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COMPARATIVE HPTLC FINGERPRINTING ANALYSIS OF DIFFERENT PHARMACEUTICAL DOSAGE FORMS OF *PUNARNAVADI KWATHA*

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

[1]PG Scholar, Department of Dravyagunavijnanam Government Ayurveda College, Tripunithura, Ernakulam, Kerala.

[2]Professor & HOD, Department of Dravyagunavijnanam Government Ayurveda College, Tripunithura, Ernakulam, Kerala.

[3]Professor & HOD, Department of Dravyagunavijnanam Government Ayurveda College, Kannur, Pariyaram, Kerala.

[4]Associate Professor, Department of Dravyagunavijnanam Government Ayurveda College, Tripunithura, Ernakulam, Kerala

Corresponding Author's Email ID: resmivijaybams@gmail.com

Abstract:

Kwatha (decoction) *Kalpana* (preparation) is most widely used dosage form in Ayurveda pharmaceutics especially in Kerala. *Punarnavadi kwatha* is one among the commonly used medicine in Ayurvedic practice, mentioned in *Chakradatta* in *sopha chikitsa.* In the modern lifestyle it is considered that *Kwatha* (decoction) *Kalpana* (preparation) has certain disadvantages in terms of shelf life, palatability etc. In order to overcome these disadvantages, different modified dosage forms are made available by the pharmaceutical companies. These includes concentrated *Kwatha*, *Kwatha* tablets, *Kwatha sookshma choornam* etc. Different pharmaceutical dosage forms of *Kwatha* are available in the market and they are extensively prescribed by the physicians. But Ayurveda practitioners are always doubtful about the efficacy of these dosage forms. So, the present study HPTLC fingerprinting analysis of different pharmaceutical dosage forms of *Punarnavadi kwatha.* aimed to assess the difference in phytoconstituents. Different pharmaceutical dosage forms include P1- classically prepared *Kwatha* (decoction), P2- Concentrated *Kwatha* (decoction), P3- *Kwatha* (decoction) tablet and P4- *Sookshma choorna Kwatha* (decoction). In the present study P1 is prepared according to traditional method and P2, P3 and P4 were purchased from GMP certified companies. The method of selection of the company was based on the lottery method. In HPTLC finger printing, maximum percentage area of phytoconstituents was in P1- classically prepared *kwatha*. The study revealed that maximum number of phytoconstituents were present in the *Kwatha* (decoction) prepared according to classical method.

Keywords- HPTLC fingerprinting, pharmaceutical dosage forms of *Punarnavadi kwatha.*

Introduction

Punarnavadi kwatha is a most commonly used polyherbal formulation in Ayurvedic medical practice. 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 & Thoms) *Daru*, (*Cedrus deodara* Roxb.) and *Abhaya* (*Terminalia chebula* Retz.).[1] Main indications of *Punarnavadi kwatha* are *Sarvanga sopha* (generalised oedema), *Udara* (ascites), *Kasa* (cough), *Soolam* (colicky pain), *Swasa* (dyspnoea) associated with *Pandu* (anaemia).[2]

In a poly herbal 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 finger printing of different pharmaceutical dosage forms of *Punarnavadi kwatha* was done to analyse the difference in phytoconstituents.

Materials and methods

a. Aim

To develop hptlc fingerprinting profile of different pharmaceutical dosage forms of *Punarnavadi Kwatha* P1-classically prepared *Kwatha* (decoction), P2- Concentrated *Kwatha* (decoction), P3- *Kwatha* (decoction) tablet and P4- *Sookshma choorna Kwatha* (decoction)

b. Materials required

HPTLC plate, Methanol, Toluene, Ethyl acetate, Formic acid, CAMAG TLC scanner, P1-classically prepared *Kwatha* (decoction), P2- Concentrated *Kwatha* (decoction), P3- *Kwatha* (decoction) tablet and P4- *Sookshma choorna Kwatha* (decoction)

c. Preparation of sample

10ml of *Punarnavadi kwatha* samples was taken and evaporated to dryness in a water bath. Residue from evaporated sample was reconstituted in 1ml of methanol, filtered through a syringe filter.

d. Solvent system

Toluene: Ethyl acetate: Formic acid (6:3:0.5)

e. HPTLC Conditions

HPTLC plate consists of 6 × 10cm, precoated with silica gel 60 F254 TLC plates (E. Merck) (0.2mm thickness) with aluminum sheet support. The spotting device was a CAMAG Linomat V Automatic Sample Spotter (CamagMuttenz, Switzerland); the syringe, 100µL (from Hamilton); the developing chamber was a CAMAG glass twin trough chamber (6 × 10cm); the densitometer consisted of a CAMAG TLC scanner 3 linked to WINCATS software.

f. Procedure

Developed the plate using the solvent system in twin trough chamber previously saturated with the solvent system for 30 minutes, wash the syringe twice with methanol. Dry the plate and place it in the scanner. Open a file and enter all parameters of scanning, integration and spectrum. For absorption reflection mode scan the plate in UV 254 nm and 366nm using Deuterium, Tungsten and Mercury lamp respectively. Scan all the tracks and then scan the UV spectrum of each scanning. Take the finger print of each track. UV spectra spots can be compared in the spectrum display. Apply 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 allow the plate to dry in air and examine under ultraviolet light 254nm&366nm.

g. Visualization

Observed the plate under UV light at 254nm, 366nm.Recorded the Rf value and colour of the resolved bands. After visualization and scanning, spray the plate with anisaldehyde sulphuric acid reagent and heat at 105^0C till the colour of the bands appear. Record the Rf value and colour of the bands.

Observation and Results

HPTLC finger printing profile of different pharmaceutical dosage forms of *Punarnavadi Kwatha,* P1-classically prepared *Kwatha* (decoction), P2- Concentrated

Kwatha, P3- *Kwatha* from tablet and P4- *Sookshma choorna Kwatha* were done. HPTLC plates were visualized at 254nm, 366nm and white light. Results obtained are tabulated below.

A. Sample P1-classically prepared *Kwatha* (decoction)

a. Area and peaks of Methanol extract of sample P1(classically prepared *Kwatha* (decoction)) at 254nm

Total 8 peaks were obtained for methanol extract of Sample P1-classically prepared *Kwatha* (decoction) at 254 nm. These 8 peaks were defined with max Rf value of 0.06 with area 685.8AU, 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.

Table No.1Area and peaks of methanol extract of sample P1(classically prepared *Kwatha* (decoction)) at 254nm

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

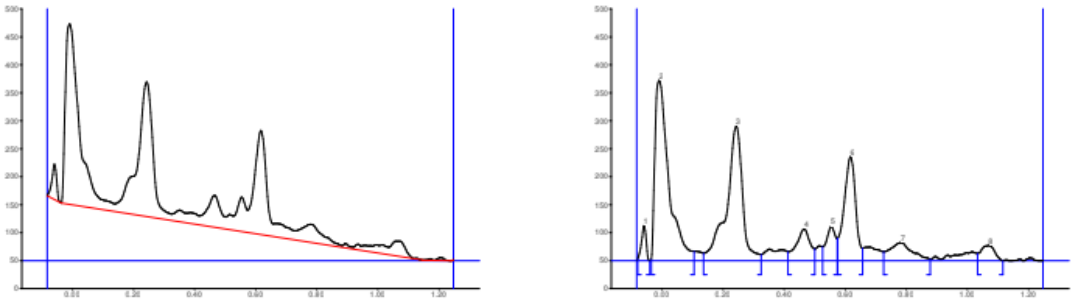


Diagram No:1 Overview graph of methanol extract of sample P1 (classically prepared *Kwatha* (decoction)) at 254nm

b. Area and peaks of Methanol extract of sample P1(classically prepared *Kwatha* (decoction)) at 366 nm

Total 12 peaks were obtained for methanol extract of *Punarnavadi Kwatha*, Sample P1- classically prepared *Kwatha* (decoction) at 366nm. The peaks were obtained with max Rf value of 0.08 with area 551.4AU, max Rf value of -0.04 with area 6045.8AU, max Rf value of 0.05 with 1777.2AU, 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 6837.2AU, 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.

Table No. 2 Area and peaks of methanol extract of sample P1(classically prepared *Kwatha* (decoction)) at 366nm

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

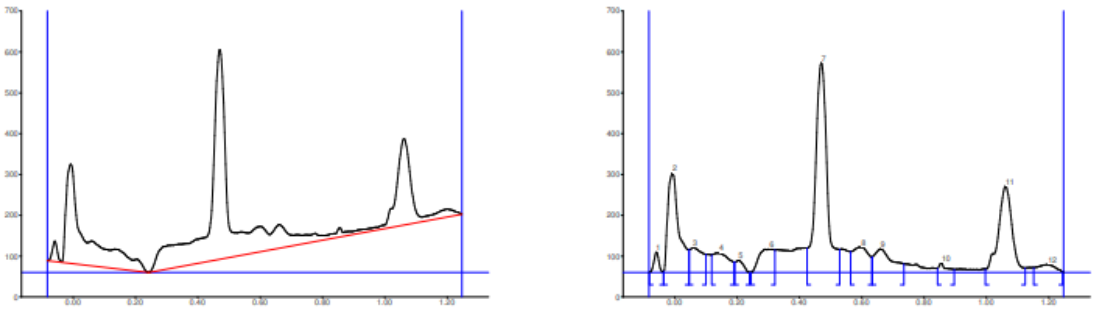


Diagram No: 2 Overview graph of methanol extract of sample P1(classically prepared *Kwatha*(decoction)) at 366nm

B. Sample P2- Concentrated *Kwatha*

a. Area and peaks of methanol extract of sample P2(Concentrated *Kwatha*) at 254nm

At 254nm, methanol extract of *Punarnavadi Kwatha,* Sample P2- Concentrated *Kwatha* (decoction) showed 7 peaks. The peaks were obtained with max Rf value of 0.06 with area 695.2 AU, max Rf value of -0.00 with area 5034.5 AU, max Rf value 0.19 with area 1840 AU, 0.24 with area 7759.1 AU, max Rf value 0.46 with area 1509 AU, max Rf value 0.61 with area 1258.6 AU and max Rf value 0.82 with area 5322.1 AU respectively, which are tabulated as follows.

Table No.3 Area and peaks of methanol extract of sample P2 (Concentrated *Kwatha* at 254nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	-0.08	-0.06	-0.04	695.2	2.97
2.	-0.04	-0.00	-0.01	5034.5	21.50
3.	0.13	0.19	0.20	1840.0	7.86
4.	0.20	0.24	0.31	7759.1	6.23
5.	0.40	0.46	0.50	1509.5	6.45
6.	0.57	0.61	0.65	1258.6	5.37
7.	0.74	0.82	0.90	5322.1	6.74

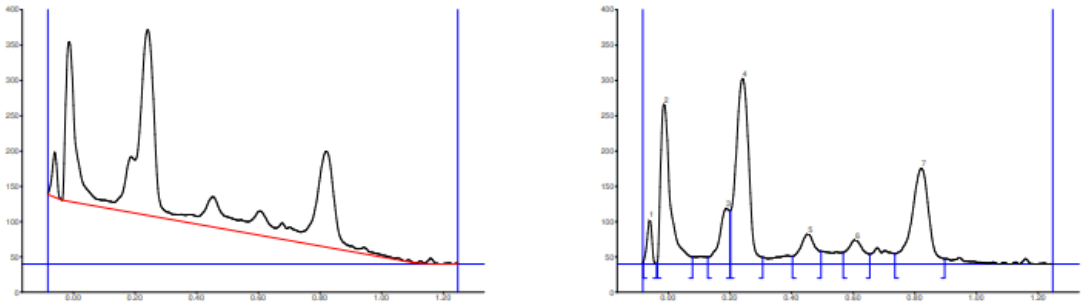


Diagram No: 3 Overview graph of methanol extract of sample P2 (Concentrated *Kwatha* at 254nm

b. Area and peaks of Sample P2(Concentrated *Kwatha*) at 366nm

Methanol extract of *Punarnavadi Kwatha,* Sample P2- Concentrated *Kwatha* showed 13 peaks at 366nm and these peaks were having max Rf value of 0.06 with area 782.2 AU, max Rf value of 0.04 with area 2752.8 AU, max Rf value of 0.01 with area 2196.5 AU, max Rf value of 0.05 with area 1253.6AU, max Rf value of 0.19 with area 443.3AU, max Rf value of 0.24 with area 1357.3 AU, max Rf value of 0.43 with area 1742.2 AU, max Rf value of 0.50

with area 2047.5AU max Rf value of 0.57 with area 1483.4 AU, max Rf value 0.71 with area 832.9AU and max Rf value 1.13 with area 1363.3 respectively are tabulated as follows.

Table No: 4 Area and peaks of methanol extract of sample P2 (Concentrated *Kwatha* at 366nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	-0.08	-0.06	0.04	782.2	3.89
2.	0.04	0.00	0.01	2752.8	13.68
3.	0.01	0.02	0.05	2196.5	10.91
4.	0.05	0.06	0.10	1253.6	6.23
5.	0.10	0.15	0.19	1606.3	7.98
6.	0.19	0.20	0.24	443.3	2.20
7.	0.24	0.30	0.31	1357.3	6.74
8.	0.43	0.46	0.49	1742.2	8.66
9.	0.50	0.54	0.56	2047.5	10.17
10.	0.57	0.57	0.62	1483.4	7.37
11.	0.63	0.68	0.70	2262.6	11.24
12.	0.71	0.72	0.77	832.9	4.14
13.	1.13	1.16	1.23	1363.3	6.77

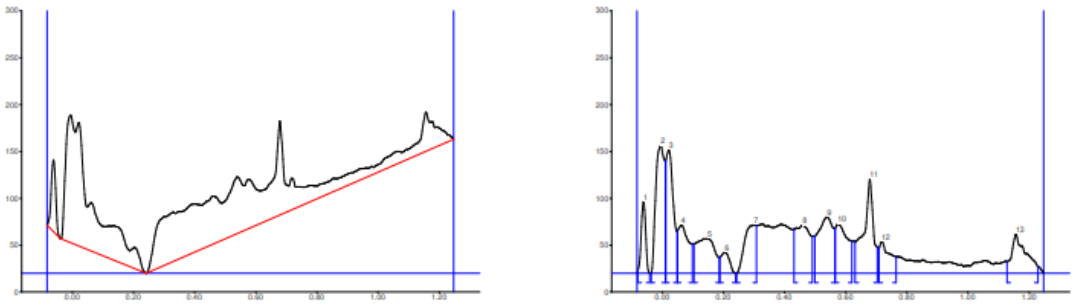


Diagram No: 4 Overview graph of methanol extract of sample P2 (Concentrated *Kwatha*) at 366nm

C. Sample P3- *Kwatha* tablet

a. Area and peaks of methanol extract of sample P3 *Kwatha* tablet at 254nm

At 254nm, methanol extract of *Punarnavadi Kwatha,* Sample P3- *Kwatha* tablet at 254nm showed 6 peaks. The peaks were obtained with max Rf value of 0.06 with area 616.3 AU, max Rf value of 0.02 with area 3575.9 AU, max Rf value 0.17 with area 4381.0 AU, max Rf value 0.24 with area 2996.5 AU, max Rf value 0.45 with area 710.4 AU respectively, which are tabulated as follows.

Table No.5 Area and peaks of methanol extract of sample P3 *Kwatha* tablet at 254nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	-0.08	-0.06	-0.04	616.3	4.84
2	-0.04	-0.02	-0.08	3575.9	28.05
3	0.08	0.17	0.21	4381.0	34.37
4	0.21	0.24	0.32	2996.5	23.51
5	0.40	0.45	0.48	710.4	5.57
6	0.81	0.81	0.86	467.4	3.67

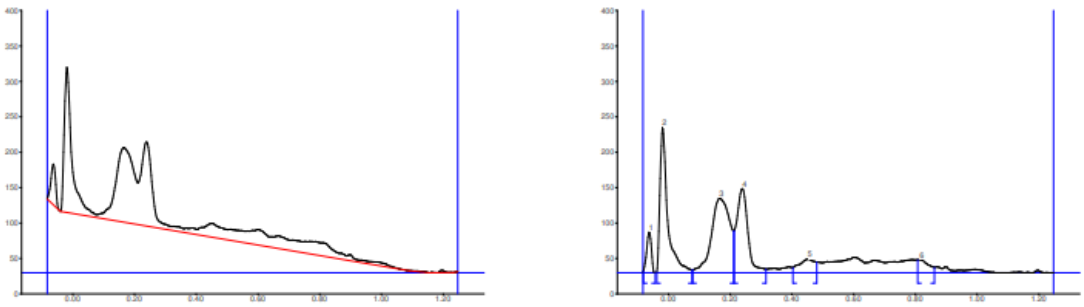


Diagram No: 5 Overview graph of methanol extract of sample P3 at 254nm

b. Area and peaks of methanol extract of sample P3 (*Kwatha* tablet) at 366nm

Methanol extract of *Punarnavadi Kwatha,* Sample P3- *Kwatha* (decoction) tablet, showed 12 peaks at 366nm and these peaks were having max Rf value of 0.06 with area 416.1AU, max Rf value of 0.02 with area 6229.9AU, max Rf value of 0. 12 with area 352.2 AU, max Rf value of 0.30 with area 1637.7 AU, max Rf value of 0.38 with area 1263.9AU, max Rf value of 0.51 with area 1106.9 AU, max Rf value of 0.58 with area 2047.5AU, max Rf value of 0.57 with area 4567.6AU, max Rf value of 0.73 with area 1489.4 AU and max Rf value of 0.73 with area 475.5AU respectively, are tabulated as follows.

Table No: 6 Area and peaks of methanol extract of sample P3 (*Kwatha* tablet) at 366nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	-0.08	-0.06	0.05	416.1	1.48
2	-0.04	-0.02	0.06	6229.9	22.19
3	0.09	0.12	0.16	863.4	3.07
4	0.18	0.21	0.24	352.2	1.25
5	0.24	0.30	0.33	1637.7	5.83
6	0.33	0.34	0.35	628.2	2.24
7	0.36	0.38	0.41	1263.9	4.50
8	0.48	0.51	0.52	1106.9	3.94
9	0.55	0.58	0.52	2047.5	10.17
10	0.57	0.57	0.62	4567.6	16.27
11	0.72	0.73	0.78	1489.4	5.30
12	1.00	0.73	0.78	475.5	1.69

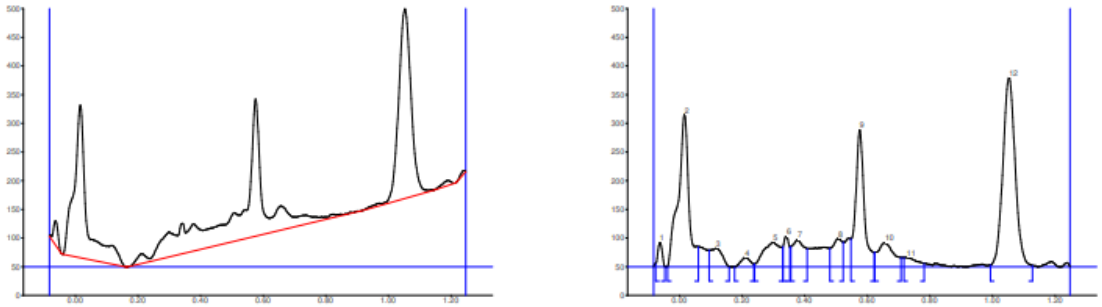


Diagram No: 6 Overview graph of methanol extract of sample P3 (*Kwatha* (decoction) tablet) at 366nm

D. Sample P4- *Sookshma choorna Kwatha*

a. Area and peaks of methanol extract of Sample P4 (*Sookshma choorna Kwatha*) at 254nm

At 254nm, methanol extract of *Punarnavadi Kwatha,* Sample P4- *Sookshma choorna Kwatha* at 254nm showed 7 peaks. The peaks were obtained with max Rf value of 0.07 with area 568.1 AU, max Rf value of 0.01 with area 5388.0 AU, max Rf value 0.24 with area 9478.3 AU, max Rf value 0.47 with area 9478.3 AU, max Rf value 0.50 with area 679.0 AU, max Rf value 0.62 with area 836.0 AU, max Rf value 0.83 with area 1447.4 AU and max Rf value 0.83 with area 4692.2 AU respectively, which are tabulated as follows.

Table No.7 Area and peaks of methanol extract of Sample P4 (*Sookshma choorna Kwatha* at 254nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	-0.08	-0.07	-0.05	568.1	2.46
2	-0.05	-0.01	-0.08	5388.0	23.34
3	0.17	0.24	0.31	9478.3	41.05
4	0.43	0.47	0.48	679.0	2.94
5	0.48	0.50	0.54	836.0	3.62
6	0.57	0.62	0.66	1447.4	6.27
7	0.75	0.83	0.90	4692.2	20.32

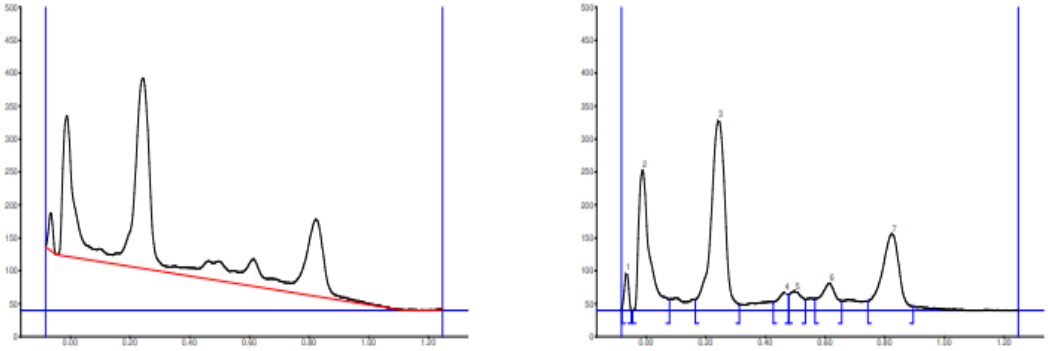


Diagram No: 7 Overview graph of methanol extract of Sample P4 (*Sookshma choorna Kwatha*) at 254 nm

b. Area and peaks of methanol extract of Sample P4 (*Sookshma choorna Kwatha*) at 366nm

Methanol extract of *Punarnavadi Kwatha,* Sample P4-*Sookshma choorna Kwatha* showed 12 peaks at 366nm and these peaks were having max Rf value of 0.07 with area 406.4AU, max Rf value of 0.01 with area 6007.2 AU, max Rf value of 0.02 with area 6485.9AU, max Rf value of 0.17 with area 2067.9AU, max Rf value of 0.17 with area 1775.2 AU, max Rf value of 0.30 with area 3119.5 AU, max Rf value of 0.40 with area 4132.9 AU, max Rf value of 0.54 with area 1738.6 AU, max Rf value of 0.58 with area 1819.1 AU max Rf value of 0.65 with area 1475.9 AU, Rf value of 0.85 with area 1038.8 AU and Rf value of 1.18 with area 1022.6 AU respectively were tabulated as follows.

Table No: 8 Area and peaks of methanol extract of Sample P4 (*Sookshma choorna Kwatha* at 366nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	-0.08	-0.07	0.05	406.4	1.31
2	-0.04	-0.01	0.01	6007.2	19.32
3	0.01	0.02	0.10	6485.9	20.86
4	0.11	0.17	0.24	2067.9	6.65

5	0.15	0.17	0.24	1775.2	5.71
6	0.25	0.30	0.36	3119.5	10.03
7	0.37	0.40	0.50	4132.9	13.29
8	0.50	0.54	0.56	1738.6	5.59
9	0.56	0.58	0.52	1819.1	5.85
10	0.63	0.65	0.70	1475.9	4.75
11	0.83	0.85	0.88	1038.8	3.54
12	1.13	1.18	1.75	1022.6	3.29

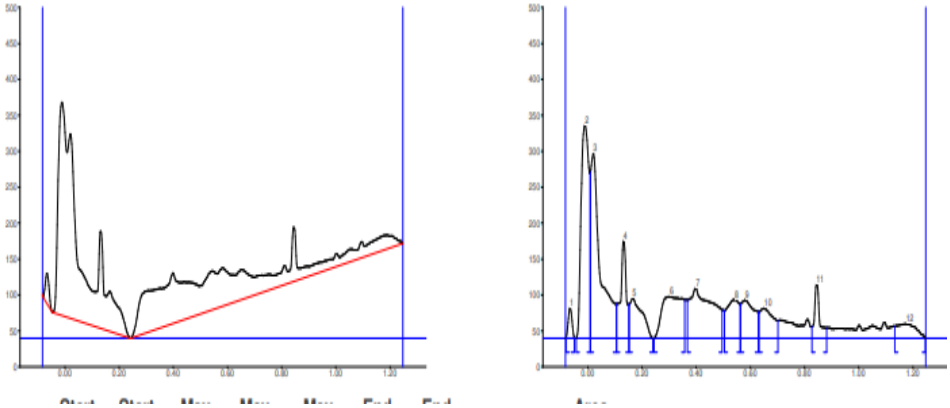
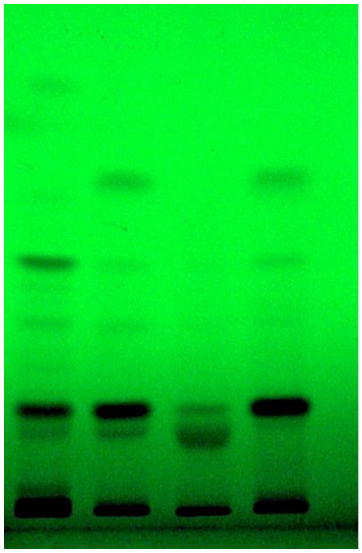
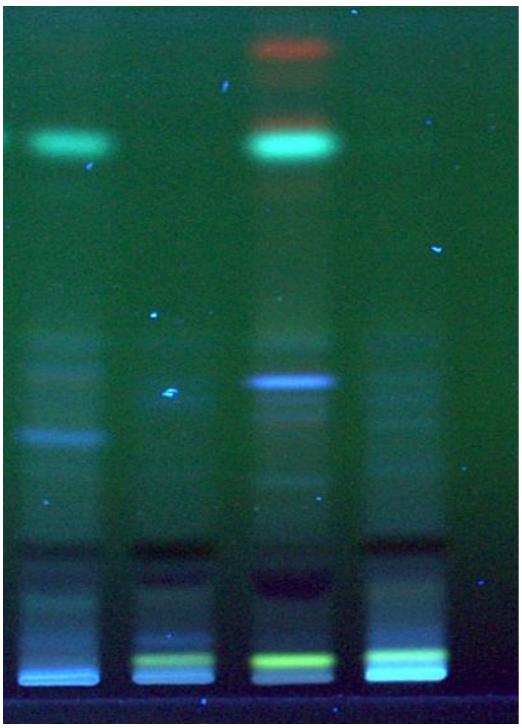


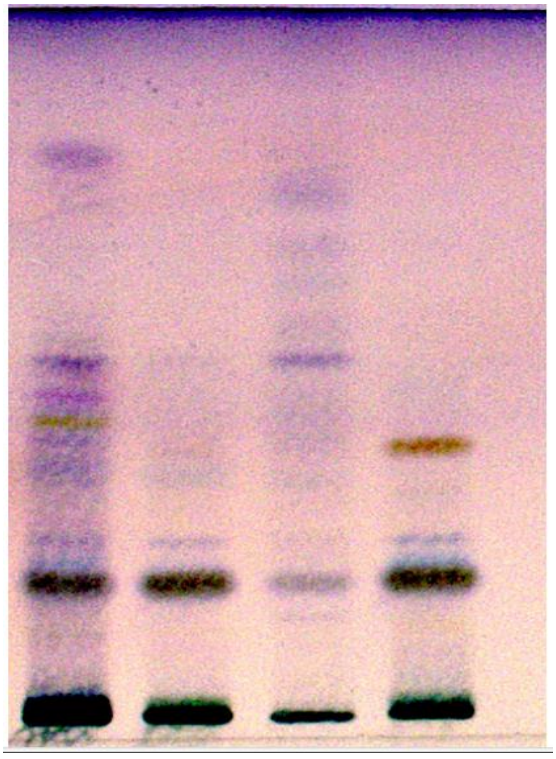
Diagram No:8 Overview graph of methanol extract of Sample P4 (*Sookshma choorna Kwatha* at 366 nm



Picture No:1 HPTLC plate of P1, P2, P3, P4 at 254nm



Picture No:2 HPTLC plate of P1, P2, P3, P4 at 366nm



Picture No:3 HPTLC plate of P1, P2, P3, P4 after derivatization in white light

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.[3] Total number of peaks, percentage area of phytoconstituents were found to be more in the sample P1-classically prepared *Kwatha* (decoction). In previous research work also maximum number of peaks obtained was for traditionally prepared one. [4] On comparing the different peaks, with Rf values, some phytoconstituents are same in the four samples. At 254nm, phytoconstituent with max Rf value 0.24 was present in both the four samples. But the area of compound was found to be more in sample P4- *Sookshma choorna kwatha*. Phytoconstituent with max Rf value 0.06 was present in three samples P1, P2 and P3. At visualization 366nm also total area of phytoconstituents were found to be more in sample P1- classically prepared *Kwatha* (decoction). Similar phytoconstituent was not seen in the four samples at visualization 366nm.Visualization of HPTLC plate after derivatization by treating with anisaldehyde sulphuric acid showed maximum number of peaks in sample P1- classically prepared *Kwatha* (decoction).

Conclusion

From the analysis it was came to know that even for the same *Kalpana* (preparation), there is wide range of difference in phytoconstituents. *Kwatha* (decoction) prepared according to the classical method, sample P1 showed more phytoconstituents than the other samples.The changes in phytoconstituents are mainly different modifications of *kwatha* (decoction) *kalpana* (preparation). More researches are needed in this regard. Quality control and standardisation of ayurvedic medicines are need of hour using modern analytical techniques.

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