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STANDARDISATION OF KALYANAKA GHRITA AND KSHEERAKALYANAKA GHRITA BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Background: *Kalyanaka Ghrita* is a formulation which has been mentioned in various classical compendium of *Ayurveda* like *Charaka Samhita*, *Sushruta Samhita*, *Ashtanga Hrudaya* etc. According to the reference in *Charaka Samhita*, it is a poly herbal preparation comprising of 28 drugs. Along with the reference of *Kalyanaka Ghrita*, *Acharya Chakrapanidatta* has quoted the reference of *Ksheerakalyanaka Ghrita*. The only difference between both the formulations is the ratio of water and the addition of milk in case of *Ksheerakalyanaka Ghrita*. Aims and Objectives: To compare, standardise and evaluate the different constituents present in *Kalyanaka Ghrita* and *Ksheerakalyanaka Ghrita*. **Methodology:** 4µl and 8µl of the above sample of *Kalyanaka ghritha* and *Ksheerakalyanaka ghritha* was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene – Ethyl acetate (9:1) and the developed plates were visualized under UV 254 and 366 nm,

and after derivatisation in vanillin-sulphuric acid spray reagent and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded. **Results:** Under Short UV, 2 and 3 peaks were observed for *Kalyanaka Ghrita* and *Ksheerakalyanaka* Ghrita respectively.4 Peaks were observed under long UV for Kalyanaka Ghrita and 3 peaks were observed for *Ksheerakalyanaka Ghrita*. Under post derivatisation, 6 peaks were observed for *Kalyanaka Ghrita* and 7 peaks were observed for *Ksheerakalyanaka Ghrita.* **Conclusion:** The peaks observed in HPTLC can be considered for fingerprint profile analysis.

KEYWORDS: *Kalyanaka Ghrita, Ksheera Kalyanaka Ghrita*, HPTLC.

INTRODUCTION

Kalyanaka Ghrita is a formulation which has been mentioned in various classical compendium of *Ayurveda* like *Charaka Samhita, Sushruta Samhita, Ashtanga Hrudaya* etc. According to the reference in *Charaka Samhita*, it is a poly herbal preparation comprising of 28 drugs. Along with the reference of *Kalyanaka Ghrita, Acharya Chakrapanidatta* has quoted the reference of *Ksheerakalyanaka Ghrita.* The only difference between both the formulations is the ratio of water and the addition of milk in case of *Ksheerakalyanaka Ghrita.*

The phytochemical constituents of polyherbal formulations like *Kalyanaka Ghrita*[1] and *Ksheerakalyanaka Ghrita*[2] is very complex and assessing the stability is very challenging also. An attempt is made to standardise *Kalyanaka Ghrita* as per the reference of *Charaka samhita* and *Ksheera kalyanaka Ghrita* as per the reference of *Chakradatta* by High Performance Thin Layer Chromatography.[3]

OBJECTIVES

To standardise and evaluate the different constituents present in *Kalyanaka Ghrita* and *Ksheerakalyanaka Ghrita*.

MATERIALS AND METHODS

Materials required: Water bath, pipette, pre-coated silica gel plate, Linomat 5 applicator, vanillin-sulphuric acid spray, alcohol, toluene, ethyl acetate, etc.

Sample preparation for HPTLC[3]

Sample obtained in the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform and was used for HPTLC.

PROCEDURE

4µl and 8µl of the above sample of *Kalyanaka ghritha* and *Ksheerakalyanaka ghritha* was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene – Ethyl acetate (9:1) and the developed plates were visualized under UV 254 and 366 nm, and after derivatisation in vanillin-sulphuric acid spray reagent and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded.

RESULTS

The R_f value of sample of *Kalyanaka Ghrita* and *Ksheerakalyanaka Ghrita* obtained after HPTLC is depicted in Table 1.

Table 1: R_f Values of Samples of Kalyanaka Ghrita (K.G) and Ksheerakalyanaka Ghrita(K.K.G).

Short UV		Long UV		Post derivatisation	
KG	**KKG**	**KG**	**KKG**	**KG**	**KKG**
-	-	-	-	0.04 (D. purple)	0.04 (D. purple)
-	-	-	-	-	0.07 (D. purple)
0.09 (L. green)	0.09 (D. green)	-	-	-	-
-	-	--	-	0.11 (D. purple)	-
-	0.13 (L. green)	-		-	0.13 (D. purple)
-	-	-	-	-	0.18 (D. purple)
-	-	-	-	-	0.23 (D. purple)
-	-	0.33 (F. blue)	-	-	-
-	-	-	-	0.35 (D. purple)	0.35 (D. purple)
-	-	-	-	0.44 (D. purple)	-
0.48 (L. green)	-	0.48 (F aqua. blue)	0.48 (F. blue)	-	-
-	-	-	-	0.52 (D. purple)	0.52 (D. purple)
-	0.55 (L. green)	-	-	-	-
-	-	0.82 (F. blue)	0.82 (F. blue)	-	-
-	-	-	-	0.87 (L. purple)	-
-	-	0.92 (F. blue)	0.92 (F. blue)	-	-

*L – light; D – dark; F - fluorescent

FIGURES

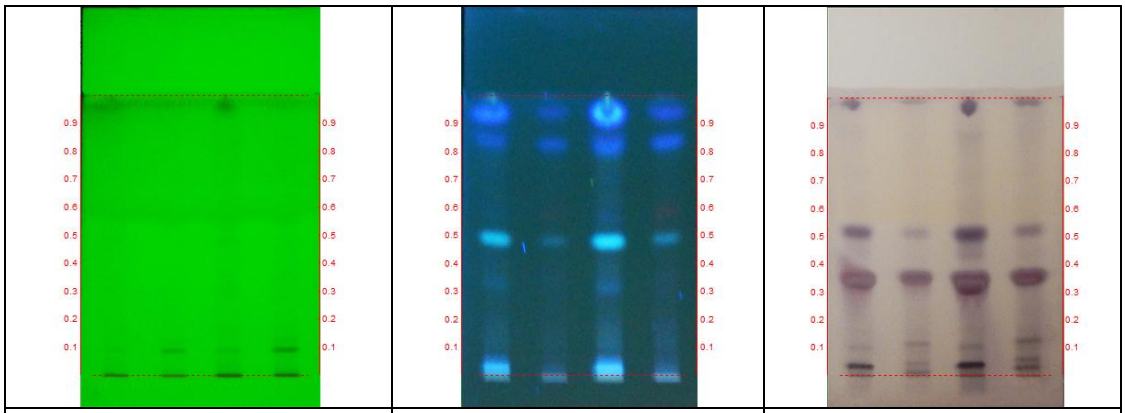

Figure 1: HPTLC Photodocumentation of sample of Kalyanaka Ghritha and Ksheera kalyanaka Ghritha.

Track 1: Kalyanaka ghritha - 4µl

Track 2: Ksheera kalyanaka ghritha - 4µl

Track 3: Kalyanaka ghritha - 8µl

Track 4: Ksheera kalyanaka ghritha - 8µl

Solvent system- Toluene: Ethyl acetate (9.0: 1.0)

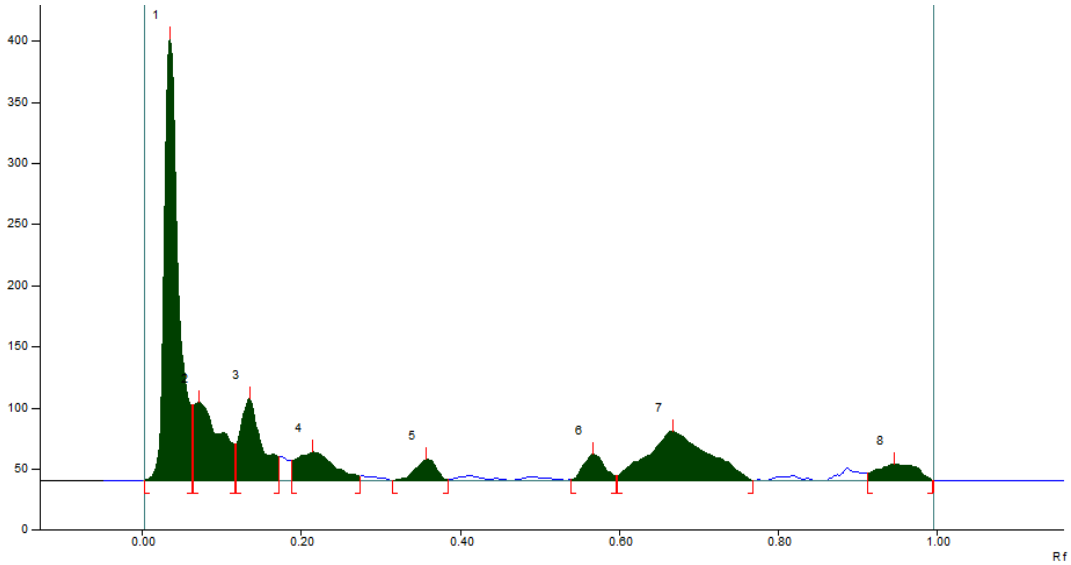

Figure 2: Densitometric scan of the sample at 254nm

Track 3, ID: Kalyanka ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.3 AU	0.04 Rf	361.4 AU	59.34 %	0.06 Rf	61.6 AU	4636.4 AU	39.55 %
2	0.06 Rf	61.8 AU	0.07 Rf	64.3 AU	10.55 %	0.12 Rf	29.6 AU	1543.5 AU	13.17 %
3	0.12 Rf	30.0 AU	0.14 Rf	67.4 AU	11.06 %	0.17 Rf	19.9 AU	1320.9 AU	11.27 %
4	0.19 Rf	16.8 AU	0.21 Rf	23.4 AU	3.84 %	0.27 Rf	3.5 AU	789.6 AU	6.74 %
5	0.31 Rf	0.2 AU	0.36 Rf	17.2 AU	2.82 %	0.39 Rf	0.7 AU	331.3 AU	2.83 %
6	0.54 Rf	1.0 AU	0.57 Rf	21.5 AU	3.53 %	0.60 Rf	3.7 AU	408.5 AU	3.49 %
7	0.60 Rf	3.9 AU	0.67 Rf	40.4 AU	6.63 %	0.77 Rf	0.1 AU	2196.9 AU	18.74 %
8	0.91 Rf	6.1 AU	0.95 Rf	13.6 AU	2.23 %	1.00 Rf	0.4 AU	494.7 AU	4.22 %

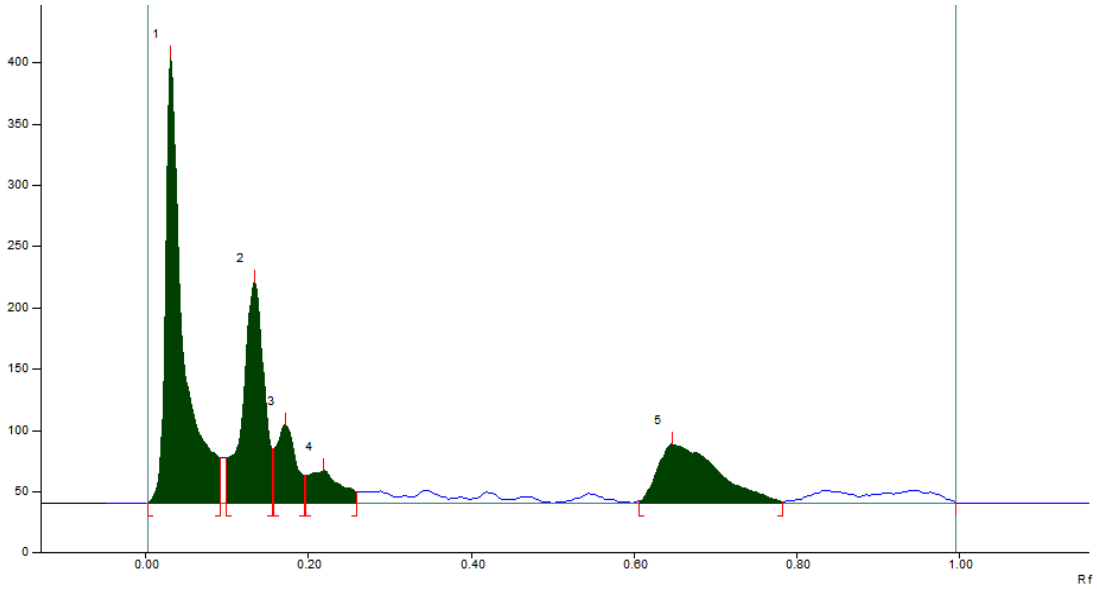

Fig 2a: Kalyanaka ghritha.

Track 4, ID: Ksheera kalyanaka ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.3 AU	0.03 Rf	363.6 AU	53.20 %	0.09 Rf	36.8 AU	5682.8 AU	42.21 %
2	0.10 Rf	37.1 AU	0.13 Rf	180.9 AU	26.47 %	0.16 Rf	44.0 AU	3379.4 AU	25.10 %
3	0.16 Rf	44.2 AU	0.17 Rf	64.2 AU	9.40 %	0.20 Rf	22.7 AU	1086.7 AU	8.07 %
4	0.20 Rf	22.8 AU	0.22 Rf	26.5 AU	3.88 %	0.26 Rf	9.5 AU	759.1 AU	5.64 %
5	0.61 Rf	1.7 AU	0.65 Rf	48.2 AU	7.05 %	0.78 Rf	1.4 AU	2555.9 AU	18.98 %

Fig 2b. Ksheerakalyanaka ghritha

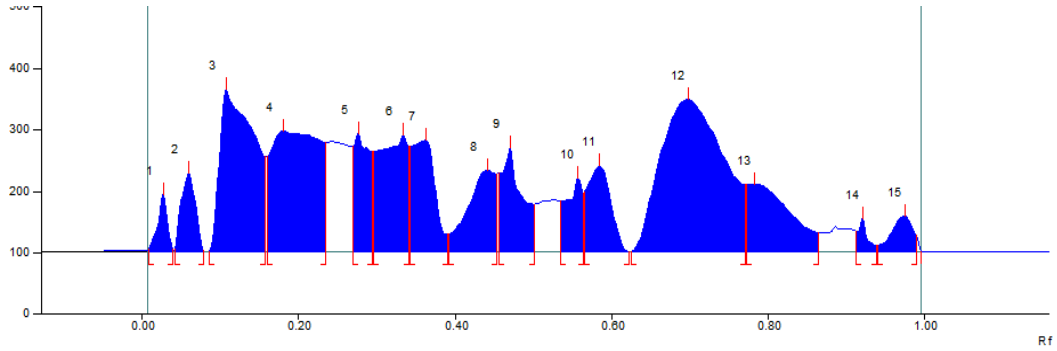

Figure 3: Densitometric scan of the sample at 366nm.

Track 3, ID: Kalyanka ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.2 AU	0.03 Rf	93.6 AU	4.11 %	0.04 Rf	3.5 AU	834.8 AU	1.32 %
2	0.04 Rf	3.7 AU	0.06 Rf	127.7 AU	5.61 %	0.08 Rf	1.8 AU	1650.7 AU	2.61 %
3	0.09 Rf	0.7 AU	0.11 Rf	263.5 AU	11.57 %	0.16 Rf	54.1 AU	8302.9 AU	13.15 %
4	0.16 Rf	154.4 AU	0.18 Rf	196.6 AU	8.63 %	0.24 Rf	77.3 AU	8896.3 AU	14.09 %
5	0.27 Rf	170.5 AU	0.28 Rf	192.4 AU	8.45 %	0.30 Rf	62.7 AU	2949.1 AU	4.67 %
6	0.30 Rf	162.8 AU	0.33 Rf	190.6 AU	8.37 %	0.34 Rf	71.7 AU	4976.1 AU	7.88 %
7	0.34 Rf	171.9 AU	0.36 Rf	181.0 AU	7.95 %	0.39 Rf	28.5 AU	3864.8 AU	6.12 %
8	0.39 Rf	28.8 AU	0.44 Rf	133.1 AU	5.84 %	0.45 Rf	27.0 AU	3352.0 AU	5.31 %
9	0.46 Rf	127.3 AU	0.47 Rf	169.0 AU	7.42 %	0.50 Rf	77.4 AU	3235.3 AU	5.12 %
10	0.54 Rf	82.6 AU	0.56 Rf	119.4 AU	5.24 %	0.56 Rf	95.9 AU	1770.6 AU	2.80 %
11	0.57 Rf	97.8 AU	0.59 Rf	139.8 AU	6.14 %	0.62 Rf	0.0 AU	2957.2 AU	4.68 %
12	0.63 Rf	0.2 AU	0.70 Rf	247.8 AU	10.88 %	0.77 Rf	09.0 AU	14415.6 AU	22.83 %
13	0.77 Rf	109.1 AU	0.78 Rf	110.5 AU	4.85 %	0.87 Rf	31.6 AU	4320.2 AU	6.84 %
14	0.91 Rf	33.9 AU	0.92 Rf	54.3 AU	2.39 %	0.94 Rf	9.6 AU	494.1 AU	0.78 %
15	0.94 Rf	9.9 AU	0.98 Rf	57.9 AU	2.54 %	0.99 Rf	26.1 AU	1112.8 AU	1.76 %

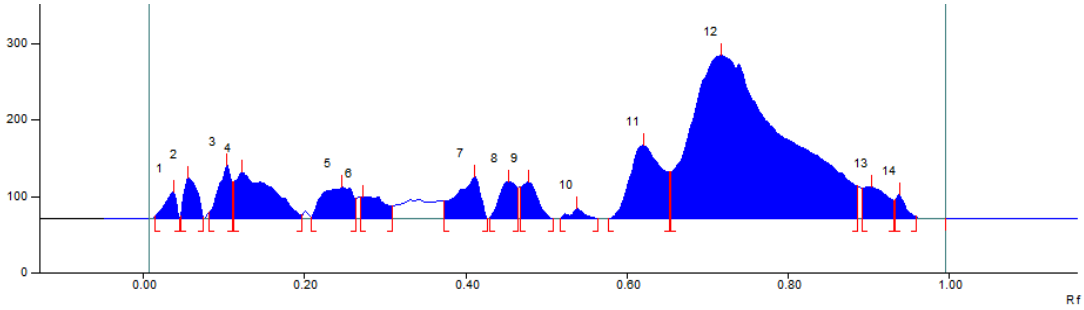

Fig 3a: Kalyanaka ghritha.

Track 4, ID: Ksheera kalyanaka ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.7 AU	0.04 Rf	34.5 AU	4.15 %	0.05 Rf	0.0 AU	350.2 AU	1.18 %
2	0.05 Rf	3.5 AU	0.05 Rf	53.4 AU	6.43 %	0.07 Rf	2.0 AU	606.4 AU	2.05 %
3	0.08 Rf	8.5 AU	0.10 Rf	69.6 AU	8.37 %	0.11 Rf	48.1 AU	797.2 AU	2.69 %
4	0.11 Rf	48.3 AU	0.12 Rf	60.4 AU	7.27 %	0.20 Rf	4.3 AU	1942.3 AU	6.56 %
5	0.21 Rf	1.6 AU	0.25 Rf	41.5 AU	4.99 %	0.26 Rf	26.5 AU	1116.6 AU	3.77 %
6	0.27 Rf	28.1 AU	0.27 Rf	28.6 AU	3.44 %	0.31 Rf	16.8 AU	630.0 AU	2.13 %
7	0.37 Rf	23.0 AU	0.41 Rf	54.6 AU	6.57 %	0.43 Rf	0.5 AU	1097.2 AU	3.71 %
8	0.43 Rf	0.8 AU	0.45 Rf	47.7 AU	5.74 %	0.47 Rf	40.4 AU	747.6 AU	2.53 %
9	0.47 Rf	41.0 AU	0.48 Rf	47.5 AU	5.71 %	0.51 Rf	0.1 AU	707.6 AU	2.39 %
10	0.52 Rf	0.3 AU	0.54 Rf	12.6 AU	1.52 %	0.56 Rf	0.0 AU	151.2 AU	0.51 %
11	0.58 Rf	0.1 AU	0.62 Rf	95.2 AU	11.45 %	0.65 Rf	60.4 AU	2661.8 AU	9.00 %
12	0.65 Rf	60.7 AU	0.72 Rf	213.3 AU	25.67 %	0.89 Rf	42.5 AU	17612.2 AU	59.52 %
13	0.89 Rf	39.8 AU	0.90 Rf	41.2 AU	4.95 %	0.93 Rf	23.9 AU	886.5 AU	3.00 %
14	0.93 Rf	24.7 AU	0.94 Rf	31.1 AU	3.74 %	0.96 Rf	2.5 AU	283.1 AU	0.96 %

Fig 3b: Ksheerakalyanaka ghritha

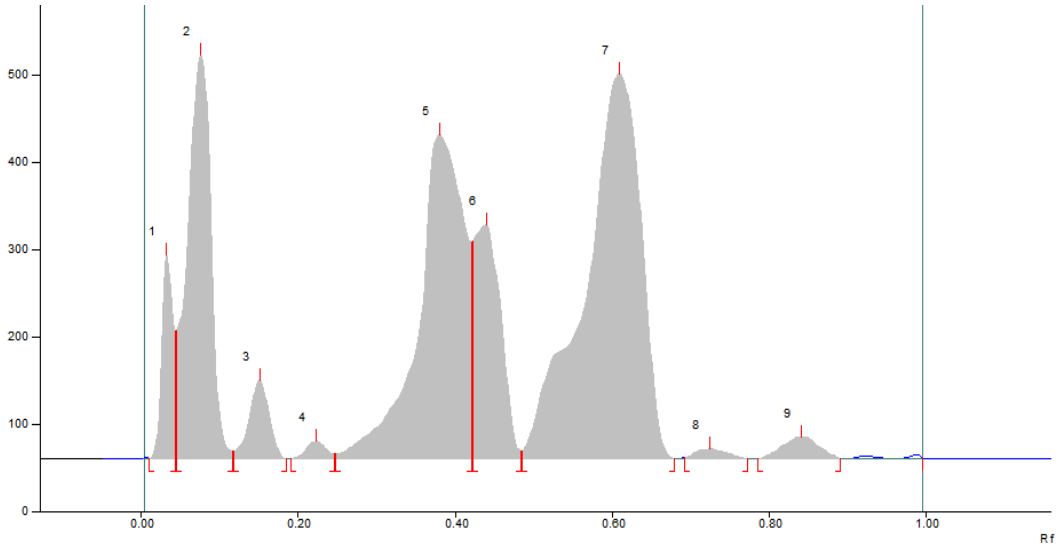

Figure 4: Densitometric scan of the sample at 620nm.

Track 3, ID: Kalyanka ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.4 AU	0.03 Rf	232.5 AU	12.12 %	0.04 Rf	45.7 AU	2408.5 AU	4.00 %
2	0.05 Rf	146.2 AU	0.08 Rf	460.9 AU	24.02 %	0.12 Rf	8.6 AU	9955.5 AU	16.55 %
3	0.12 Rf	8.7 AU	0.15 Rf	90.0 AU	4.69 %	0.19 Rf	0.1 AU	1632.9 AU	2.71 %
4	0.19 Rf	0.0 AU	0.22 Rf	19.6 AU	1.02 %	0.25 Rf	5.2 AU	360.9 AU	0.60 %
5	0.25 Rf	5.3 AU	0.38 Rf	370.9 AU	19.33 %	0.42 Rf	47.5 AU	15614.6 AU	25.96 %
6	0.42 Rf	248.4 AU	0.44 Rf	268.1 AU	13.98 %	0.48 Rf	9.2 AU	6606.5 AU	10.98 %
7	0.49 Rf	9.4 AU	0.61 Rf	439.9 AU	22.93 %	0.68 Rf	0.2 AU	22425.1 AU	37.28 %
8	0.69 Rf	1.4 AU	0.73 Rf	11.4 AU	0.60 %	0.77 Rf	0.2 AU	321.7 AU	0.53 %
9	0.79 Rf	0.1 AU	0.84 Rf	25.2 AU	1.31 %	0.89 Rf	0.1 AU	833.7 AU	1.39 %

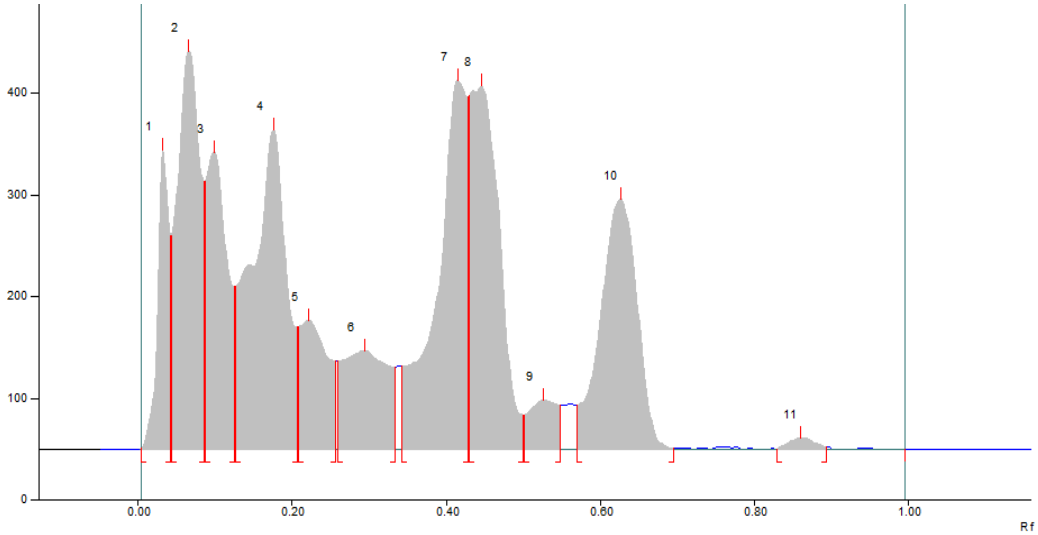

Fig 4a. kalyanaka ghritha.

Track 4, ID: Ksheera kalyanaka ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.7 AU	0.03 Rf	294.0 AU	11.58 %	0.04 Rf	09.7 AU	3096.5 AU	4.75 %
2	0.04 Rf	210.1 AU	0.07 Rf	391.0 AU	15.40 %	0.09 Rf	62.8 AU	8376.1 AU	12.84 %
3	0.09 Rf	262.9 AU	0.10 Rf	291.9 AU	11.50 %	0.13 Rf	59.5 AU	5884.2 AU	9.02 %
4	0.13 Rf	160.1 AU	0.18 Rf	313.2 AU	12.34 %	0.21 Rf	20.3 AU	10208.9 AU	15.65 %
5	0.21 Rf	120.5 AU	0.22 Rf	126.5 AU	4.98 %	0.26 Rf	86.6 AU	3398.9 AU	5.21 %
6	0.26 Rf	86.2 AU	0.30 Rf	96.9 AU	3.82 %	0.33 Rf	80.6 AU	4196.4 AU	6.43 %
7	0.34 Rf	81.8 AU	0.42 Rf	362.1 AU	14.26 %	0.43 Rf	45.8 AU	10282.1 AU	15.76 %
8	0.43 Rf	346.7 AU	0.45 Rf	357.2 AU	14.07 %	0.50 Rf	33.9 AU	9725.2 AU	14.91 %
9	0.50 Rf	33.9 AU	0.53 Rf	48.4 AU	1.91 %	0.55 Rf	43.4 AU	1307.4 AU	2.00 %
10	0.57 Rf	43.8 AU	0.63 Rf	245.8 AU	9.68 %	0.70 Rf	0.7 AU	8464.3 AU	12.98 %
11	0.83 Rf	0.2 AU	0.86 Rf	11.8 AU	0.46 %	0.90 Rf	2.2 AU	283.3 AU	0.43 %

Fig 4b. Ksheerakalyanaka ghritha.

DISCUSSION

On analysing the HPTLC reports,it was observed that at 254nm(Fig.2) two peaks were observed at R_f value of 0.09 and 0.48 for *Kalyanaka Ghrita* and three peaks were observed for *Ksheerakalyanaka Ghrita* at R_f value 0.09,0.13 and 0.55.At 366nm(Fig.3) for *Kalyanaka Ghrita*, four peaks were observed at R_f value 0.33,0.48,0.82 and 0.92 and three peaks were observed for *Ksheerakalyanaka Ghrita* at R_f value of 0.48,0.82and 0.92.At 620nm(Fig 4), six peaks were observed at R_f value of 0.04,0.11,0.35,0.44,0.52 and 0.87 for *Kalyanaka Ghrita* and seven peaks were observed at R_f value of 0.04,0.07,0.13,0.18,0.23,0.35 and 0.52 for *Ksheerakalyanaka Ghrita.*

CONCLUSION

The peaks observed in HPTLC can be considered for fingerprint profile analysis in order to get standard markers of the sample drug. To get a clear picture of the composition, there should be standard markers and test should be conducted with different solvent system which is out of the limit of the study. It is difficult to compare and interpret the results and it requires much detailed study with trial and error method. The addition of milk as an ingredient to the formulation can bring about changes in physico-chemical parameters as well as therapeutic properties.

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