

# High Performance Thin Layer Chromatographic Standardization and Quantification of Marker Compounds in an Ayurvedic Polyherbal Formulation *Padmakadi Churna*

Ravichand Meshram<sup>1</sup>, Umakant Sahu<sup>2</sup>, Arun K S Parihar<sup>2</sup>, Prashant K Gupta<sup>1,3</sup>, Nagendra Singh Chauhan<sup>2,\*</sup>

<sup>1</sup>Shri Narayan Prasad Awasthi Government Ayurved College, Raipur, Chhattisgarh, INDIA.

<sup>2</sup>Drugs Testing Laboratory Avam Anusandhan Kendra, G.E. Road, Raipur, Chhattisgarh, INDIA.

<sup>3</sup>Department of Kaumarabhritya, All India Institute of Ayurveda, New Delhi, INDIA.

## ABSTRACT

The aim of this study was to find out a simple, accurate and sensitive HPTLC method for the detection and quantification of marker molecule Gallic and Piperine in *Padmakadi churna* for standardization. Ethanolic extraction was performed for analysis. HPTLC was done using gallic acid and Piperine as a standard. The mobile phase was a mixture of Toluene:ethyl acetate:Ethanol:Formic acid (6:3:1:0.3 v/v/v) and detection at 254 nm. The  $R_f$  was detected at - 0.39 for Gallic acid and  $R_f$  - 0.55 for Piperine was identified in *Padmakadi Churna*. The simple and sensitive HPTLC method was successfully developed for determination of Gallic acid and Piperine in *Padmakadi Churna*. The proposed HPTLC method was found to be simple, fast and inexpensive, and can be used for the routine quality control of raw materials.

**Keywords:** *Padmakadi Churna*, Physicochemical, HPTLC, Gallic acid, Piperine, Standardization.



www.ijnponline.com

DOI : 10.5530/ijnp.2022.1.4

## INTRODUCTION

*Padmakadi Churna* or *Padmakadileha* is a Ayurveda formulation which is commonly used in *Kasa* (Cough). *Padmakadi Churna* is used to Treatment of *Kasa* (cough), its various components have *Rasayana guna* (immune modulatory properties) and also regulate *Dhatvagni* (Metabolism) and promote the health of the child. *Padmakadi Churna* is unique concept of *Ayurveda*. It has described about *charak samhita* in *kasa roga chikitsa*. It prevent to all type of cough (*kasa roga*).<sup>[1]</sup> *Padmakadi Churna* is commonly used for the management of all type of *kasa roga* having 11 herbal drugs *Prunus cerasoides*, *Terminalia chebula*, *Emblia officinalis*, *Terminalia bellirica*, *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Embelia ribes*, *Cedrus deodara*, *Sida cordifolia* and *Pluchea laccolata*.

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Yoga, Siddha and Unani. India can emerge as the major country and play the lead role in production of standardized, therapeutically effective Ayurvedic formulations. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analysed using sophisticated modern techniques of standardization. However, one of the impediments in the acceptance of the Ayurvedic formulations is the lack of standard quality control profiles. Herbal medicines are considering an enhance can be well target to paediatrics age group clinical practice also substantiate the inclination of society towards Ayurveda for common paediatrics ailments. Due to the complex nature and inherent variability of the chemical constituents of the plant-based drugs, it is difficult to establish quality control parameters, therefore the modern analytical techniques are expected to help in circumventing this problem. Physicochemical Parameter of *Padmakadi Churna* was performed as reported for churna using method given Ayurvedic Pharmacopoeia of India.<sup>[2]</sup>

Herbal medicine is in the huge demand across the globe as far as primary healthcare is concerned. They have wide spectrum biological activities, highly economical, very low ADR and good safety profile.<sup>[3]</sup> The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards.<sup>[1]</sup> Pharmaceutical analysis helps to confirm the quality of finished product. The pharmaceutical analysis is such a branch of chemistry, which includes the series of process like identification, determination, quantization, and purification. That is in particular used for the separation of the components from the mixture and for the determination of the structure of the compounds.<sup>[4]</sup> Analytical study of any drug is essential to check the quality of the drug and to standardize it. In the present era of increasing global demand for Ayurvedic medicines, quality control for efficacy and safety of herbal products is of paramount importance.<sup>[5,6]</sup> The authenticity, quality, and purity of herbal drugs are established by references given in pharmacopoeia.<sup>[7,8]</sup> In the light of above background, the present study was undertaken to ascertain the quality standards of formulations. Standardization of *Padmakadi Churna* is not reported till date. So, this study was conducted with the aim to standardize this formulation with respect to its physicochemical properties, HPTLC quantification and fingerprint study.<sup>[9,10]</sup>

## MATERIALS AND METHODS

All chemicals and reagent used were analytical grade. Standard marker drugs gallic acid and Piperine procured from Sigma-aldrich Merck Bangalore, India.

### Preparation of *Padmakadi Churna*

*Padmakadi Churna* has eleven ingredient *Prunus cerasoides*, *Terminalia chebula*, *Emblia officinalis*, *Terminalia bellirica*, *Zingiber officinale*, *Piper*

**Correspondence:** Dr. Nagendra Singh Chauhan, Senior Scientific Officer and Government Analyst, Drugs Testing Laboratory Avam Anusandhan Kendra, Raipur-492 010, Chhattisgarh, INDIA. Email id: chauhan.nagendra@gmail.com.

*nigrum*, *Piper longum*, *Embelia ribes*, *Cedrus deodara*, *Sida cordifolia* and *Pluchea laccolata* which were purchased from Raipur market and authenticated by Prof. P.K Joshi department of *Dravyaguna*. After investigation, in collaboration with the department of *Rashstra* and *Bhaishajya Kalpana*, very fine powder was prepared by *Churna Kalpana* method mentioned in Ayurveda Table 1. This formulation was prepared by taking all the herbs in equal proportion. The sieve no. 120 was used to prepare *Padmakadi Churna* and finally kept in air tight container.

### Physicochemical parameters

*Padmakadi Churna* analysis according to the general parameters for *churna* given in the Ayurvedic Pharmacopoeia of India (API,2011), particle fitness, loss on drying (%), total ash (%), Acid insoluble ash (%), alcohol-soluble extractive(%), water-soluble extractive(%), pH(10 % aqueous solution).

### High-Performance Thin Layer Chromatographic Analysis<sup>[11]</sup>

#### Preparation of Ethanolic extract

Each 100 mg of *Padmakadi Churna* sample in volumetric flask capacity of 10 ml and dissolved with 5 ml ethanol separately and sonicated for 10 min, after that the volume was makeup upto 10ml by using ethanol. Filter the solution with 0.45µ membrane filter to get clear solution which was used in HPTLC study.

#### Preparation of standard solution

10 mg of standard Gallic acid and Piperine each was dissolved in 5ml ethanol in two different 10 ml volumetric flask and sonicated for 10 min, after that the volume was makeup upto 10 ml by using ethanol. Filtered the solution by using 0.45 µ membrane filter to remove any type of get clear solution.

### Chromatographic Conditions

Precoated silica gel 60F<sub>254</sub> was taken as stationary phase and applied the band of different concentration by using Linomate 5 sample applicator. This plate was developed in solvent system Toluene: Ethyl acetate: Ethanol: Formic acid (6: 3: 1:0.3) up to 7mm from the solvent front. After development of TLC plate it was scanned in scanner 4 and quantified the amount of gallic acid and piperine present in *Padmakadi Churna* by densitometric method.

**Table 1: Ingredients of Padmakadi Churna.**

Sl. no.	Herb name	Botanical name	Part to be used	Ratio
1.	Padmaka	<i>Prunus cerasoides</i> D. Don	Twak	1 part
2.	Haritaki	<i>Terminalia chebula</i>	Phala	1 part
3.	Amlaki	<i>Embelia officinalis</i>	Phala	1 part
4.	Vibhitaki	<i>Terminalia bellirica</i>	Phala	1 part
5.	Shunthi	<i>Zingiber officinale</i>	Kanda	1 part
6.	Marich	<i>Piper nigrum</i>	Phala	1 part
7.	Pippli	<i>Piper longum</i>	Phala	1 part
8.	Vidang	<i>Embelia ribes</i>	Phala	1 part
9.	Deodaru	<i>Cedrus deodara</i>	Kandsara	1 part
10.	Bala	<i>Sida cordifolia</i>	Mula	1 part
11.	Rasna	<i>Pluchea laccolata</i>	Patra	1 part

### Preparation of calibration curve

On Precoated silica gel 60F<sub>254</sub> stationary phase 5 band of different concentration of each standard Gallic acid (0.3 µg, 0.9 µg, 1.5 µg, 2.1 µg, 2.7 µg and 3.0 µg) and Piperine (0.3 µg, 0.9 µg, 1.5 µg, 2.1 µg, 2.7 µg and 3.0 µg) was applied to prepare calibration curve and band of ethanolic extract of sample *Padmakadi Churna* of was a by using Linomate 5 sample applicator.

The standard solutions of Gallic acid (0.3, 0.9,1.5, 2.1, 2.7 and 3.0 µL/spot) and Piperine (0.3, 0.9,1.5, 2.1, 2.7 and 3.0 µL/spot) were applied on TLC plate and further it was developed and scanned as per the chromatographic conditions mentioned above. The peak areas were recorded. Calibration curve of Gallic acid and Piperine was prepared by plotting peak area against concentration of Gallic acid and Piperine Figure 1.

### Quantification of Gallic acid and Piperine content in Padmakadi Churna

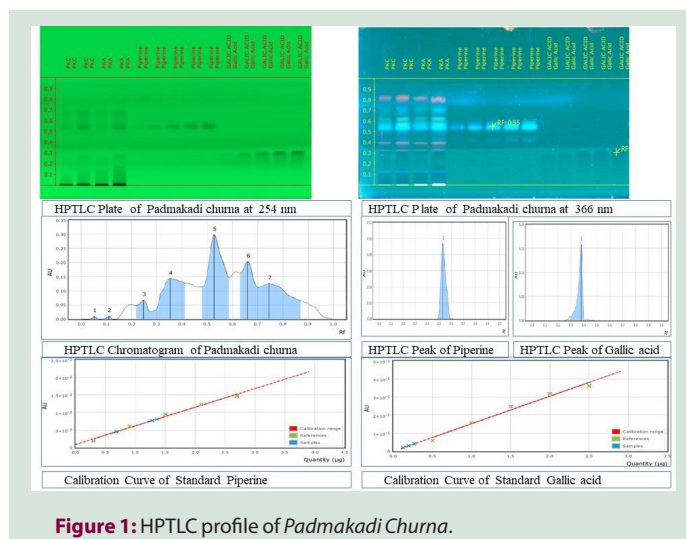
Gallic acid and Piperine content in ethanolic extract of sample *Padmakadi Churna* formulation were determined. After development in Solvent system the plate were scanned at 254 nm. The amount of Gallic acid and Piperine content were calculated from their respective Calibration curves by using densitometric method.

## RESULTS AND DISCUSSION

Ayurvedic tradition medicine is going more and more popular around the world nowadays. The practices of ayurveda deal with diseases and illness with herbs. The physicochemical value of *Padmakadi Churna* was reported in Table 2. HPTLC technique is one of the most sophisticated

**Table 2: Result of physico-chemical analysis of the Padmakadi Churna.**

S I.no.	Test name	Result
1.	Loss on drying(%)	6
2.	Total Ash(%)	7.8
3.	Acid-insoluble ash (%)	1.66
4.	Water soluble extractive(%)	7.1
5.	Alcohol soluble extractive(%)	17.24
6.	pH value(10% aqueous solution)	4.8



**Figure 1: HPTLC profile of Padmakadi Churna.**

techniques available for the standardization and quantification of herbal drugs. *Padmakadi Churna* is a polyherbal ayurvedic medicine which is known for the management of the *Kasa* (cough). Present study was design to determine the quantities of Gallic acid and Piperine present in poly herbal ayurvedic formulation *Padmakadi Churna*. Sample is extracted with ethanol and sonicated for 10 min and filtered with 0.45  $\mu$  membrane filter, filtrate was used for HPTLC study. Solvent system, Toluene: Ethyl acetate: Ethanol: Formic acid (6: 3: 1:0.3 v/v/v) showed better separation for Gallic acid and Piperine, the plate was scanned at 254 nm for densitometry chromatographic evaluation on a Camag Scanner IV using visionCATS software. All the corresponding spectra is overlapping with markers confirm the presence and quantified the amount of markers present in sample. The quantities of the marker compound Gallic acid and piperine in ethanolic extract of *Padmakadi Churna* was found to be 241  $\mu$ g and 1.320  $\mu$ g respectively in 100 gm of sample.

## CONCLUSION

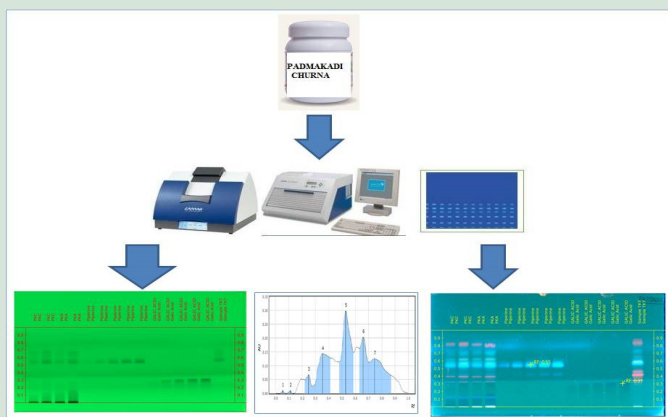
A HPTLC method has been developed for determination and quantification of Gallic acid and Piperine in *Padmakadi Churna*, a poly herbal ayurvedic formulation. From the above-mentioned results, it can be concluded that HPTLC technique is low cost, fast, precise and accurate which can be successfully employed for the quantification of plant markers. Developed HPTLC method can be contently used for

routine standardization/ quality control or analysis of the entire two marker compound for *Padmakadi Churna*.

## REFERENCES

1. Dash B, Sharma BK, Samhita Charak, Sanskrit C. Series. Office, Varanasi, India. 7<sup>th</sup> ed. 2001.
2. Anonymous. The ayurvedic pharmacopoeia of India, Part 2. 1<sup>st</sup> ed. New Delhi: Ministry of Health and Family welfare, Department of AYUSH Government of India; 2008;1.
3. Shrikumar S, Ravi TK. Approaches towards development and promotion of herbal drugs. *Pharm Rev.* 2007;1:180-4.
4. Sudha CPD. *Pharmaceutical analysis*. London: Pearson Education, New Delhi; 2012;3.
5. Anonymous. Guidelines on quality of herbal medicinal products/traditional medicinal products, EMEA/CVMP/81400 review. London: European Agency for the Evaluation of Medicinal Products (EMA) publications; 2005.
6. Anonymous. The Use of Essential Drugs, Eighth report of the WHO Expert committee. Geneva: World Health Organization publications. 1990.
7. Anonymous. Quality control methods for medicinal plant materials. Geneva: World Health Organization; 1998.
8. Anonymous. Parameters for qualitative assessment of Ayurveda and Siddha drugs, Part A. New Delhi: CCRAS; 2005;31.
9. Anonymous. The Ayurvedic Pharmacopoeia of India. 1<sup>st</sup> ed. Vol. III(III) (Formulations). New Delhi: Department of AYUSH; 2008;161. Appendix 2.
10. Laboratory guide for the analysis of Ayurveda and Siddha formulations. Central council for research in Ayurveda and Siddha. New Delhi: Department of AYUSH, Ministry of Health and Family welfare, Government of India-110058. 30-1.
11. Stahl E. *Thin-layer chromatography*. 2<sup>nd</sup> ed. Springer-Verlag New York, Inc 175 5<sup>th</sup> Ave. New York. 1969;125-33.

## GRAPHICAL ABSTRACT



## ABOUT AUTHORS



**Dr. Ravichand Meshram MD** (Paediatrics) from Shri Narayan Prasad Awasthi Government Ayurved College, Raipur, Chhattisgarh, India. Currently working as Ayurved Medical Officer Balod Chhattisgarh.



**Mr. Umakant Sahu**, M.Pharm, (Ph.D persuing ) presently working as Scientific Officer Chemistry in Drugs testing Laboratory Avam Anusandhan Kendra Raipur (C.G.) under Directorate of Ayush, Health and Family Welfare, Govt. of Chhattisgarh. He is eminent scientist and academician, expertise in various sophisticated instruments handling and analysis, standardization, quality control of classical and other class of ayush/ allopathic drug as per pharmacopoeial monographs. Published various research worked in reputed journals and has served as Assistant Professor in different organization having 13 Years of academic and research experience.

## SUMMARY

India has the potential to become a significant nation and take the lead in the creation of standardised, medically effective Ayurvedic medicines. India must investigate the crucial herbs for medicine. Only if the herbal items are examined and analysed utilising advanced current standardising techniques will this be possible. The absence of standardised quality control profiles is one barrier to the Ayurvedic medicines' acceptability, though. It is challenging to develop quality control guidelines for plant-based medications due to their complex nature and inherent variability, hence contemporary analytical techniques are used instead. The Indian Ayurvedic Pharmacopoeia technique was used to perform the physical-chemical parameters of the Padmakadi Churna. As far as primary healthcare is concerned, herbal medicine is in extremely high demand all around the world. They offer a broad range of biological activities, are very cost-effective, have a very low ADR, and have a favourable safety profile. The WHO assembly has stressed the necessity for quality control of medicinal plant products by adopting cutting-edge methods and appropriate standards in a number of decisions. Padmakadi Churna standardisation has not yet been recorded. In order to standardise this formulation with respect to its physicochemical characteristics, HPTLC quantification, and fingerprint study, this study was carried out.



**Arun Kumar Singh Parihar** is young researcher currently working as Scientific Officer at Drugs testing Laboratory Avum Anusandhan Kendra, Raipur (C.G.). He did his B. Pharm Guru Ghashidas Vishwavidalaya, Bilaspur and M.Pharm from University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.). He has nearly 6 years of research experience, 2 years of teaching experience and 2 years of worked as Gov. Pharmacist. He has to his credit more than 7 research/review papers and 2 book chapters published in national and international journals. He has delivered invited talks at several conferences/workshops as subject expert and resource person. His areas of thrust include development of Quality standardaridzation of Ayurvedic various formulation.



**Dr. Prashant Kumar Gupta** is working as an Associate Professor-Kaumarabhritya, All India Institute of Ayurveda, New Delhi, earlier associated with Shri NPA Govt Ayurveda College, Raipur as Reader and Head- Kaumarabhritya. He is a clinician and researcher and has more than 50 publications, 2 patents, 3 books, 5 chapters, and 2 national fellowships of DHR and INSA. He has contributed to various institutional and national policy drafts of AYUSH.



**Dr. Nagendra Singh Chauhan** obtained his M.Pharm, Ph.D from the Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar in 2006 and 2011. He is currently working as a Senior Scientific Officer Grade-II and Government Analyst at the Drugs Testing Laboratory Avam Anusandhan Kendra, Raipur, Chhattisgarh, India. He has professional expertise in natural product isolation and phytopharmacology. He has written more than 75 articles published in national and international journals, 22 book chapters and edited five books.

**History:** Submission Date: 06-11-2022; Review Completed: 29-11-2022; Accepted Date: 15-12-2022

**Cite this article:** Meshram R, Sahu U, Parihar AKS, Gupta PK, Chauhan NS. High Performance Thin Layer Chromatographic Standardization and Quantification of Marker Compounds in an Ayurvedic Polyherbal Formulation *Padmakadi Churna*. Indian J Nat Prod. 2022;36(1):24-7.