Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2024; 13(4): 01-06
Received: 02-04-2024
Accepted: 06-05-2024

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Validation of ancient principles of Bhaishajya Sangraha Kala using HPTLC technique: A comparative study of vasa (*Justicia adhatoda*) and saptaparna (*Alstonia scholaris*) leaves

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DOI: https://doi.org/10.22271/phyto.2024.v13.i4a.14988

Abstract
Background: Ayurveda emphasizes specific times for the collection of medicinal plants to ensure optimum potency and therapeutic efficacy. This study aimed to validate ancient principles of Bhaishajya Sangraha Kala (time of drug collection) by comparing the HPTLC analysis of leaves from Vasa (*Justicia adhatoda* L.) and Saptaparna (*Alstonia scholaris* R.Br) collected during different seasons.
Methods: Classical Ayurvedic texts were reviewed for guidelines on plant collection methods. Leaves of Vasa and Saptaparna were collected monthly from an herbal garden, dried, powdered, and subjected to HPTLC analysis. The methanolic extracts were prepared, applied onto silica gel plates, and developed using specific mobile phases. Rf values were obtained and interpreted. Days required for drying and the number of permissible peaks in HPTLC analysis were recorded.
Results: The study found variations in the Rf values of phytoconstituents in Vasa and Saptaparna leaves collected at different times of the year. The highest number of permissible peaks was observed during June-July and October-November, corresponding to the Warsha and Hemant ritu (seasons) respectively. The drying time of samples varied across seasons, likely influenced by factors such as temperature and humidity.
Discussion: Ayurvedic texts prescribe specific collection times for different plant parts, reflecting ancient wisdom on maximizing medicinal potency. The observed variations in Rf values indicate changes in phytochemical composition over time, suggesting a potential correlation with seasonal changes. These findings underscore the importance of traditional knowledge in guiding cultivation and collection practices.
Conclusion: The study validates the significance of specific collection times prescribed in Ayurvedic texts through HPTLC analysis, demonstrating variations in phytoconstituents of Vasa and Saptaparna leaves collected at different times. These findings provide a scientific basis for understanding the impact of Bhaishajya Sangraha Kala on medicinal potency and support the integration of traditional knowledge into modern herbal cultivation practices.

Keywords: Ayurveda, Bhaishajya Sangraha Kala, HPTLC, phytoconstituents, medicinal plants

Introduction
Ayurveda has given four important pillars of successful treatment under the heading of Chikitsa Chatushpada, which includes Bhishak (Physician), Dravya (Medicines), Upasthata (Nursing staff) and *Rogi* (Patient) [1]. Thus, availability of medicines having optimum quality is of utmost importance. Classical texts of Ayurveda i.e. Brihat-trayi has mainly dealt with the application of Herbal drugs, Thus, they have mentioned the process of herbal drug collection, storage and processing in very detail [2]. Whether collected from wild or cultivated, plant collection follows a particular method and are based on various criteria like plant part, season, time, method, purpose and drug potency.
Various Acharyas have mentioned specific time period for collection of different plant parts. The time of collection may be dependent on the possibility of presence of highest amount of active principle during the specific time period [3]. Thus, it is advisable to collect the part in the designated time period only. Practically it may not be possible to collect the parts according to the directions given by the Samhitas. This may hamper the expected therapeutic outcome of the herb/drug. Thus, it is needed to evaluate the plants for the presence of Active principles in various seasons throughout the year.
High performance thin layer chromatography (HPTLC) is the sophisticated method designed as a tool for identification, authentication and quality control of herbal medicines. It works on the principle of separation based on adsorption.

HPTLC has wide application as an analytical tool due to its simplicity, minimum sample-cleanup requirement, and ability to analyze a number of samples simultaneously [4]. It is mainly used to separate and identify the phytochemical constituents present in the plant sample [5].

Vasa (botanically identified as *Adhatoda vasica* L.) and Saptaparna (botanically identified as *Alstonia scholaris* R.Br) are two widely used herbs mainly in the treatment of respiratory and dermatological disorders. Leaves of both plants have been selected for the present study because of their easy availability around the year. Present study aims at the comparison of HPTLC analysis of methanolic extract of leaves of Vasa and Saptaparna collected from the identical source during the various seasons of the year.

Materials and Methods

Review study

Classical Ayurveda texts were screened for the references regarding methods for collection of plant materials.

Collection of Plant Materials and Authentication

The leaves of Vasa and Saptaparna (without galls) were collected from herbal garden of All India Institute of Ayurveda New Delhi and authentication done at department of Dravyaguna.

Drying of Plant Materials

The sample collected were washed under running tap water. The leaves were then kept for shade dry till it was dried completely. The dried sample was then powdered and passed through 80 no. sieve and stored in airtight container for further evaluation. Same process was repeated in each month.

Results and Discussion

Studies on combining ability revealed that the lines, L7, L1 and L3 were the good general combiners for total yield per plant in order of merit. Similarly, the line L7 for days to first flowering, early harvesting, plan for total yield per plant in order of merit. Similarly, T-height fruit length, fruit diameter and total number of fruits. L2 days to first flowering, for total yield per plant in order of merit.

HPTLC analysis

Sample preparation

One gram of dried powder mixed with 10 ml of methanol; it was then exposed to intermittent stirring for 6 hrs and then kept steady overnight. After 18 hrs, the sample was sonicated for 30 minutes and then filtered using Whatmann filter paper number 1. After filtration, the obtained filtrate was centrifuged at 25 °C for 10 minutes at 14000 rpm.

Stationary phase

Sample application

Sample application by linomat-5 automatic spotter machine by using Hamilton 100 µl syringe by spraying it using nitrogen gas. The HPTLC was performed on 10 x 10 cm aluminum silica gel 60 F254 plates. Amount of sample application of Vasa and Saptaparna was 10 µl and 5 µl respectively with same band length of 0.8 mm.

Sample drying

After sample application the plates are kept for drying by using HPTLC drier on temperature 40 degree Celsius. (5min).

Mobile phase

Vasa-Ethyl acetate: Chloroform: Ethanol: Ammonia (6:3:1:1)
Saptaparna-Hexane: Ethyl acetate: Methanol: Formic acid (5:4.5:0.5:0.5).

Mobile phase was prepared in volumetric flask of 100ml and then transferred to twin trough chambers separately. Then chamber was allowed to saturate for 20 minutes. After that the plates are kept in respective chambers for development. (Till the mark of 7cm) After proper development drying procedure was repeated and plated were scanned using CAMAG Scanner under 254 and 366 nm wavelength.

Observations

Table 1: *Aushadha sangarahan kala* (~time of drug collection) as per various classics

Sr. No.	Prayojyanga (Useful part)	Charak [6]	Sushruta [7]	Ashtang Sangraha [8]	Raj Nighantu [9]	Bruhat Nighantu ratnakarar
1.	Kanda (tuber)	Sharad	--	Sharad	Hima	Sharad
2.	Ksheera (latex)	Sharad	--	Sharad	--	Sharad
3.	Moola (root)	Greeshama Shishira	Pravrutta	Greeshma	Shishira	Grishma Shishira
4.	Patra (leaves)	Varsha, Vasant	--	Varsha,Vasant	Nidagha	Varsha, Vasant
5.	Phala (fruit)	Yatha rutu	Greeshma	Yatha rutu	--	Yatha rutu
6.	Pushpa (flower)	Yatha rutu		Yatha rutu	Vasanta	Yatha rutu
7.	Shakha (branches)	Varsha, Vasant	--	Varsha,Vasant	--	--
8.	Sara (heartwood)	Hemant	Vasant	Hemant	--	Hemant

Table 2: Days required for drying of each sample.

Days for drying	Vasa	Saptaparna
January	9	10
February	8	9
March	6	8
April	7	7
May	6	7
June	6	6
July	7	7
August	8	9
September	8	9
October	9	10
November	10	10
December	10	11

Table 3: Rf value of Vasa at 254 nm

January	0.61	0.74			
February	0.62	0.77			
March	0.28	0.54	0.68		
April	0.27	0.54	0.65		
May	0.32	0.43	0.55	0.71	
June	0.21	0.4	0.53	0.69	
July	0.42	0.64	0.76		
August	0.31	0.35	0.54	0.59	0.64
September	0.28	0.4	0.67	0.82	
October	0.28	0.40	0.67	0.62	
November	0.31	0.46	0.52	0.59	0.72
December	0.19	0.43	0.52	0.64	0.72

Table 4: Rf value of Vasa at 366 nm

January	0.11	0.61	0.74	0.86			
February	0.59	0.65	0.73	0.88			
March	0.43	0.54	0.57	0.68	0.80		
April	0.18	0.52	0.62	0.81			
May	0.20	0.31	0.44	0.56	0.65	0.69	
June	0.17	0.21	0.40	0.53	069		
July	0.13	0.22	0.32	0.46	0.55	0.61	0.74
August	0.12	0.35	0.56	0.65			
September	0.10	0.31	0.40	0.54	0.67		
October	0.35	0.46	0.55	0.68			
November	0.20	0.32	0.45	0.58	0.67	0.85	
December	0.19	0.43	0.52	0.64	0.72	0.81	

Table 5: Rf value of Saptaparna at 254 nm

January	0.48	0.63	0.76							
February	0.43	0.54	0.61	0.65	0.78					
March	0.27	0.59	0.69	0.73	0.80					
April	0.23	0.32	0.39	0.42	0.48	0.59	0.69			
May	0.23	0.44	0.52	0.58	0.66	0.73				
June	0.16	0.21	0.26	0.30	0.35	0.41	0.51	0.58	0.67	
July	0.16	0.30	0.37	0.44	0.59	0.67	0.75			
August	0.12	0.27	0.33	0.38	0.46	0.49	057	0.61	0.67	
September	0.32	0.41	0.53	0.56	0.63	0.68				
October	0.34	0.43	0.56	0.59	0.65	0.74	0.79			
November	0.12	0.16	0.21	0.26	0.31	0.36	0.44	0.48	0.56	0.61
December	0.13	0.52	0.64	0.69	0.76					

Table 6: Rf value of Saptaparna at 366 nm

January	0.49	0.55	0.61	0.69	0.80		
February	0.41	0.57	0.65	0.73	0.77		
March	0.48	0.59	0.73	0.80			
April	0.21	0.34	0.41	0.49	0.59	0.69	0.75
May	0.16	0.44	0.58	0.73	0.81		
June	0.18	0.24	0.32	0.38	0.41	0.52	0.57
July	0.17	0.23	0.30	0.43	0.60	0.67	0.75
August	0.31	0.39	0.49	0.57	0.67		
September	0.17	0.31	0.37	0.50	0.63	0.68	
October	0.20	0.34	0.45	0.63	0.73	0.86	0.92
November	0.13	0.15	0.21	0.26	0.36	0.55	0.70
December	0.20	0.38	0.50	0.58	0.63	0.69	

Number or permissible peaks

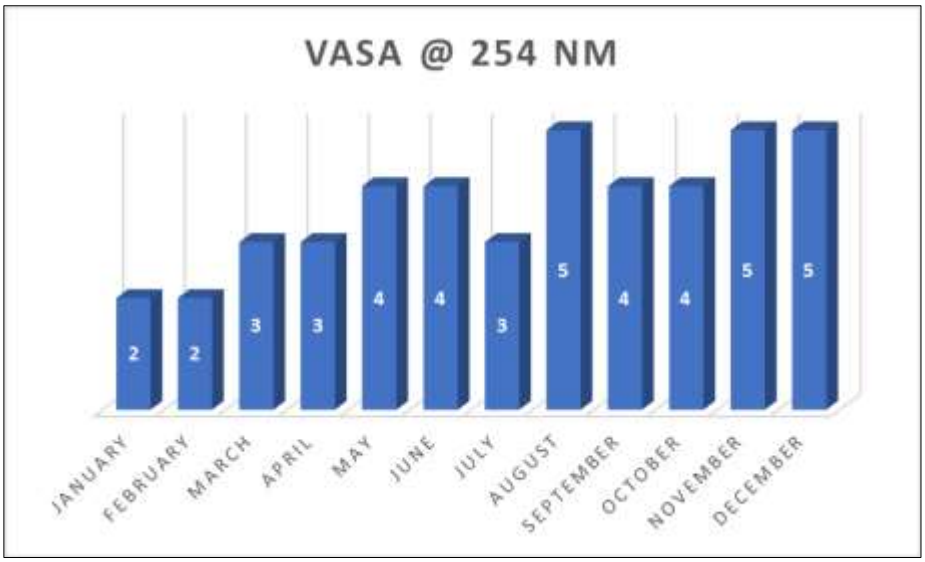

Fig 1: Vasa at 254 nm

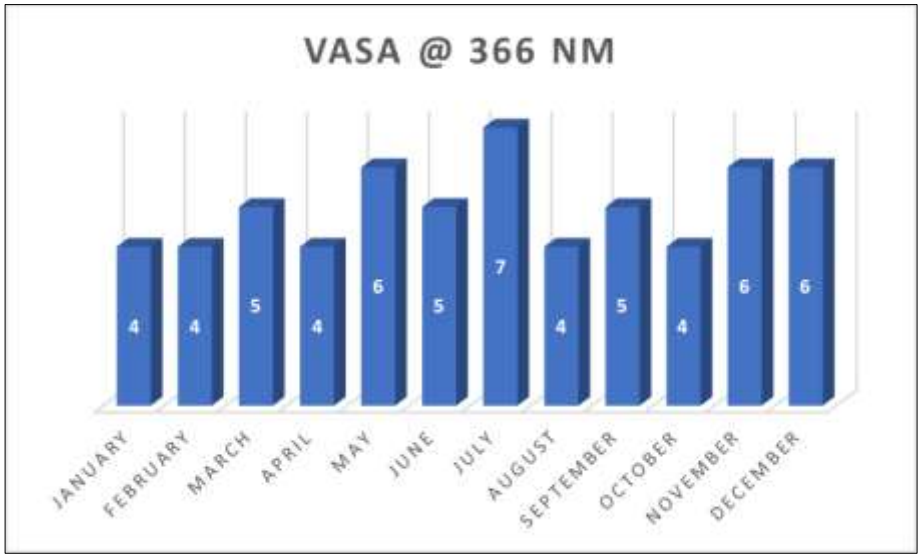

Fig 2: Vasa @ 366 nm

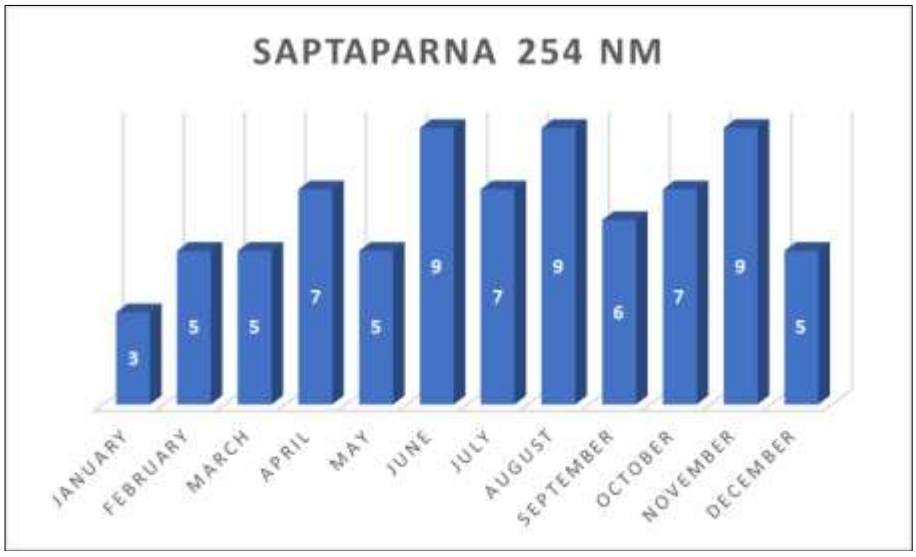

Fig 3: Saptaparna @ 254 nm

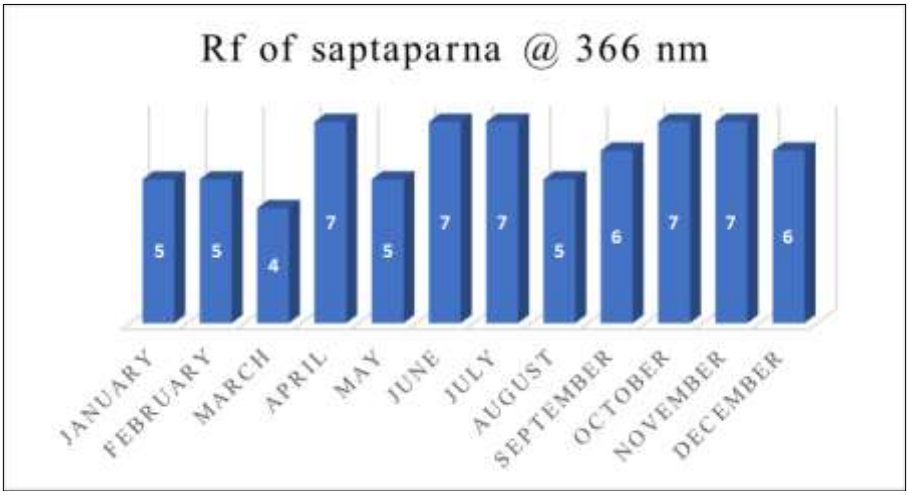

Fig 4: Saptaparna @ 366 nm

Analysis

The scanning of both samples was done by using TLC scanner at wavelength 254 nm (deuterium lamp) and 366 nm (mercury lamp). Then finds rf values and interpret the result.

Discussion

Quality, safety and efficacy are the three important aspects of any therapy used in Ayurvedic sciences. Therapeutic efficacy of any herb depends upon the presence of pharmacologically active constituents present and quality as well as quantity of secondary metabolites depends upon various factors [10]. Modern guidelines of Good Agricultural and Collection Practices (GACP) by World Health Organisation (WHO) which indicates that scholars of Ayurveda were quite aware about such practices and were practicing them for cultivation and collection of herbs. Ayurveda literature has minutely elaborated methods of collection and preservation of herbal medicine. Aushadha sangarahan kala (~time of drug collection) were elaborated by Acharya Charaka, Acharya Sushruta, Ashtang sangraha, Raj Nighantu and Bruhat Nighantu ratnakarar [11]. They all describe the part used to collected in that typical session and can be preserved properly for therapeutic use (Table no 1). In depth analysis shows that Bruhat Nighantu ratnakarar and Ashtang sangraha follows the charak Samhita, Acharya Sushruta has only discussed about Moola, Phala and sara in Pravrutta, Greeshama and Vasant respectively. Raj Nighantu gives his separate opinion regarding leaves and flower collections, it says the leaves should be collected in Nidagh Rutu that is Greeshma Rutu and flowers in Vasant Rutu. The exact scientific basis of these methods is elaborated neither by any Acharya nor by any commentator. Thus concerning with the contemporary concepts of pharmacology, the increased potency of the drugs can be attributed to increased quality and/or quantity of active phytoconstituents present in the plant.

High performance thin layer chromatography (HPTLC) is a sophisticated method designed as a tool for identification, authentication and quality control of herbal medicines. It works on the principle of separation based on adsorption and has wide application as an analytical tool due to its simplicity, minimum sample-cleanup requirement, and ability to analyze a number of samples simultaneously. Phytochemicals present in the extract move on the stationary phase due to capillary action, the distance travelled by the constituent in proportion to that of solvent (mobile phase) is given is terms of Rf, calculated as distance traveled by component/distance traveled by solvent. Thus, it can be assumed that each specific permissible Rf represents a specific active phytoconstituent present in the extract [12]. Hence, number of permissible Rf values obtained after scanning the plate at 254 and 366 nm wavelength, represent the probable number of active phytoconstituents present in that extract (Fig no 1-4). In the present study, method of extraction, stationary as well as mobile phase were kept constant for all the 12 studies, thus presence of variation in Rf values is indicative of presence of variation in separated phytoconstituents (Table no 3-6). Thus, HPTLC analysis of similar plant sample collected at different time may have difference in Rf values, which indicates the variation in presence of phytoconstituents over the period of time.

The sample collection at morning time is given by classical Texts of ayurveda. The concept of *Chhayashushkata* (~shade drying) is also described in classics of Ayurveda. The time required for drying of samples are different according to season which are given in table. The probable reason behind the variation of time required for drying was may be temperature difference, humidity, water content of plant etc. From the HPTLC studies it can be noted that, highest number of permissible Rf values were obtained during the months of June-july and October-November. From the available correlation between Gregorian calendar with ancient Indian system of Ritu, it can be inferred that, Months of June-July correspond to Warsha while October-November correspond to Hemant ritu. Higher number of permissible Rf values in these months, suggest presence of higher number of phytoconstituents present in the sample. Further sophisticated analysis like Gas chromatography and Mass spectrometry studies may be done to quantify the phytoconstituents obtained during each month.

Conclusion

Variation in HPTLC analysis of herb sample collected from same source during different time interval over the year is indicative of changes in phytochemical constitution of the herb. So this study can be used as a basis for further analysis regarding importance of specific time of drug collection and will concrete the basis of good cultivation practices in Ayurveda.

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