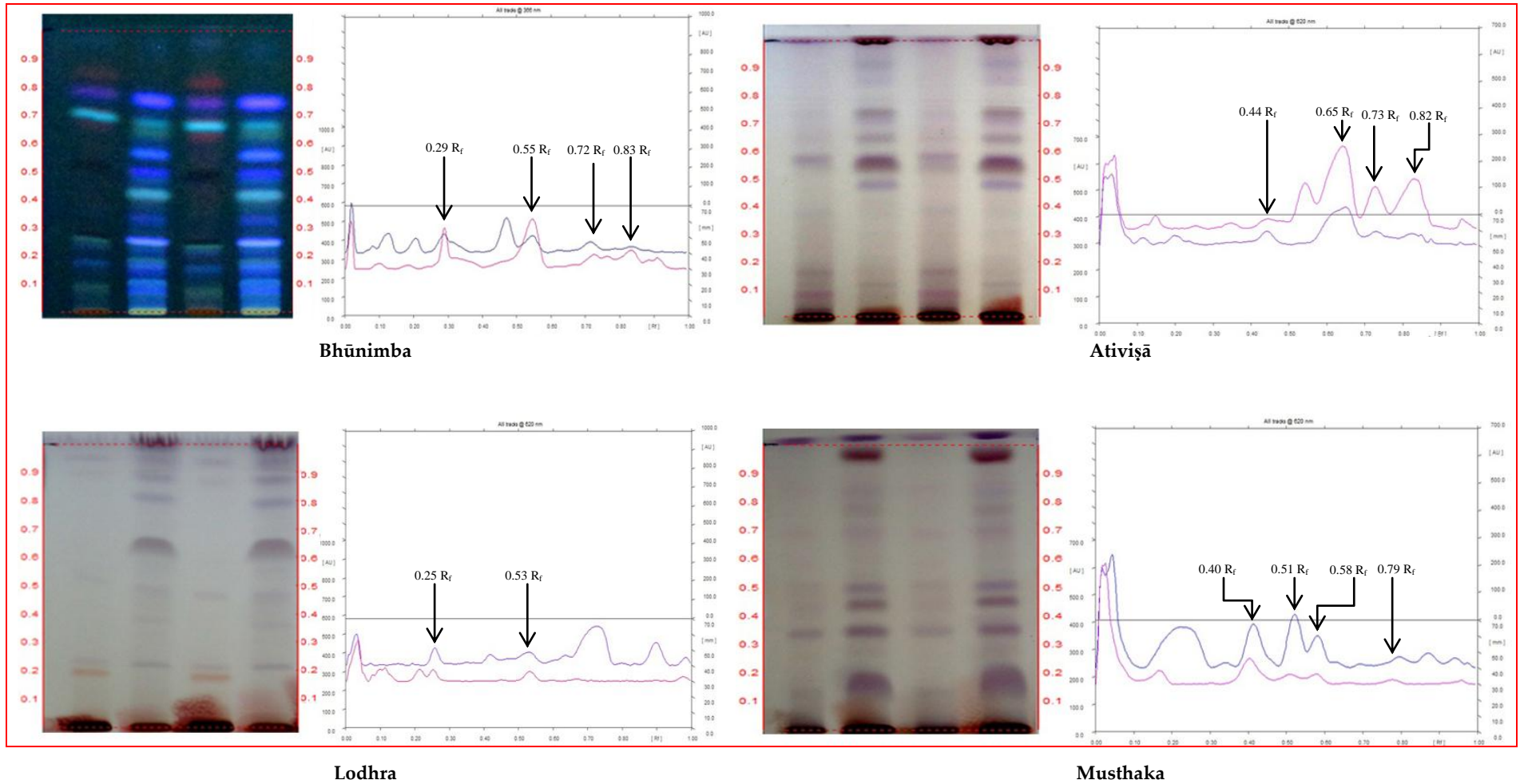
There were 7 peaks in BKC (5.4, 3.6, 3.5, 2.1, 1.6, 1.3, and 0.9 ppm), 11 in Ativiṣā (5.3, 4.3, 3.9, 3.8, 3.7, 3.6, 3.5, 3.2, 3.1, 3.1 and 1.3 ppm), 15 in Bilva (8.6, 7.9, 7.6, 6.9, 5.3, 3.9, 3.8, 3.6, 3.5, 3.3, 2.3, 2.2, 2.0, 1.7 and 1.3 ppm), 12 in Dhānyaka (5.1, 5.0, 4.7, 3.4, 3.2, 3.1, 2.9, 2.3, 2.0, 1.8, 1.0 and 0.6 ppm), 11 in Bālaka (9.4, 7.7, 7.2, 6.9, 5.7, 4.7, 2.1, 1.7, 1.5, 1.3 and 1.0 ppm), 24 in Indrayava (7.5, 7.1, 7.0, 6.8, 6.6, 6.3, 5.4, 5.3, 4.3, 4.2, 4.0, 3.9, 3.8, 3.6, 3.5, 3.4, 3.3, 3.2, 2.8, 2.2, 2.1, 1.5, 1.3 and 1.0 ppm), 13 in Lodhra (5.2, 4.9, 4.3, 3.9, 3.8, 3.7, 3.6, 3.5, 3.2, 3.15, 3.08, 2.9 and 1.3 ppm), 14 in Musthaka (9.48, 7.0, 5.4, 5.0, 4.8, 4.4, 3.8, 3.7, 3.6, 3.5, 3.3, 3.2, 1.3 and 0.9 ppm) and 14 in Bhūnimba (7.6, 7.0, 4.9, 4.6, 4.3, 4.1, 4.0, 3.7, 3.6, 3.5, 3.3, 3.2, 1.3 and 0.9 ppm). The peak at 5.4 ppm was seen in both Indrayava and Musthaka. Peak at 3.6 and 3.5 were seen in all ingredients except Dhānyaka and Bālaka. Peak at 1.3 ppm were seen in all ingredients except Dhānyaka. Peak at 0.9 ppm was seen in Musthaka and Bhūnimba. Peak at 3.6, 3.5 and 1.3 ppm were the peaks of Ativiṣā seen in BKC, peaks at 5.3, 4.3, 3.9, 3.8, 3.7, 3.2 and 3.1 were distinctive for Ativiṣā. Peak at 3.6, 3.5 and 1.3 ppm were the peaks of Bilva seen in BKC, peaks at 8.6, 7.9, 7.6, 6.9, 5.3, 3.9, 3.8, 3.3, 2.3, 2.2, 2.0 and 1.7 were distinctive for Bilva. There was no distinct peak of Dhānyaka seen in BKC. Peak at 2.1 and 1.3 ppm were the peaks of Bālaka seen in BKC, peaks at 9.4, 7.7, 7.2, 6.9, 5.7, 4.7, 1.7, 1.5 and 1.0 ppm were distinctive for Bālaka. Peak at 5.4, 3.6, 3.5, 2.1 and 1.3 ppm were the peaks of Indrayava seen in BKC, peaks at 7.5, 7.1, 7.0, 6.8, 6.6, 6.3, 5.3, 4.3, 4.2, 4.0, 3.9, 3.8, 3.4, 3.3, 3.2, 2.8, 2.2, 1.5 and 1.0 were distinctive for Indrayava. Peak at 3.6, 3.5 and 1.3 ppm were the peaks of Lodhra seen in BKC, peaks at 5.2, 4.9, 4.3, 3.9, 3.8, 3.7, 3.2, 3.15, 3.08 and 2.9 were distinctive for Lodhra. Peak at 5.4, 3.6, 3.5, 1.3 and 0.9 ppm were the peaks of Musthaka seen in BKC, peaks at 9.48, 7.0, 5.0, 4.8, 4.4, 3.8, 3.7, 3.3 and 3.2 were distinctive for Musthaka. Peak at 3.6, 3.5, 1.3 and 0.9 ppm were the peaks of Bhūnimba seen in BKC, peaks at 7.6, 7.0, 4.9, 4.6, 4.3, 4.1, 4.0, 3.7, 3.3 and 3.2 were distinctive for Bhūnimba (Table 1).

There are several PMs mentioned in the classics of Ayurveda, Siddha and Unani for different disorders. During routine quality control checking of PMs ingredients and the PM are fingerprinted using HPLTC using a single solvent system as reported in a earlier study on this PM.^[10] Mapping of each ingredient along with PM in a suitable solvent system will help more in spotting the ingredients more accurately. Analysis of presence and absence of ingredients in a PM is a challenging work though ¹H-NMR has been in use for detection of adulteration and substitution in herbal ingredients.^[11]

Table 1. ¹H NMR spectroscopy of in

BKC	Ativiṣā	Bilva	Dhānyaka	Hrivera	Indrayava	Lodhra	Musta	Bhūnimba
				9.4			9.48	
		8.6						
		7.9		7.7				
		7.6			7.5			7.6
				7.2	7.1			
					7		7	7
		6.9		6.9	6.8			
					6.6			
					6.3			
				5.7				
5.4					5.4		5.4	
	5.3	5.3			5.3	5.2		
			5.1					
			5				5	
						4.9		4.9
							4.8	
			4.7	4.7				4.6
							4.4	
	4.3				4.3	4.3		4.3
					4.2			
					4			4.1
								4
	3.9	3.9			3.9	3.9		
	3.8	3.8			3.8	3.8	3.8	
	3.7					3.7	3.7	3.7
3.6	3.6	3.6			3.6	3.6	3.6	3.6
3.5	3.5	3.5			3.5	3.5	3.5	3.5
			3.4		3.4			
		3.3			3.3		3.3	3.3
	3.2		3.2		3.2	3.2	3.2	3.2
	3.1					3.15		
	3.1		3.1			3.08		
			2.9			2.9		
					2.8			
		2.3	2.3					
		2.2			2.2			
2.1				2.1	2.1			
		2	2					
		1.7	1.8	1.7				
1.6				1.5	1.5			
1.3	1.3	1.3		1.3	1.3	1.3	1.3	1.3
0.9			1	1	1		0.9	0.9
			0.6					

Figure 1. HPTLC photo documentation and 3D display of Bhūnimbadi Kwatha Churna and its Ingredients



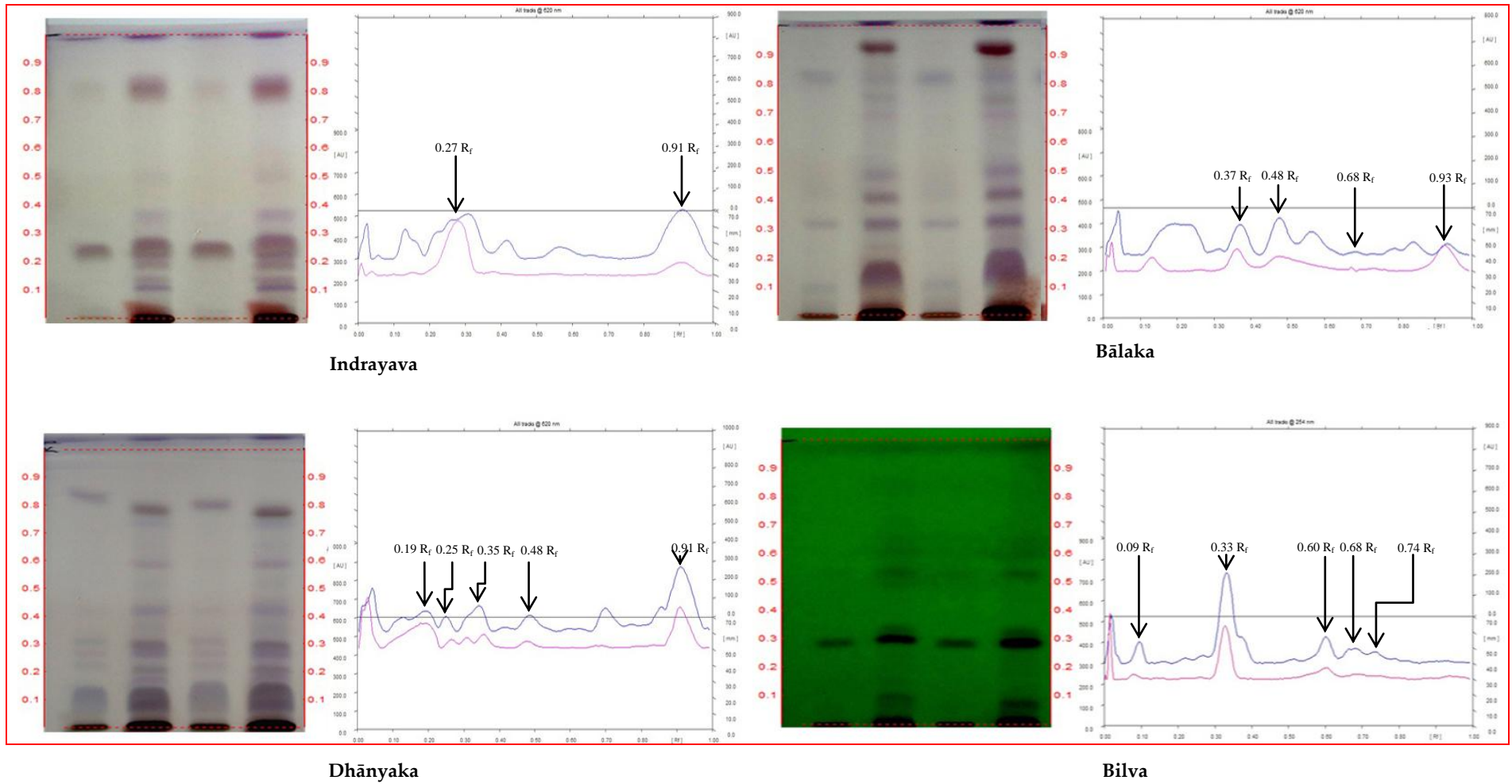
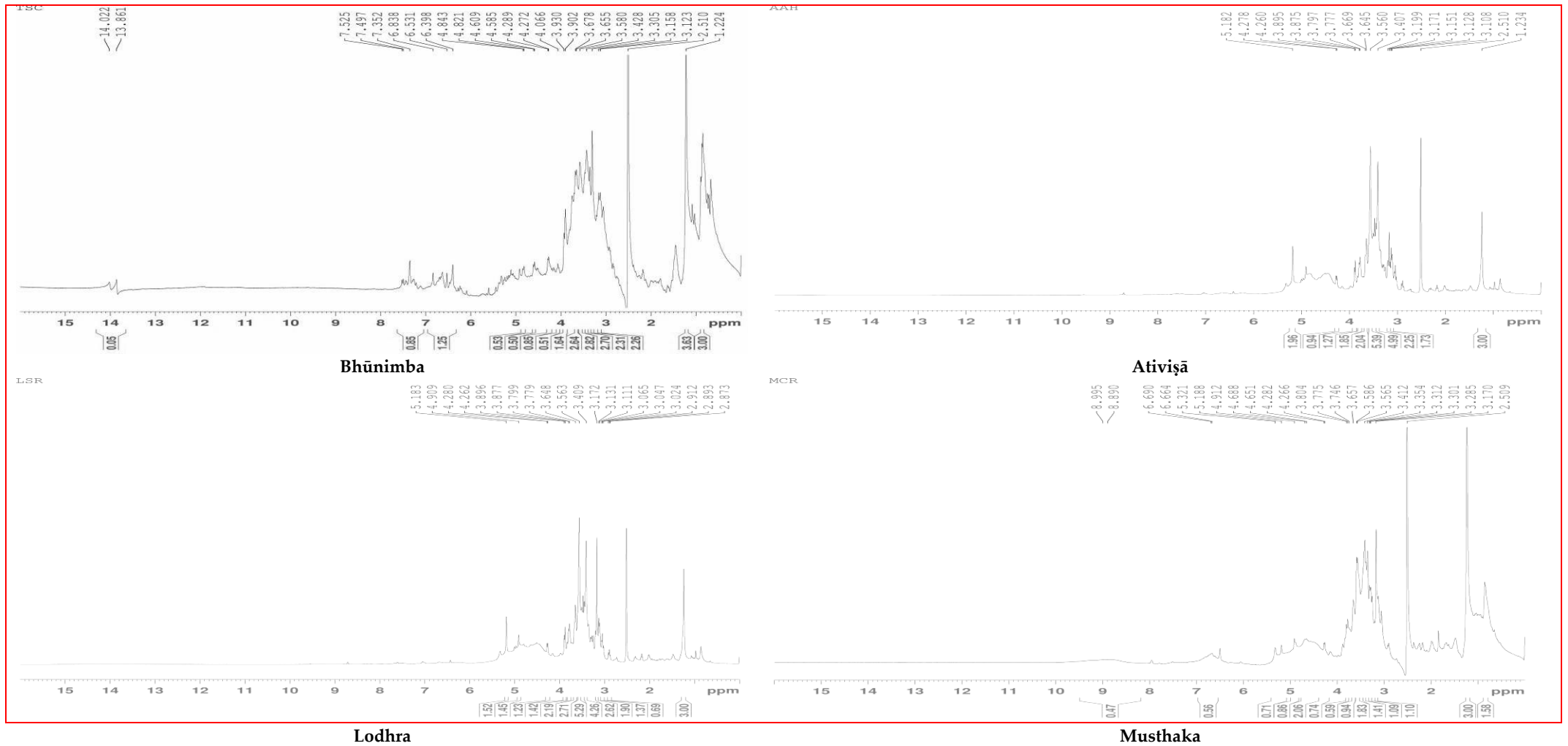
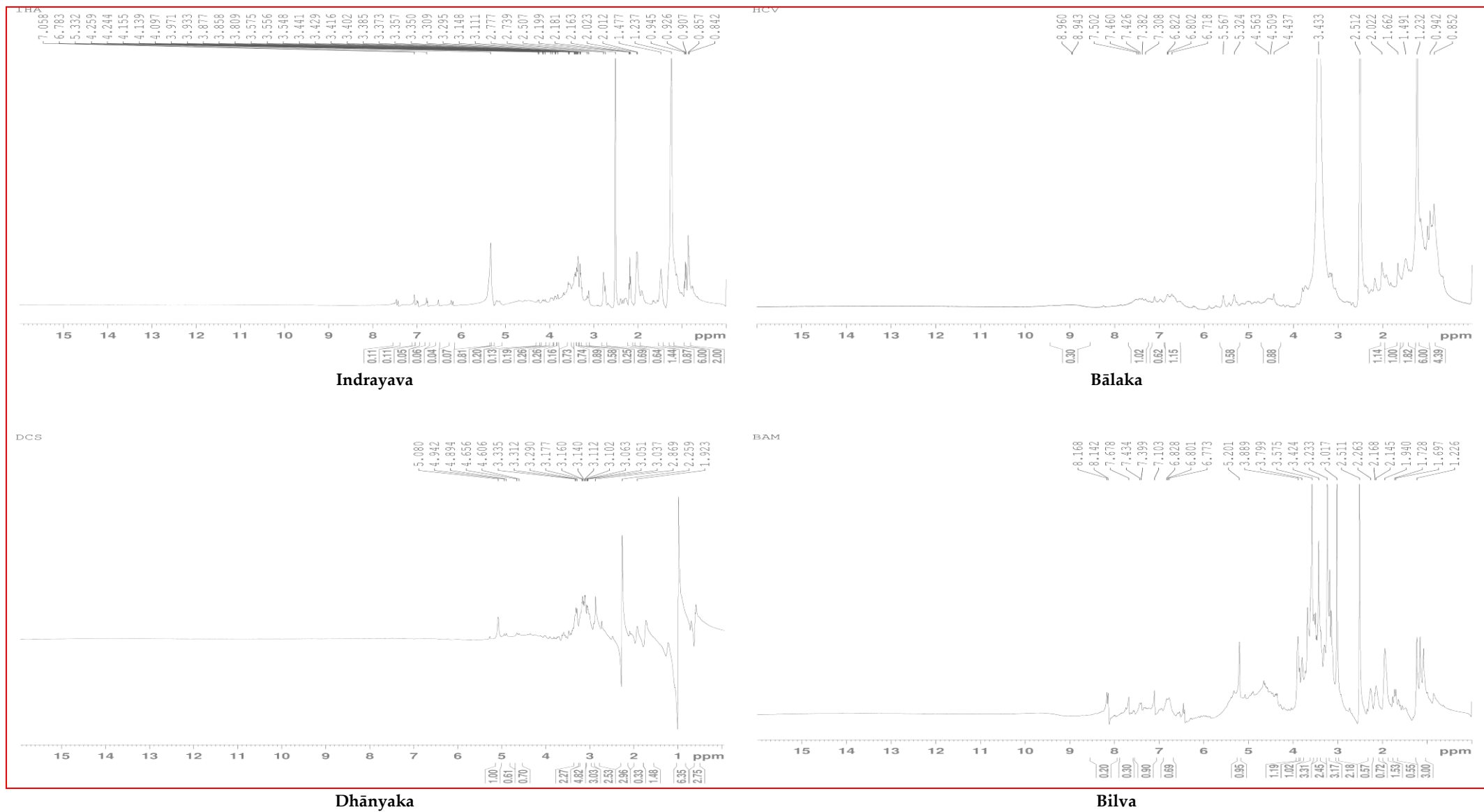
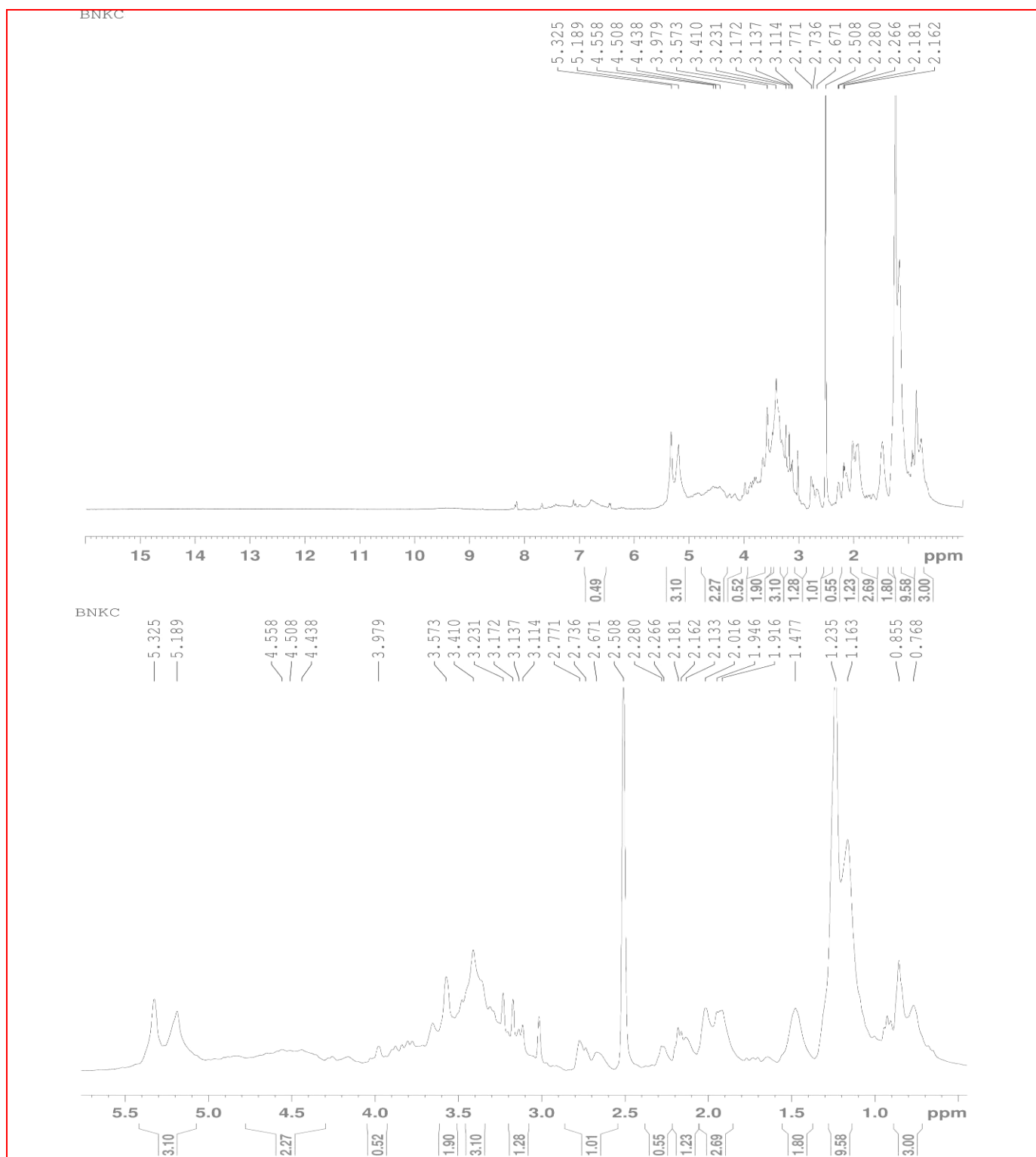


Figure 2. ¹H NMR spectrum of Bhūnimbadi Kwatha Churna and its Ingredients







Bhūnimbadi Kwatha Churna

CONCLUSION

The data obtained in the current study with multivariate analysis would help in drawing a novel standardisation platform for multiherb formulations used in traditional systems of medicine.

ACKNOWLEDGEMENT

This work was supported by the scheme of Seed Money for Young Scientists for Research (SMYSR) Project (No. VGST/P-3/SMYSR/GRD-291/2013-14 dated January 28, 2013) by Vision Group on Science and Technology (VGST), Govt. of Karnataka. Heartfelt thanks to Dr. S. Ananth Raj, Consultant, VGST, for the kind support. Authors are also grateful to Dr. D. Veerendra Heggade, President, SDME Society, Ujire for the support. Guidance from Dr. R. Muralidhar, Senior Manager, SDM Ayurveda Pharmacy is duly acknowledged.

CONFLICTS OF INTEREST

Nil

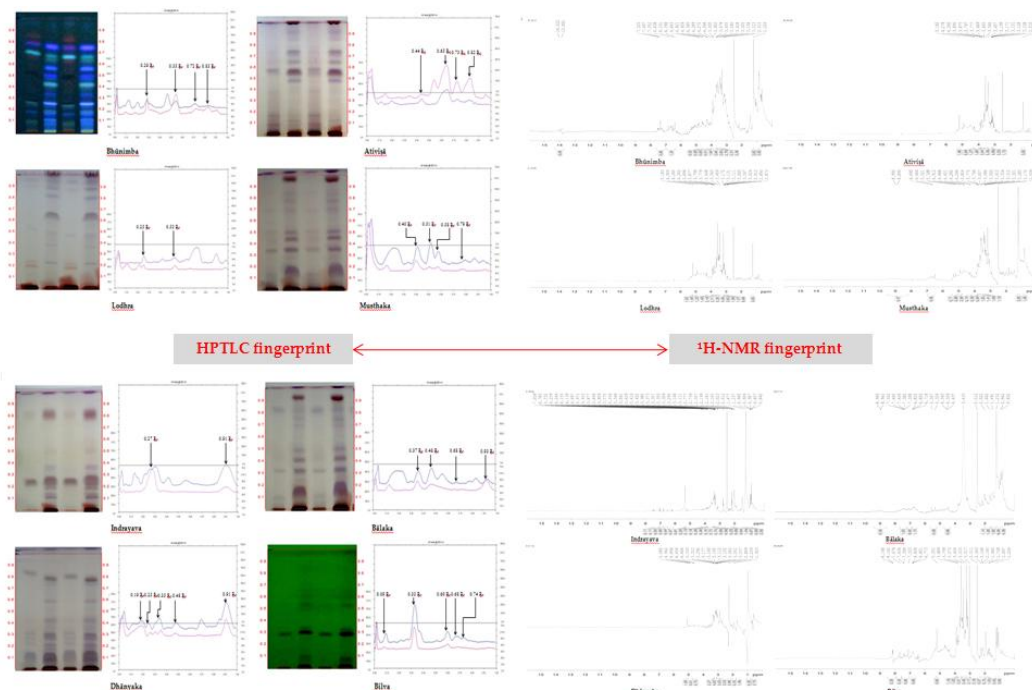
REFERENCE

1. Shailajan S, Menon SN. Polymarker based standardization of an Ayurvedic formulation, Lavangadi vati using high performance thin layer chromatography. J Pharmacy Res. 2011; 4:467-70.
2. Mueen Ahmed KK. Introduction to isolation, identification and estimation of lead compounds from natural products. In: Hiremath SR. Textbook of industrial pharmacy. Chennai, India: Orient Longman Private Ltd; 2008, p. 345-379.
3. Hota D. Evaluation of plant extracts. In: Bioactive medicinal plants. New Delhi: Gene-Tech Books; 2010, p. 86-87.
4. Anthony Booker, Debora Frommenwiler, Deborah Johnston, Chinenye Umealajekwu, Eike Reich, Michael Heinrich. Chemical variability along the value chains of turmeric (*Curcuma longa*): A comparison of nuclear magnetic resonance spectroscopy and high performance thin layer chromatography. Journal of Ethnopharmacology 152(2014)292-301
5. Ayurvedic Formulary of India. 1st ed., Part-III. New Delhi: Government of India, Ministry of Health and Family Welfare, Department of and Homoeopathy (AYUSH); 2011. p.91.
6. Sethi PD. High Performance Thin Layer Chromatography. 1st ed., CBS Publishers and Distributors, New Delhi, 10; 996. p. 1-56.
7. Stahl I. Thin layer Chromatography: a laboratory hand book. Springer-verlag, Berlin; 1969. p. 52-86.
8. Wagner H, Bladt S. Plant Drug Analysis. 2nd ed. Springer-verlag Berlin Hiedelber; 1996.
9. Kim, H.K., Verpoorte, R., 2010. Sample preparation for plant metabolomics. Phytochem. Anal. 21, 4-13.
10. Veronique Gilard, Stephane Balayssac, Aurelie Tinaugus, Nathalie Martins, Robert Martino, Myriam Malet-Martino. Detection, Identification and Quantification by 1H NMR of Adulterants in 150 Herbal Dietary Supplements Marketed for Improving Sexual Performance. Journal of Pharmaceutical and Biomedical Analysis 2014;102C:476-93.
11. Koppala Narayana Sunil Kumar, Priyadarshini, Basaviah Ravishankar, Betkeri Yashovarma. Quality standards for Bhūnimbādi Kvātha Chūrṇa. J Ayu Med Sci 2016;1(1):19-33. DOI: 10.5530/jams.2016.1.4

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GRAPHICAL ABSTRACT



Cite this article as: Sunil Kumar KN, Priyadarshini, Ravishankar B. HPTLC and ¹H-NMR as Fingerprints to Spot Ingredients of a Polyherbal Medicine Bhunimbadi Kvatha Churna. J Ayu Med Sci 2016;1(2):63-71. DOI:10.5530/jams.2016.1.10

