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Research Article

SELECTIVE HPTLC METHOD FOR ESTIMATION OF ASPARTIC ACID/VITEXIN IN *VIGNA MUNGO* - A NUTRITIONAL FOOD

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ABSTRACT

A simple sensitive HPTLC method developed for the Quantification of aspartic acid and vitexin in the plant *Vigna mungo* crude drug, lab extract and commercial extract. The stationary phase was precoated aluminium silica gel G F $_{254}$ Plates. The mobile phase for aspartic acid and vitexin were n-Butenol: Glacial acetic Acid: Water (50: 10: 40) and Glacial acetic Acid: Water (100: 11: 11: 26) respectively. The plate was scanned and quantified at 544 nm for aspartic acid and 366 nm for vitexin. The amount of aspartic acid and vitexin were estimated by the comparing the peak area of standard and the same were present in the crude drug, lab extract and commercial extract. The content of aspartic acid was found to be 0.0625% w/w, 0.7052% w/w & 0.2862% w/w and the content of vitexin was found to be 0.1624% w/w, 0.2007% w/w & 0.1633% w/w in *Vigna mungo* crude drug, lab extract and commercial extract respectively. In order to obtain precission and accuracy the recovery study were performed and result obtained with mean value 99.89 % and 101.44 % for aspartic acid and vitexin respectively, which prove reproducibility of the result. The mean of % RSD value was found to be 1.54 and 1.13 for aspartic acid and vitexin respectively. This estimation technique is very much useful for the estimation of aspartic acid and vitexin present in the various medicinal plants.

Keywords: Accuracy, Aspartic acid, Precission, Vitexin, HPTLC.

INTRODUCTION

Vigna mungo belonging to family Papilionaceae, black gram originated in India where it has been cultivation from ancient times and it has also been introduced to other tropical areas mainly by Indian immigrants. It is having Rheumatism, nervous disorders, aphrodisiac, liver diseases, diuretic and health food [1, 2, 3, 4, 5].

Vigna mungo seeds contain proteins, vitamins, amino acids and lipids. Seeds also contain phytoalexin, phaseollin, vitexin and iso-vitexin (flavonoids) and lectin.

High Performance Thin Layer Chromatography (HPTLC) is emerging as a versatile, high throughput & cost-effective technology that is uniquely suited to assessing the identity and quality of botanical materials [6, 7, 8].

The aim of the present work is to develop a method for estimation of aspartic acid and vitexin by HPTLC technique simultaneously.

MATERIALS AND METHODS

Plant materials

Vigna mungo seed material was collected at Ooty and authentified by Dr. S. Rajan, Field Botanist, Medicinal Plant Collection and Survey Unit, Department of Ayush, Emerald, Ooty. Commercial extract of *Vigna mungo* was obtained from Amsar Pvt. Ltd., Indore, and (M.P.) .The marker compound was obtained from Natural Remedies Pvt. Ltd., Bangalore, India.

Preparation of the plant extract

Coarse powder of the dried material of *Vigna mungo* seed extraction was carried out by maceration method (For 7 days) by using ethanol 70% as a solvent.

Method development of HPTLC

Standard preparation

5 mg of aspartic acid and vitexin was dissolved in 5 ml of methanol individually at (1mg/ml concentration).

Sample preparation (extracts)

Crude drug preparation: 1000 mg of powdered *Vigna mungo* crude drug was dissolved in 10 ml of methanol and slightly warmed on water bath and filtered through Whatman filter paper, and the same solution was used for HPTLC analysis (100 mg/ml concentration).

Extract preparation: 1000 mg of lab extract was dissolved in 10 ml of methanol and slightly warmed on water bath and filtered through Whatman filter paper, and the same solution was used for HPTLC analysis (100 mg/ml concentration). The same procedure was followed for the preparation of commercial extract.

Chromatographic conditions for determination of aspartic acid

Stationary phase	: Precoated Silica gel F $_{254}$ Plates (MERCK)
Mobile phase	: n-Butenol: Glacial acetic Acid: Water (50: 10: 40)
Saturation	: 60 mins
Development chamber	: CAMAG twin trough development chamber
Applicator	: CAMAG Linomat IV applicator
Scanner	: CAMAG Scanner III CATS (4.06), Switzerland
Mode of scanning	: Absorption (deuterium)
Detection wavelength	: 544 nm
Volume applied (standard)	: 5 µl
Volume applied (sample)	: 7 µl each sample

Chromatographic conditions for determination of vitexin

Stationary phase	: Precoated Silica gel F $_{254}$ Plates (Merck)
Mobile phase	: Ethyl acetate: Formic acid: Glacial acetic Acid: Water (100: 11: 11: 26)
Saturation	: 60 mins
Development chamber	: CAMAG twin trough development chamber
Applicator	: CAMAG Linomat IV applicator
Scanner	: CAMAG Scanner III CATS (4.06), Switzerland
Mode of scanning	: Absorption (deuterium)
Detection wavelength	: 366 nm
Volume applied (standards)	: 2 µl
Volume applied (samples)	: 10 µl each sample

Procedure

Before spotting, the plates were pre-washed with methanol. Standard and samples solutions were applied to the plates as sharp bands by means of CAMAG Linomat IV applicator. The spots were

dried in a current of air. The mobile phase (20 ml) was poured into a twin trough glass development chamber was left to equilibrate for 30 minutes and the plate was placed in the chamber [10, 11]. The plate was then developed until the solvent front had travelled at a distance of 75 mm above the base of the plate. The plate was then removed from the chamber and dried in a current of air. Detection and quantification was performed with CAMAG Scanner III at a wavelength of 364 nm.

Linearity

Linearity was performed by applying standard solution at different concentration range from 100 to 500 ng/spot and 500 to 2500 ng/spot aspartic acid and vitexin respectively, on 20 x 20 cm HPTLC plates, precoated silica gel G F_{254} Plates (Merck) in the form of sharp 7 mm bands; the distance between two adjacent band was 8 mm. the plates were developed in a solvent system of n-Butenol: Glacial acetic Acid: Water (50: 10: 40) and Ethyl acetate: Formic acid: Glacial acetic Acid: Water (100: 11: 11: 26) for aspartic acid and vitexin respectively, up to a distance 75 mm, at room temperature [9, 11]. The plates were dried in air.

The detector response for aspartic acid and vitexin were measured for each band at wavelength of 544 nm and 366 nm respectively, using CAMAG TLC Scanner and win Cat software [10]. The peak area of aspartic acid and vitexin were recorded for each concentration. The linearity curve of aspartic acid and vitexin were obtained by plotting agraph of peak area of aspartic acid and vitexin *vs* applied concentration of aspartic acid and vitexin (ng) respectively.

Method validation

The method was validated for precission, repeatability and accuracy. The precission was checked by repeated sacannig of same spot of aspartic acid (250 ng) and vitexin (1500 ng) respectively, three times each and was expressed as relative standard deviation (% RSD) [12, 13]. The repeatability of the method was confirmed by analyzing 100 ng, 250 ng and 500 ng/spot and 500 ng, 1500 ng and 2500 ng/spot of standard aspartic acid and vitexin respectively, solution (n = 3) and was expressed as % RSD. The precision of the method was studied by analyzing aliquots of standard solution of aspartic acid (100 ng, 250 ng and 500 ng/spot) and vitexin (500 ng, 1500 ng and 2500 ng/spot) respectively on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % RSD.

To study the accuracy, the recovery experiment was performed by the method of standard addition. The recovery of the added amount of standard was analyzed at three different levels. Each level of addition was repeated three times on three different days and the recovery of the add amount of standard was calculated.

Limit of detection and limit of quantitation was also calculated by the proposed method. [12, 13]

RESULTS AND DISCUSSION

The amount of aspartic acid and vitexin present in the crude drug, lab extract and commercial extract of *Vigna mungo* were estimated by using HPTLC technique by comparing with the peak area of standard and sample. The results are given in table 1. The results reveals that the R_f of the sample *Vigna mungo* crude drug, lab extract and commercial extract were matching with the standard R_f of marker compound aspartic acid and vitexin and the amount of marker compound present in the samples were calculated. The content of aspartic acid was found to be 0.0625% w/w, 0.7052% w/w & 0.2862% w/w and the content of vitexin was found to be 0.1624% w/w, 0.2007% w/w & 0.1633% w/w in *Vigna mungo* crude drug, lab extract and commercial extract respectively (Fig. 1, 2, 3, 4, 5 & 6).

The calibration curve was linear in the range of 100 to 500 ng/spot and 500 to 2500 ng/spot aspartic acid and vitexin respectively and the correlation coefficient was determined. The correlation coefficient was found to be 0.9899 and 0.9982 for aspartic acid and vitexin respectively.

The limit of quantification was found to be 120 ng and 450 ng for aspartic acid and vitexin respectively and the limit of detection was 40 ng and 150 ng respectively. The method was validated in terms of precission and reproducible expressed as % RSD which were found to be less than 2% and less than 2% for aspartic acid and vitexin respectively. The recovery values obtained were 99.49 to 100.24 % and 98.82 to 105.15 % showing accuracy of the method for aspartic acid and vitexin respectively. The average percentage recovery was found to be 99.88 % and 101.44 % for aspartic acid and vitexin respectively given in table 2.

Table 1: HPTLC quantification of aspartic acid and vitexin in *Vigna mungo*

S. No.	Sample	Marker compounds	Standard R_f values	Sample R_f values	Amount of Marker Compound (%w/w)
1	*Vigna mungo* raw	Aspartic acid	0.26	0.25	0.0625
		Vitexin	0.75	0.75	0.1624
2	*Vigna mungo* labExtract	As Aspartic acid	0.26	0.24	0.7052
		Vitexin	0.75	0.75	0.2007
3	*Vigna mungo*CommercialExtract	A Aspartic acid	0.26	0.24	0.2862
		Vitexin	0.75	0.74	0.1633

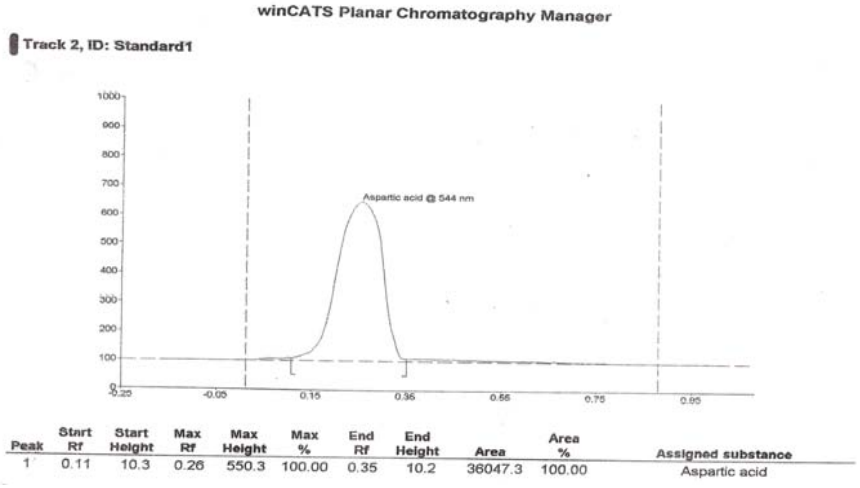
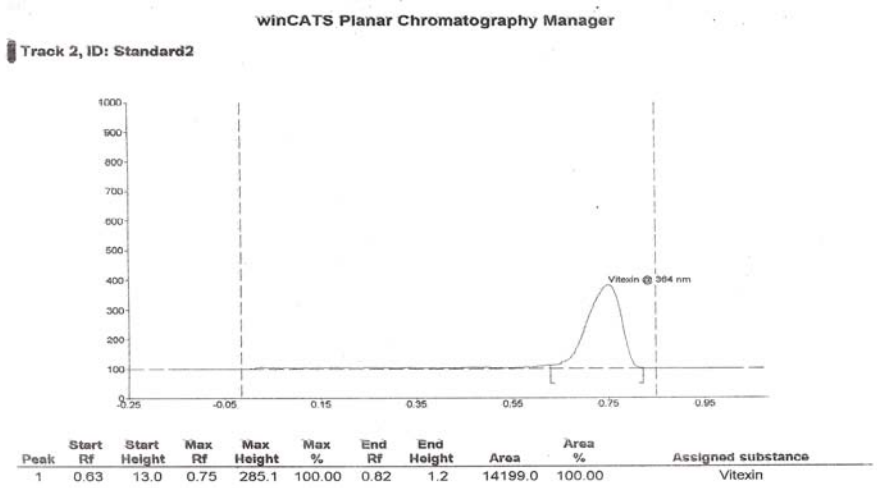


Fig. 1: HPTLC Chromatogram of standard aspartic acid

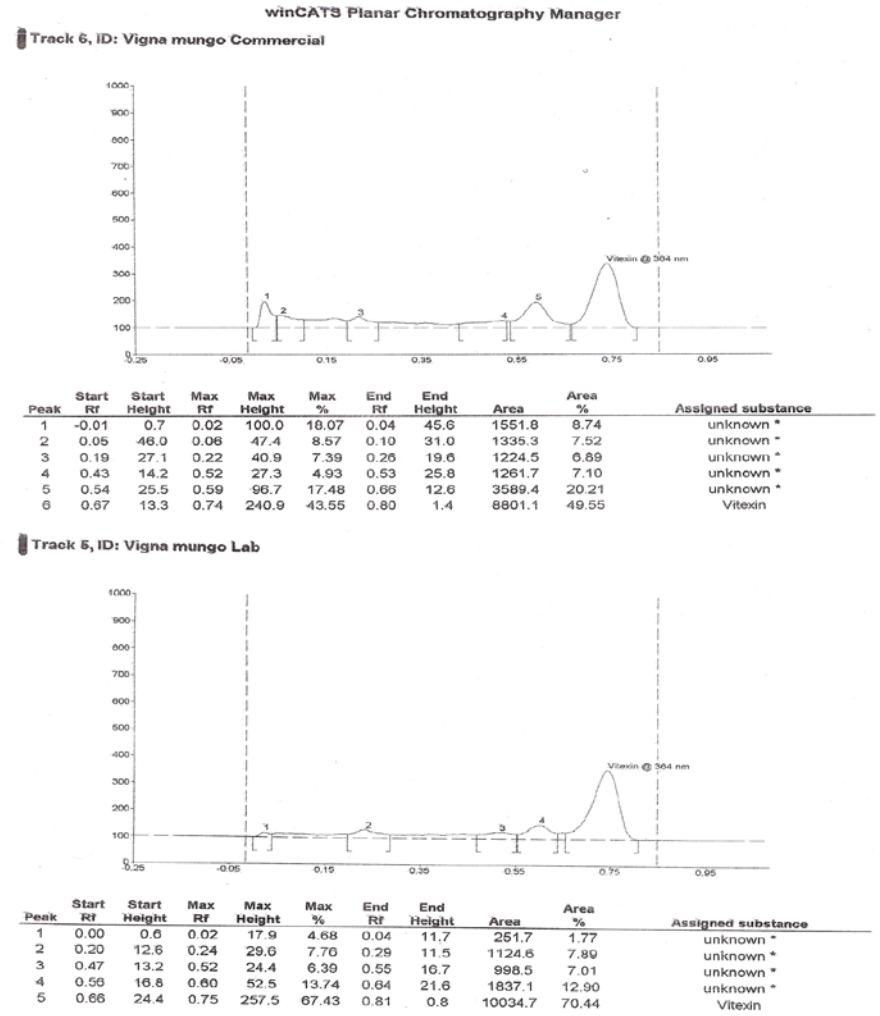
Table 2: Validation parameters for quantification of aspartic acid and vitexin by HPTLC

Parameters	Aspartic acid	Vitexin
Precission (% RSD)	< 2 %	< 2%
Linearity	100 to 500 ng/spot	500 to 2500 ng/spot
Limit of detection	40 ng/spot	150 ng/spot
Limit of quantification	120 ng/spot	450 ng/spot
Accuracy	99.49 to 100.24 %	98.82 to 105.15 %



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.63	13.0	0.75	285.1	100.00	0.82	1.2	14199.0	100.00	Vitexin

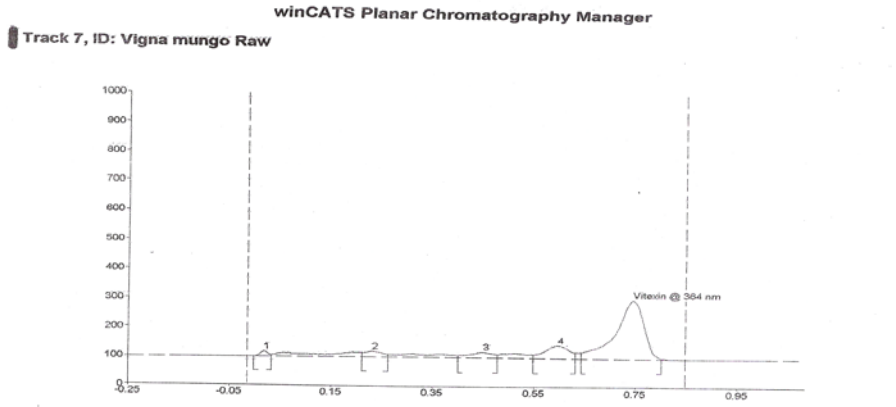
Fig. 2: HPTLC Chromatogram of standard vitexin



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	0.7	0.02	100.0	18.07	0.04	45.6	1551.8	8.74	unknown *
2	0.05	46.0	0.06	47.4	8.57	0.10	31.0	1335.3	7.52	unknown *
3	0.19	27.1	0.22	40.9	7.39	0.26	19.6	1224.5	6.89	unknown *
4	0.43	14.2	0.52	27.3	4.93	0.53	25.8	1261.7	7.10	unknown *
5	0.54	25.5	0.59	96.7	17.48	0.66	12.6	3589.4	20.21	unknown *
6	0.67	13.3	0.74	240.9	43.55	0.80	1.4	8801.1	49.55	Vitexin

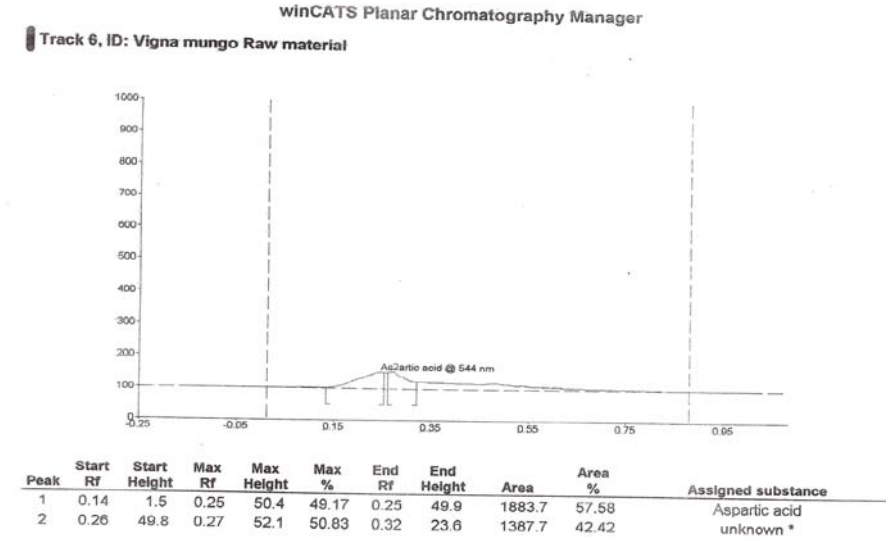
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.6	0.02	17.9	4.68	0.04	11.7	251.7	1.77	unknown *
2	0.20	12.6	0.24	29.6	7.76	0.29	11.5	1124.6	7.89	unknown *
3	0.47	13.2	0.52	24.4	6.39	0.55	16.7	998.5	7.01	unknown *
4	0.56	16.8	0.60	52.5	13.74	0.64	21.6	1837.1	12.90	unknown *
5	0.66	24.4	0.75	257.5	67.43	0.81	0.8	10034.7	70.44	Vitexin

Fig. 3: HPTLC Chromatogram of *Vigna mungo* commercial extract (Track No. 6) and lab extract (Track No. 5) for Vitexin



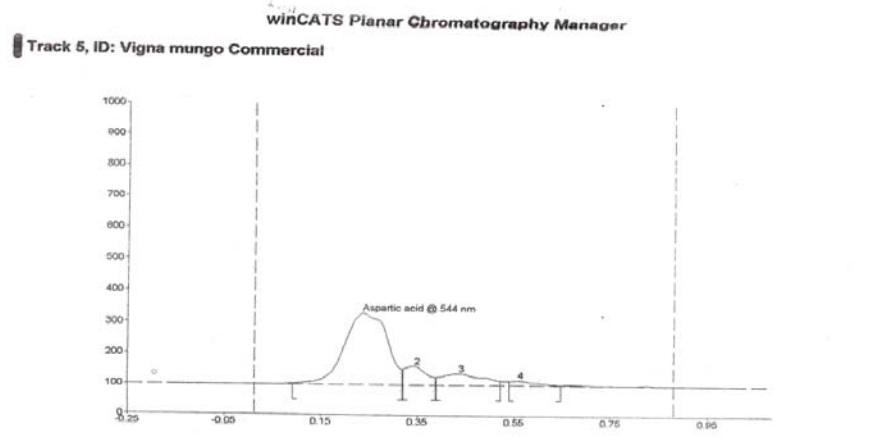
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.00	0.8	0.02	16.9	5.57	0.03	6.5	200.2	1.84	unknown *
2	0.21	15.3	0.23	20.1	6.60	0.26	11.5	529.6	4.85	unknown *
3	0.40	9.0	0.45	20.5	6.75	0.48	13.0	719.1	6.59	unknown *
4	0.55	13.7	0.60	44.4	14.61	0.63	21.3	1532.7	14.05	unknown *
5	0.65	22.5	0.75	202.1	66.47	0.80	2.3	7929.7	72.67	Vitexin

Fig. 4: HPTLC Chromatogram of *Vigna mungo* raw material for vitexin

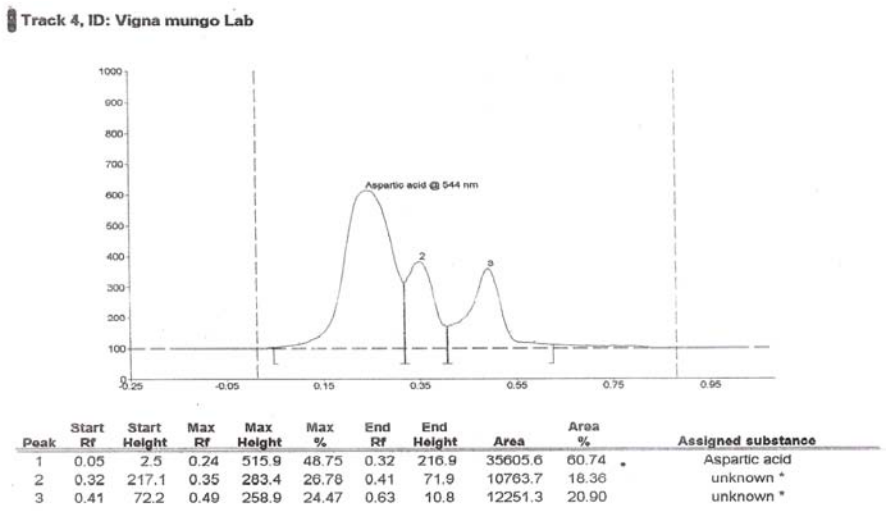


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.14	1.5	0.25	50.4	49.17	0.25	49.9	1883.7	57.58	Aspartic acid
2	0.26	49.8	0.27	52.1	50.83	0.32	23.6	1387.7	42.42	unknown *

Fig. 5: HPTLC Chromatogram of *Vigna mungo* raw material for aspartic acid



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	2.7	0.24	231.6	66.51	0.32	53.2	14449.5	74.39	Aspartic acid
2	0.32	53.4	0.34	61.8	17.74	0.39	26.4	1972.2	10.15	unknown *
3	0.39	26.5	0.44	38.5	11.05	0.52	15.1	2368.4	12.19	unknown *
4	0.54	14.9	0.56	16.4	4.70	0.65	1.8	633.4	3.26	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	2.5	0.24	515.9	48.75	0.32	216.9	35605.6	60.74	Aspartic acid
2	0.32	217.1	0.35	283.4	26.78	0.41	71.9	10763.7	18.36	unknown *
3	0.41	72.2	0.49	258.9	24.47	0.63	10.8	12251.3	20.90	unknown *

Fig. 6: HPTLC Chromatogram of *Vigna mungo* commercial extract (Track No. 5) and lab extract (Track No. 4) for aspartic acid

CONCLUSION

The developed HPTLC method was simple accurate, precise, economic and can be utilised for estimation of aspartic acid and vitexin in *Vigna mungo* could be used as a valuable analytical tool in the routine analysis. Aspartic acid and vitexin can be used as one of the appropriate analytical markers present in the various medicinal plants.

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