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SIMULTANEOUS ESTIMATION OF FLAVONOIDS AND PHENOLIC ACID IN MARKETED SINGLE HERBAL FLOWER POWDERS OF *CASSIA AURICULATA* BY HPTLC TECHNIQUE

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ABSTRACT
The aim of the study is to detect simultaneously in single mobile phase the rutin, quercetin and gallic acid in anti diabetic commercial herbal formulations.HPTLC method was adopted to confirm the presence of these common secondary metabolites in the tested commercial herbal formulations. Results of the study clearly revealed the presence of rutin, quercetin, responsible for antidiabetic activity.The developed HPTLC method can be employed for the routine investigations of above revealed markers in commercial herbal formulations.

KEYWORDS: *Cassia auriculata,* Rutin, quercetin , gallic acid and HPTLC technique.

INTRODUCTION
C. auriculata (family: Cesalpinaceae) is a common plant in Asia, profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes,conjunctivitis and opthalmia.[1] It is a fast growing branched tall, evergreen shrub with reddish brown branches.[2] It is one of the principle constituents of 'Avaarai panchaga chooranam'- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level.[3] And well known for its phytochemical compositions,[4,5] pharmacological applications and therapeutic potential.[6] *Cassia* species are rich sources of polyphenols, anthraquinone derivatives, flavonoids and polysaccharides.[7] Each part of this plant has medicinal value.[8] *C. auriculata* flowers are Irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, and crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes). The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones. The petals also number 5, are free, imbricate and crisped along the margin, bright yellow veined with orange. The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules.[9] The flowers are used in throat troubles, urinary disorders and as astringent .[10] An aqueous extract of the leaves and flower possesses hypoglycemic activity [11] and antipyretic activity.[12] Recently it has been reported as a potent anticancer herb.[13]

MATERIALS AND METHODS
Collection of Marketed herbal formulations
Three cassia flower powders were procured.

Evaluation of Collected Marketed Herbal Formulations
Preparation of standards and extracts from the commercial herbal Formulations: One gram of the each formulation was taken and sonicated with 10 ml of methanol. Filtered and the filtrate solution was used for HPTLC analysis. Standard marker compounds were prepared using methanol to get a concentration 1 mg/1ml.

Table. 1: Organoleptic evaluation and pH of formulations.

Name of the formulation	Colour	Odour	Nature of particles	Taste	P^H of 1% solution of formulation
SKM Avaramboo	Yellowish Brown	aromatic	Fine powder	Bitter with mucilagenous	6.9
Cannan herbals avaramboo	Brown	aromatic	Fine powder	Bitter with mucilagenous	6.4
SVK herbals avaramboo	Brown	aromatic	Fine powder	Bitter with mucilagenous	6.6

Equipment: A Camag HPTLC system comprising of Linomat 5 applicator and Camag TLC scanner and single pan balance of Shimadzu model was used, for weighing the samples.

Chemicals and solvents: Rutin,Quercetingallic acid caffeicacid,ferulic acid and mangiferin were procured from Sigma Chemical Company Inc., USA. Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. HPTLC was carried out using Merck aluminium sheet coated with silica gel GF 254 (0.2 mm).

Preparation of extracts from the commercial herbal Formulations: One gram of the each formulation was taken and sonicated with 10 ml of methanol. Filtered and the filtrate solution was used for HPTLC analysis. Standard marker compounds were prepared using methanol to get a concentration 1 mg/1ml.

Application of sample: The sample solutions were spotted in the form of bands of width 6 mm with a Hamilton 100 µl syringe on precoated plate 60 F254 (10 cm × 10 cm with 0.2 mm m thickness, E.Merck) using a CamagLinomat V applicator. The slit dimension was kept mm × 0.45 mm. Five µl of each sample and standard solutions were applied on to the plate.

Development: The chromatogram was developed in Camag glass twin -trough chamber (10-10 cm) previously saturated with the mobile phase toluene: ethyl acetate: formic acid: methanol [3:6:1.6:0.4] for 10 min (temperature 25.2°C, relative humidity 40%). The migration distance was 80 mm. TLC plates were air dried with air dryer. Densitometric scanning was performed using Camag TLC Scanner -III at 254 nm and 366 nm operated by a Wincat software.

Detection: The plate was scanned at UV 254 and 366 nm using CamagTLC Scanner-3 and Linomat V. R$_f$value of each compound which were separated on p late and data of peak area of each band was recorded.

RESULTS AND DISCUSSION

The following different solvent compositions were tried for monitoring the elution of components in herbal formulations.[14]

1 Ethyl acetate: glacial acetic acid formic acid: water (100:3:3:28).
2 Ethyl Acetate: Methanol: Water Toluene (100:13:10:13).
3 Chloroform: ethyl acetate: methanol (6:4:0.3).
4 Ethyl Acetate: Methanol: Water Toluene (100:15.5:13.5:2).
5 Ethyl acetate: methanol:water (100:15.5:13.5).

Among the 5 mobile phases attempted, Toluene: ethyl acetate: formic acid: methanol in the ratio of 3:6:1.6:0.4 gave better elution for all the formulations tested and hence it was used as mobile phase for detection of constituents in herbal formulations. The optimized chamber saturation time for mobile phase was 3.0 min at room temperature (25 ± 1°C). The densitometric analysis was performed at 256 nm in reflectance mode.[15]

The Rf values of the marker compounds were in the range of 0.09 to 0.79 (Fig 1, Fig 2 and Table 3). SKM Avaramboo showed Rf values same as that of rutin 0.08, and quercetin0.86 . Cannan herbals avaramboo showed Rf value equal to rutin 0.10 and gallic acid 072 and SVK herbals avaramboo showed Rf values same as that of rutin 0.08, gallic acid 074 and quercetin0.86 . The chromatogram of the SKM Avaramboo, Cannan herbals avaramboo and SVK herbals avaramboo were shown in the Fig 2 to Fig 9.Rutin was present in all the three formulations In the present paper the ideal method, first time we followed was simultaneously detection of standard markers rutin, quercetin and, gallic acid present in anti oxidant and antidiabetic commercial herbal formulations by HPTLC technique.

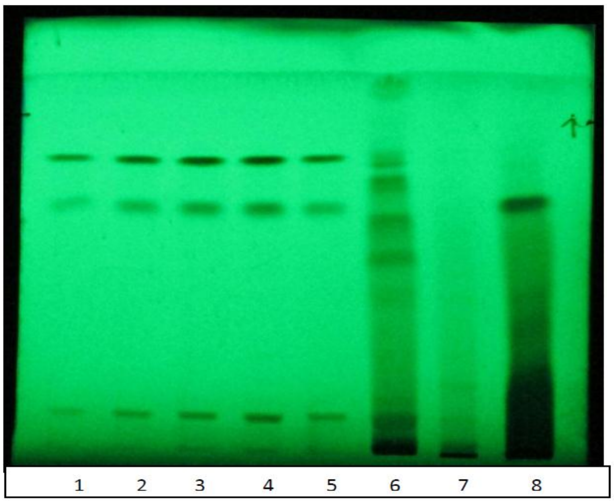
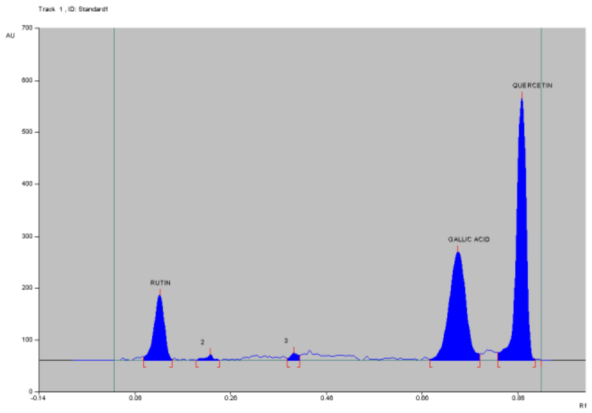


Fig. 1: Chromatogram of five concentrationsof standards and three commercial single powder formulations after development in mobile phase.

1 Track 1 RUTIN 0.08 (1 µL) Gallic acid0.73 (1 µL) Quercetin 0.87(1 µL)
2 Track 2 RUTIN 0.10 (2 µL) Gallic acid0.72 (2 µL) Quercetin 0.86(2 µL)
3 Track 3 RUTIN 0.10 (3 µL) Gallic acid0.72 (3 µL) Quercetin 0.86(3 µL)
4 Track 4 RUTIN 0.09 (4 µL) Gallic acid0.72 (4 µL) Quercetin 0.86(4 µL)
5 Track 5 RUTIN 0.10 (5 µL) Gallic acid0.72 (5 µL) Quercetin 0.87(5 µL)
6. Track 6 SKM Avaramboo (8µL) -12 peaks
7. Track 7Cannan herbals avaramboo (8µL)-10 peaks
8. Track 8 SVK herbals avaramboo (8µL)-8 peaks.

Table. 3: R_f values of Rutin, Gallic acid, Quercetin in herbal flower powder of cassia.

Tracknumber/ Name of formulation	Amount of sample applied in µl	Number of peak	No.of compounds & its R_f values	Name of marker present in formulation
Track-1 STD1	3.0 µl	3	**0.11,,0.73,0.87**	Rutin, Gallic acid, Quercetin
Track-2 STD2	6.0 µl	3	**0.10,,0.72, 0.86**	Rutin, Gallic acid, Quercetin
Track-3 STD3	9.0 µl	3	**0.10, 0.72,0.86**	Rutin, Gallic acid, Quercetin
Track-4 STD4	12.0 µl	3	**0.09, 0.72,0.86**	Rutin, Gallic acid, Quercetin
Track-5 STD5	15.0 µl	3	**0.10,0.72,0.87**	Rutin, Gallic acid, Quercetin
Track-6 SKM Avaramboo	8.0 µl	12	**0.08**,0.15,0.20,0.24,0.28,0.37, 0.47,0.57,0.69,0.80,**0.86**,0.89	Rutin, Qercetin
Track-7 Cannan herbals avaramboo	8.0 µl	10	**0.10**,0.19,0.30,0.39,0.43,0.45, 0.52,0.67,**0.72**,0.79	Rutin, Gallic acid
Track-8 SVK herbals avaramboo	8.0 µl	8	**0.08**,0.20,0.38,0.47,0.62,**0.74**, 0.79,**0.86**	Rutin, Gallic acid, Quercetin



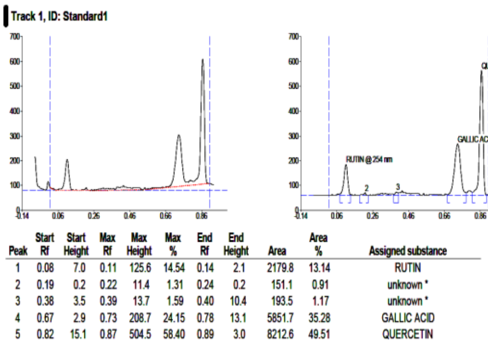
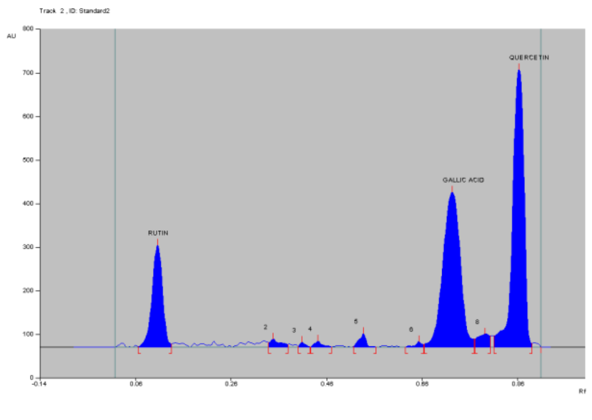


Fig. 2: Chromatogram of Track-1.



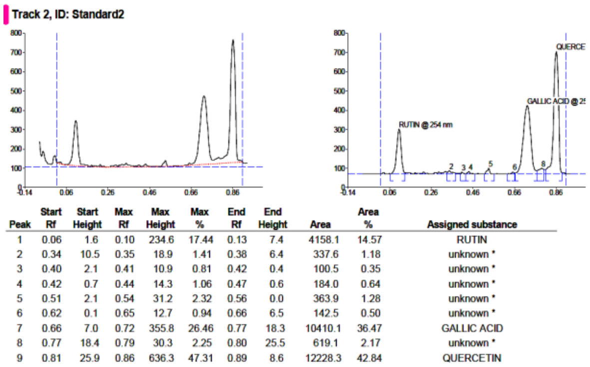
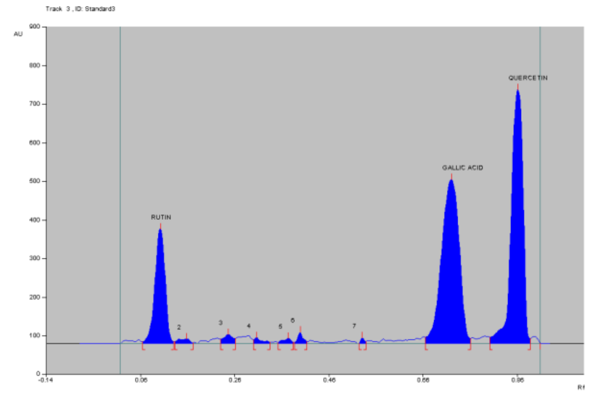


Fig. 3: Chromatogram of Track-2.



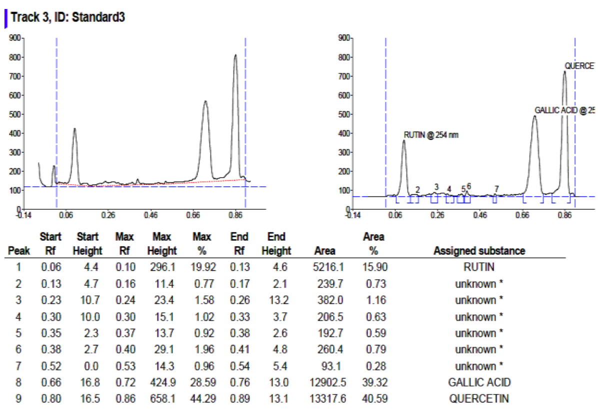
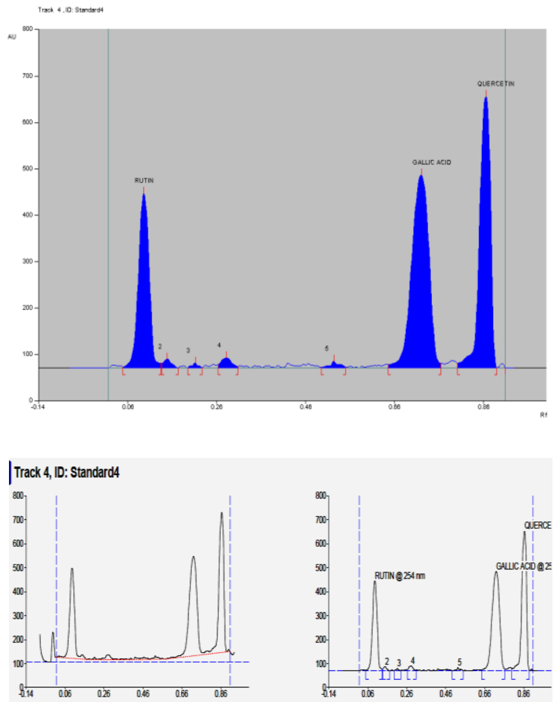
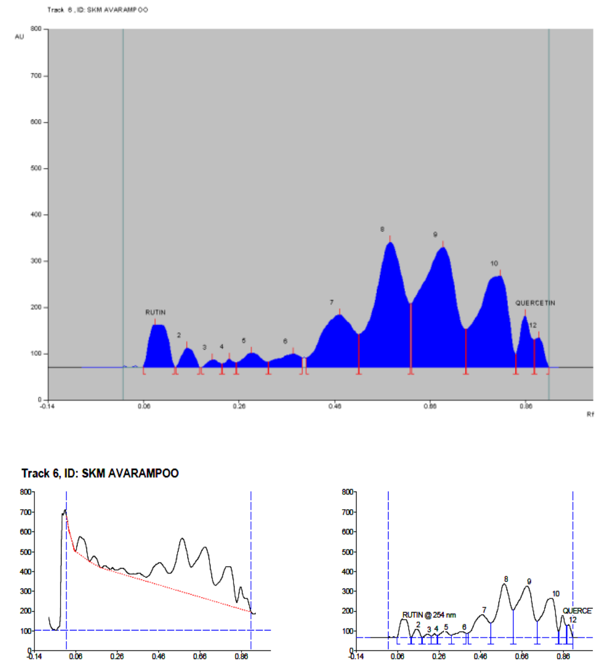


Fig. 4: Chromatogram of Track-3.



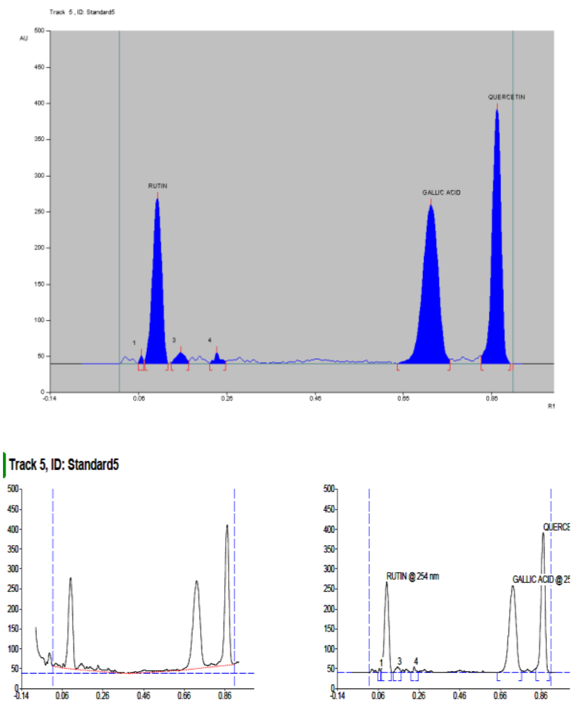
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	1.9	0.09	376.5	26.12	0.13	9.8	7381.8	22.28	RUTIN
2	0.13	9.8	0.15	18.7	1.30	0.17	0.7	273.0	0.82	unknown *
3	0.19	0.6	0.21	10.7	0.75	0.23	0.7	111.3	0.34	unknown *
4	0.26	7.0	0.28	21.5	1.49	0.31	2.7	414.4	1.25	unknown *
5	0.49	1.3	0.52	14.0	0.97	0.55	2.1	228.6	0.69	unknown *
6	0.64	6.8	0.72	415.5	28.82	0.76	9.9	13081.2	39.47	GALLIC ACID
7	0.80	9.8	0.86	584.7	40.56	0.89	0.1	11649.0	35.15	QUERCETIN

Fig. 5: Chromatogram of Track-4.



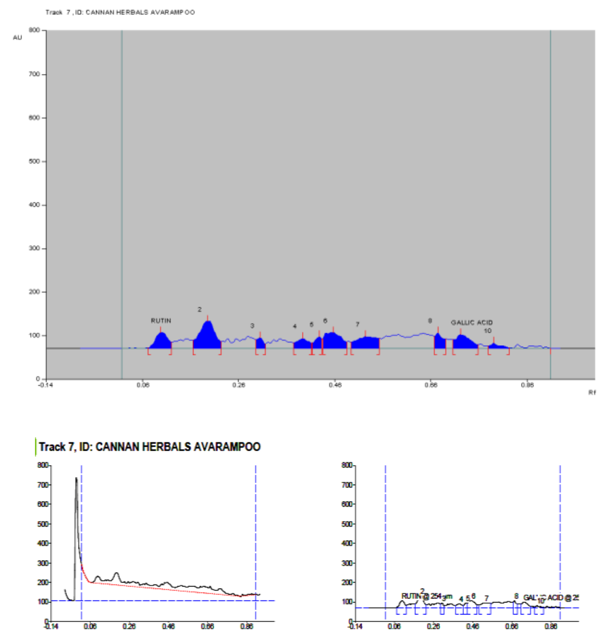
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	1.1	0.08	91.7	7.40	0.12	0.9	2850.3	5.31	RUTIN
2	0.13	0.2	0.15	41.2	3.32	0.18	0.5	865.0	1.61	unknown *
3	0.18	0.0	0.20	16.6	1.33	0.22	7.7	309.7	0.58	unknown *
4	0.22	7.9	0.24	18.0	1.45	0.25	9.8	258.7	0.48	unknown *
5	0.25	10.0	0.28	30.9	2.49	0.32	11.1	976.2	1.82	unknown *
6	0.32	11.2	0.37	28.8	2.32	0.39	20.8	1086.4	2.02	unknown *
7	0.40	20.5	0.47	113.4	9.15	0.51	70.9	6121.6	11.40	unknown *
8	0.51	71.1	0.57	270.0	21.77	0.62	137.0	13276.3	24.72	unknown *
9	0.62	137.7	0.69	258.5	20.84	0.73	81.8	15045.5	28.01	unknown *
10	0.73	82.1	0.80	197.2	15.90	0.84	28.0	9951.5	18.53	unknown *
11	0.84	28.8	0.86	110.8	8.93	0.88	59.2	2038.9	3.80	QUERCETIN
12	0.88	59.4	0.89	63.3	5.10	0.91	0.0	925.9	1.72	unknown *

Fig. 7: Chromatogram of Track-6 SKM Avaramboo.



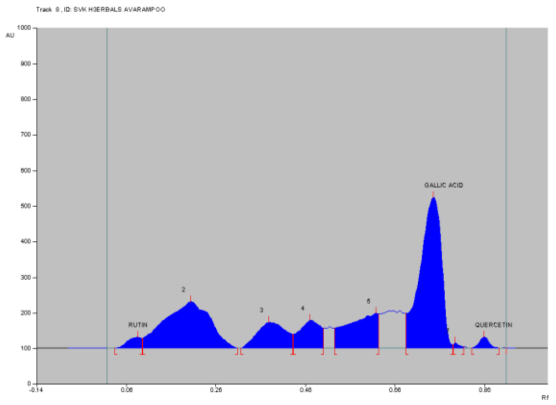
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	0.5	0.06	11.6	1.38	0.07	4.0	66.5	0.41	unknown *
2	0.07	4.6	0.10	229.2	27.19	0.12	0.6	3835.2	23.58	RUTIN
3	0.13	1.8	0.15	15.5	1.84	0.17	3.5	262.7	1.61	unknown *
4	0.22	0.5	0.24	15.1	1.79	0.26	3.3	166.1	1.02	unknown *
5	0.64	0.1	0.72	219.8	26.07	0.76	5.8	6187.3	38.03	GALLIC ACID
6	0.83	10.9	0.87	351.9	41.74	0.90	0.0	5749.9	35.35	QUERCETIN

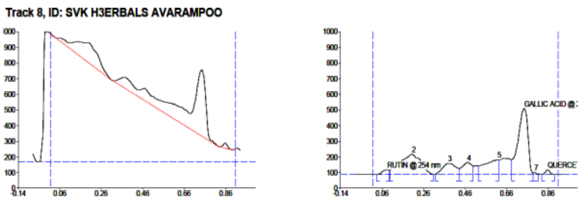
Fig. 6: Chromatogram of Track-5.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	1.1	0.10	36.2	11.46	0.12	14.1	762.0	11.01	RUTIN
2	0.16	19.1	0.19	62.5	19.75	0.22	16.0	1648.6	23.81	unknown *
3	0.29	20.1	0.30	24.8	7.85	0.31	10.2	286.2	4.13	unknown *
4	0.37	13.1	0.39	22.5	7.10	0.41	12.1	462.3	6.68	unknown *
5	0.41	12.3	0.43	26.8	8.47	0.43	21.8	311.3	4.50	unknown *
6	0.43	22.4	0.45	36.4	11.52	0.48	14.8	1041.9	15.05	unknown *
7	0.49	14.3	0.52	26.8	8.48	0.55	23.2	944.6	13.64	unknown *
8	0.67	27.7	0.67	36.2	11.45	0.69	21.2	466.4	6.74	unknown *
9	0.70	20.5	0.72	31.5	9.95	0.76	8.0	800.4	11.56	GALLIC ACID
10	0.78	5.8	0.79	12.5	3.96	0.82	1.7	199.1	2.88	unknown *

Fig. 8: Chromatogram of Track-7 Cannan herbals Aavaramboo.





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	0.1	0.08	30.1	3.44	0.09	26.9	764.7	1.88	RUTIN
2	0.09	27.3	0.20	130.0	14.84	0.31	0.1	10735.6	26.44	unknown *
3	0.31	0.2	0.38	71.5	8.16	0.43	38.7	3761.5	9.26	unknown *
4	0.43	38.9	0.47	78.4	8.95	0.50	55.4	2939.6	7.24	unknown *
5	0.52	56.6	0.62	97.6	11.15	0.62	95.3	5309.2	13.07	unknown *
6	0.68	96.8	0.74	423.0	48.29	0.79	9.2	16411.6	40.41	GALLIC ACID
7	0.79	9.9	0.79	14.0	1.60	0.81	4.1	152.2	0.37	unknown *
8	0.83	0.1	0.86	31.3	3.57	0.89	0.2	534.6	1.32	QUERCETIN

Fig. 9: Chromatogram of Track-8 SVK herbals Avaramboo.

Table. 4: Estimation of rutin in herbal flower powder of cassia auriculata.

Track Number/Name of the formulation	Amount of Sample applied in µl	R_f value of peak	Area of peak	Amount of marker present in applied µl of Sample in µg	Percentage of standard marker present in each 100mg of powder
Track-1 STD1	3.0 µl	0.11-Rutin	2179.8	1 µg	100%
		0.73-Gallic acid	5851.7	1 µg	100%
		0.87-Quercetin	8212.6	1 µg	100%
Track-2 STD2	6.0 µl	0.10-Rutin	4158.1	2 µg	100%
		0.72-Gallic acid	10410.1	2 µg	100%
		0.86-Quercetin	12228.3	2 µg	100%
Track-3 STD3	9.0 µl	0.10-Rutin	5216.1	3 µg	100%
		0.72-Gallic acid	12902.5	3 µg	100%
		0.86-Quercetin	13317.6	3 µg	100%
Track-4 STD4	12.0 µl	0.09-Rutin	7381.8	4 µg	100%
		0.72-Gallic acid	13081.2	4 µg	100%
		0.86-Quercetin	11649.0	4 µg	100%
Track-5 STD5	15.0 µl	0.10-Rutin	3835.2	5 µg	100%
		0.72-Gallic acid	6187.3	5 µg	100%
		0.87-Quercetin	5749.9	5 µg	100%
Track-6 SKM Avaramboo	8.0 µl	0.08-Rutin	2850.3	3.71 µg	3.7%
		0.86-Quercetin	2038.9	0.45 µg	0.45%
Track-7 cannan herbals avaramboo	8.0 µl	0.10-Rutin	762.0	0.99 µg	0.9%
		0.72-Gallic acid	800.4	0.24 µg	0.24%
Track-8 SVK Herbal avaramboo	8.0µl	0.08-Rutin	764.7	0.99 µg	0.9%
		0.074-Gallic acid	16411.6	5.01 µg	5.0%
		0.86-Quercetin	534.6	0.12 µg	0.12%

CONCLUSION

It can be concluded that rutin, quercetin and gallic acid were simultaneously detected in the three antioxidant and anti diabetic commercial formulations. Presence of rutin and and quercetin in6 SKM Avaramboo ,rutin and gallic acid in cannan herbals avaramboo and SVK herbal avaramboo contains all the three anti oxidant markers .Rutin was present inall the three anti diabetic commercial formulations . The markercompound selected for detection was well established for anti diabetic activity. The developed HPTLC method may be adopted for routine detection of rutin, quercetin, and gallic acid in commercial formulations bysimultaneous detection.

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