International Research Journal of Ayurveda & Yoga Vol. 6(11), pp. 25-29, November, 2023
Available online at http://irjay.com
ISSN: 2581-785X
DOI: 10.47223/IRJAY.2023.61105

ORIGINAL RESEARCH ARTICLE

High-performance Thin-layer Chromatography Fingerprinting Analysis of *Punarnavadi Kwatha*

Resmi Vijayan[1]*, P. Y. Ansary[2], Sara Moncy Oommen[3], V. V. Shincymol[4]

[1]PG Scholar, Department of Dravyaguna Vijnanam, GAVC, Tripunithura, Kerala, India.
[2]HOD and Professor, Department of Dravyaguna Vijnanam, GAVC, Tripunithura, Kerala, India.
[3]Professor and HOD, Department of Dravyaguna Vijnana, Government Ayurveda College, Kannur, Kerala, India.
[4]Associate Professor, Department of Dravyaguna Vijnanam, GAVC, Tripunithura, Kerala, India.

ARTICLE INFO

Article history:
Received on: 10-10-2023
Accepted on: 23-11-2023
Published on: 30-11-2023

Key words:
High-performance thin-layer chromatography
fingerprinting
Punarnavadi kwatha,
Peaks,
Phytoconstituents

ABSTRACT

Introduction: *Punarnavadi kwatha* is one among the commonly used medicine in Ayurvedic practice, mentioned in *Chakradatta* in *sopha chikitsa.* Main indications of *Punarnavadi kwatha* are *Sarvanga sopha* (generalised oedema), *Udara* (ascites), *Kasa* (cough), *Soolam* (colicky pain), and *Swasa* (dyspnoea) associated with *Pandu* (anaemia). For the purpose of quality assurance and uniformity, scientific verification of Ayurvedic medications is important. Usage of modern analytical techniques helps in the proper authentication of medicines. In the present study, high-performance thin-layer chromatography (HPTLC) fingerprinting of *Punarnavadi kwatha* aimed to assess the different phytoconstituents.

Materials and Methods: Methanolic extract of *Punarnavadi kwatha* was subjected to HPTLC fingerprinting using the solvent system toluene:ethyl acetate:formic acid (6:3:0.5). Toluene is a least polar solvent, ethyl acetate is mid polar solvent and formic acid is highly polar so in this solvent system, maximum separation of compounds took place.

Results and Discussion: The number of peaks obtained at visualization 254 nm was 8 and total area of compounds in is 30309.5. Visualization at 366 nm, number of peaks was 12 and total area is 42473.2.

Conclusion: Thus, the study revealed that HPTLC fingerprinting of formulations can be used in the quality control of ayurvedic medicaments. The maximum separation of compounds occurred at this solvent system.

1. INTRODUCTION

Punarnavadi kwatha is a most commonly used polyherbal formulation in Ayurvedic medical practice. There are many references for the formulation in ancient scriptures of Ayurveda. In *Chakradatta* (*Chikitsa Samgraha*), a unique textbook of Ayurveda, first documented description about *Punarnavadi kwatha* is seen while explaining *Chikitsa* (treatment) of *Sopham* (oedema).[1] Later, *Bhaishajya Ratnavali* explains *Punarnavadi kwatha* in *Udara chikitsa* (treatment of ascites).[2] In *Sarngadhara Samhita* in the context of Ayurvedic dosage form *kwatha* (decoction), *Punarnavadi kwatha* is mentioned.[3] In *Sahasrayogam*, a malayalam compilation book describes the formulation *Punarnavadi kwatha* in both *Pandu* (anaemia) and *Udara* (ascites) *chikitsa* (treatment).[4] The Ayurvedic formulary of India referred the formulation mentioned in *Chakradatta.*[5]

The ingredients of this polyherbal formulation as per Ayurvedic Formulary of India are *Punarnava* (*Boerhaavia diffusa* Linn.), *Nimba* (*Azadirachta indica* A. Juss), *Patola* (*Trichosanthes dioica* Roxb.) *Sundi* (*Zingiber officinale* Rosc.), *Tiktha* (*Picrorhiza kurroa* auct Non-Royle.), *Amrita* (*Tinospora cordifolia* (Wild). Miers ex Hook. f and Thoms) *Daru*, (*Cedrus deodara* Roxb.), and *Abhaya* (*Terminalia chebula* Retz.). Main indications of *Punarnavadi kwatha* are *Sarvanga sopha* (generalised oedema), *Udara* (ascites), *Kasa* (cough), *Soolam* (colicky pain), and *Swasa* (dyspnoea) associated with *Pandu* (anaemia).

Corresponding author:
Dr. Resmi Vijayan, PG Scholar,
Department of Dravyaguna Vijnanam, GAVC,
Tripunithura, Kerala, India. Mobile: +91-8547071613.
Email: resmivijaybams@gmail.com

In a polyherbal formulation, pharmacological actions are mainly contributed by the combination of ingredient drugs. The scientific validation of Ayurvedic medicines is a need of hour for the quality control and standardization. In the present study, high-performance thin-layer chromatography fingerprinting *Punarnavadi kwatha* was done to analyze the different phytoconstituents.

2. MATERIALS AND METHODS

10 ml of *Punarnavadi Kwatha* samples was taken and evaporated to dryness in a water bath. Residue from evaporated sample was reconstituted in 1 ml of methanol, filtered through a syringe filter. Solvent system used was toluene: ethyl acetate: formic acid.[6] High-performance thin-layer chromatography (HPTLC) plate consists of 6 × 10 cm, precoated with silica gel 60 F254 thin-layer chromatography (TLC) plates (E. Merck) (0.2 mm thickness) with aluminum sheet support. The spotting device was a CAMAG Linomat V Automatic Sample Spotter (CamagMuttenz, Switzerland); the syringe, 100 µL (from Hamilton); the developing chamber was a CAMAG glass twin trough chamber (6 × 10 cm); the densitometer consisted of a CAMAG TLC scanner 3 linked to WINCATS software.

Developed the plate using the solvent system in twin trough chamber previously saturated with the solvent system for 30 min, washed the syringe twice with methanol. Dried the plate and placed it in the scanner. For absorption reflection mode scan the plate in ultraviolet (UV) 254 nm and 366 nm using Deuterium, Tungsten, and Mercury lamp, respectively. Scanned all the tracks. Took the finger print of each track. UV spectra spots can be compared in the spectrum display. Applied 10 µl of the extract on HPTLC plate and develop the plate to a distance of 8 cm using toluene: ethyl acetate: formic acid (6:3:0.5). After development allowed the plate to dry in air and examined under ultraviolet light 254 nm and 366 nm. Observed the plate under UV light at 254 nm and 366 nm. Recorded the Rf value and color of the resolved bands. After visualization and scanning, sprayed the plate with anisaldehyde sulfuric acid reagent and heat at 105°C till the color of the bands appears. Record the Rf value and color of the bands.

3. OBSERVATION AND RESULTS

HPTLC finger printing profile *Punarnavadi Kwatha* was done using methanolic extract Visualized the plates under 254nm and 366nm. Results obtained are tabulated below.

3.1. Area and Peaks of Methanol Extract of *Punarnavadi Kwatha* at 254 nm

Total 8 peaks were obtained for methanol extract of *Punarnavadi Kwatha* at 254 nm. These 8 peaks were defined with max Rf value of 0.06 with area 685.8 AU, max Rf value of 0.01 with area 10185.2, max Rf value of 0.24 with area 8752.9 AU, max Rf value of 0.47 with area 1824.2 AU, max Rf value of 0.56 with area 1332.6 AU, max Rf value of 0.58 with area 4949.7 AU, max Rf value of 0.78 with area 1711.3 AU, and max Rf value of 1.07 with area 867.8, respectively, which are tabulated as follows in Chart 1, Table 1 and Figure 1.

3.2. Area and Peaks of Methanol Extract of *Punarnavadi Kwatha* at 366 nm

Total 12 peaks were obtained for methanol extract of *Punarnavadi Kwatha* at 366 nm. The peaks were obtained with max Rf value of 0.08 with area 551.4AU, max Rf value of −0.04 with area 6045.8 AU, max Rf value of 0.05 with 1777.2 AU, max Rf value of 0.12 with area 1769.0 AU, max Rf value of 0.19 with 532.0 AU, max Rf value of 0.25 with area 6837.2 AU, max Rf value 0.48 with area 4379.5 AU, max Rf value of 0.58 with area 3979.0AU, max Rf value 0.56 with area 13102.4 AU, max Rf value of 0.63 with area 2320.7, max Rf value of 0.85 with area 411.3 AU, max Rf value with 1.00 with 6395.6, and max Rf value with 1.15 with 791.0 AU, respectively, which are tabulated as follows in Chart 2 and Figure 2.

4. DISCUSSION

High-performance thin-layer chromatography fingerprinting of *Punarnavadi kwatha* was not available in the previous literature. In a previous study of Hptlc finger printing of *Pathyashadanga kwatha,* similar methodology was adopted.[6] Total 8 peaks were obtained for methanol extract of *Punarnavadi Kwatha* at 254 nm. While analyzing the percentage area of compounds, max Rf value of 0.01 with area 10185.2, max Rf value of 0.24 with area 8752.9 AU and max Rf value of 0.58 with area 4949.7 AU were the compounds identified with maximum percentage area. At visualization 366 nm, total 12 peaks were obtained. Max Rf value of 0.82 with area 16729.5, max Rf value 0.56 with area 13102.4 AU, and max Rf value of 0.25 with area 6837.2 AU were the major identified compounds. After the process of derivatization also, the maximum separation of bands was visible as shown in Figure 3. In the quality control and standardization procedures, HPTLC fingerprinting can be used as a tool.

5. CONCLUSION

From the analysis, it was came to know that using the HPTLC fingerprinting, total 8 peaks were obtained for methanol extract of *Punarnavadi Kwatha* at 254 nm. At visualization 366nm, total 12 peaks were obtained. Development of HPTLC finger-printing profile of the pharmacologically active specific chemical constituent of herbal formulations plays key role in correct identification and to confirm the quality as well as purity of the formulations. Quality control and standardization of ayurvedic medicines are need of hour using modern analytical techniques.

6. ACKNOWLEDGMENT

With deep sense of respect and love, I express my sincere gratitude toward my guide, Dr. P.Y. Ansary MD, PhD (Ay), Professor and HOD, Department of Dravyagunavijnanam, Govt. Ayurveda College, Tripunithura, for his timely and meticulous guidance, supervisions, and suggestions to bring in perfection to every bit of this work. I am immensely grateful to Dr. Sara Monsy Oommen MD (Ay), Professor and HOD, Department of Dravyagunavijnanam, Govt. Ayurveda College, Kannur and Dr. Shincymol V.V MD (Ay), Associate Professor, Department of Dravyagunavijnanam, Govt. Ayurveda College, Tripunithura, for the valuable guidance, timely advice, and encouragement throughout the completion of this work. I would like to thank Dr.Reshma P John MD (Ay), Assistant Professor, Dr. Honey Thomas MD (Ay), Assistant professor, Department of Dravyagunavijnanam, Govt. Ayurveda College, Tripunithura for their valuable suggestions and cooperation throughout the study. I am sincerely grateful to Dr. Mridula M K MD (Ay), Dr. Jilu Joy MD (Ay) and Dr. Sethu R MD (Ay) Former Assistant professors, Department of Dravyagunavijnanam, Govt. Ayurveda College, Tripunithura for valuable suggestions, support and timely advices, and cooperation throughout the study.

7. AUTHORS' CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

Nil.

9. ETHICAL APPROVALS

This study not required ethical clearance as it is laboratory study.

10. CONFLICTS OF INTEREST

Nil.

11. DATA AVAIBALITY

This is an original manuscript and all data are available for only review purposes from principal investigators.

12. PUBLISHERS NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Sen S, editor. Chakrapani. Chakradatta. Trans-Pandita Kulapathy VA Upadhikaadikari. Sotha Chikitsa, Sloga no. 11. Varanasi: Choukhambha Orientalia; 1959. p. 470.
2. Das G. Bhaishajya Ratnavali. In: Choudhary AK, editor. Trans Kanjiv Lochan. Vol. 2., Ch. 40. Udara Roga, Sloka 34-35. Varanasi: Chaukambha Sanskrit Series Office; 2014. p. 571.
3. Himasagarachandra Murthy P. Sarngadhara. Sarngadhara Samhita. Madhyama khandam. Ch. 2. Kwathakalpana Adhyaya Sloka 76-77. Varanasi: Choukhambha Sanskrit Series Office; 2013. p. 124.
4. Krishnan Vaidyan KV, Gopala Pillai S, editors. Sahasrayogam Vidyarambham. Udara Chikitsa. Alappuzha: Vidyarambham Publishers; 2013. p. 279.
5. Ministry of Health and Family Welfare. The Ayurvedic Formulary of India. 1st ed. Part 1, A. New Delhi: Government of India; 2016. p. 182-3.
6. Abraham A, Samuel S, Mathew L. Phytochemical analysis of Pathyashadangam kwath and its standardization by HPLC and HPTLC. J Ayurveda Integr Med 2020;11:153-8.

How to cite this article:
Vijayan R, Ansary PY, Oommen SM, Shincymol VV. High-performance Thin-layer Chromatography Fingerprinting Analysis of *Punarnavadi Kwatha*. IRJAY. [online] 2023;6(11);25-29.
Available from: https://irjay.com
DOI link- https://doi.org/10.47223/IRJAY.2023.61105

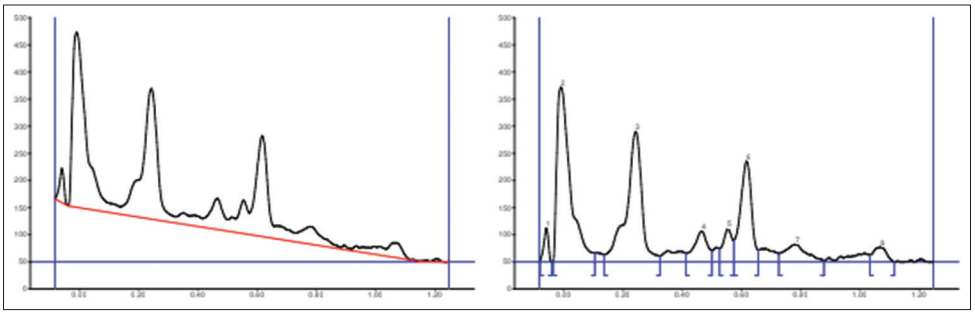

Chart 1: Overview graph of methanol extract of Punarnavadi Kwatha at 254 nm

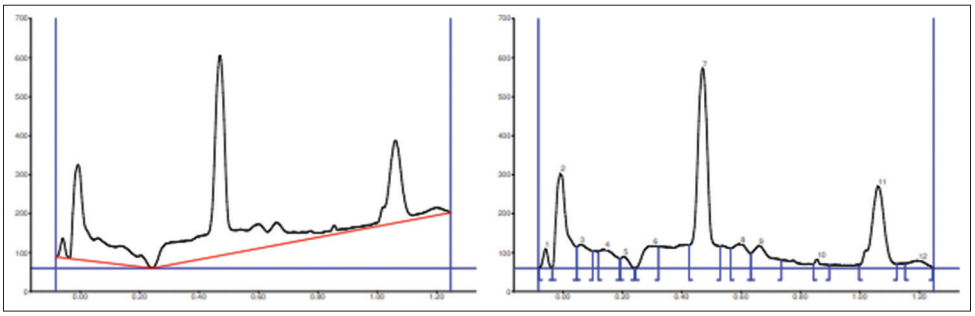

Chart 2: Overview graph of methanol extract of Punarnavadi Kwatha at 366 nm

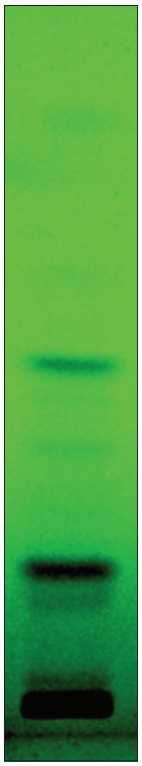

Figure 1: Visualization at 254 nm

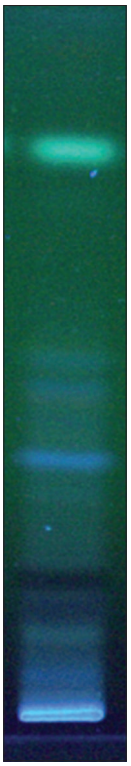

Figure 2: Visualization at 366 nm

Figure 3: Visualization after derivatization in white light

Table 1: Area and peaks of methanol extract of *Punarnavadi Kwatha* at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	−0.08	−0.06	0.04	685.8	2.26
2	−0.04	−0.01	0.10	10185.2	33.60
3	0.14	0.24	0.32	8752.9	28.88
4	0.41	0.47	0.50	1824.2	6.02
5	0.53	0.56	0.57	1332.6	4.40
6	0.58	0.62	0.66	4949.7	16.33
7	0.73	0.78	0.88	1711.3	5.65
8	1.03	1.07	1.12	867.8	2.86

Table 2: Area and peaks of methanol extract of *Punarnavadi Kwatha* at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	−0.08	−0.06	0.04	551.4	1.45
2	−0.04	−0.01	0.05	6045.8	15.95
3	0.05	0.06	0.10	1777.2	4.69
4	0.12	0.14	0.19	1769.0	4.67
5	0.19	0.20	0.24	532.0	1.40
6	0.25	0.31	0.32	6837.2	12.87
7	0.43	0.47	0.53	1939.6	5.12
8	0.56	0.60	0.63	13102.4	34.57
9	0.63	0.66	0.74	2320.7	6.12
10	0.85	0.82	0.90	16729.5	1.09
11	1.00	1.06	1.13	6395.6	16.87
12	1.15	1.20	1.25	791.0	2.09