HPTLC FINGERPRINT PROFILE OF ORMOCARPUM SENNOIDES LEAVES

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ABSTRACT

Objective:To develop the finger print of medicinally and economically important leaves of Ormocarpum Sennoides .Method: Ethanol extract of the leaves developed in mobile phase of Chloroform: Methanol (6:4) using standard procedures and scanned under UV at 254nm, 366nm. Result: HPTLC finger printing of Ethanol extract has shown several peaks with different Rf values. Conclusion: Since there is no previous hptlc study on this drug, this fingerprint would help in the identification and authentication of this species and provide referential information for standardization of the Ormocarpum Sennoides for curing different bone related issues.

Keywords : HPTLC Fingerprint Chromatography, Ormocarpum Sennoides,Kattumurungai, Elumbotti, Lumbago, Plant Authentication

I. INTRODUCTION

The recent advances in modern medicine have evolved from folk medicine and traditional system by probing through the various phytochemical and pharmaceutical screening is necessary for the evolving trends in modern medicine. It has estimated that 56% of lead compounds for medicines in British national formulary and natural products [1]. There are about 1250 Indian medicinal plants, which used for formulating therapeutic preparation according Ayurveda and other traditional system of medicine [2].

Standardization of plant material is essential for identification and quantification of active constituents in the plant material for drug formulation using chromatographic and spectral fingerprints.

HPTLC methods are more proficient, faster and outcomes are more reliable and reproducible. When combining HPTLC with digital scanning profiling, It also provides accurate Rf values and quantifiable analysis of samples by in situ scanning densitometry aided by the formation of easily detective derivatives by post chromatography chemical reactions as required as well as record of separation in the form of chromatography with fractions represented as peaks with defined parameters including observance (Intensity), Rf height and area [3]. The pictorial fluorescence image of HPTLC coupled with digital scanning profile is more attractive to herbal analysts for constructing an herbal chromatography fingerprint for means ofHPTLC [7], [8].

1.1. Ormocarpum Sennoides

Kattumurungai is a shrub with membranous leaflets, yellow flowers and moniliform pods found in Orissa, Deccan and South India. The roots are considered tonic, stimulant and are used in the treatment of Lumbago, an

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application prepared by rubbing the root bark, oil is used in paralysis[4]. The leaves are used in traditional Indian medicine system especially by the Irula Tribes for fracture healing in Kanchipuram district,Tamil Nadu,India.

II. MATERIALS AND METHODS

2.1. Instrumentation

A Camag HPTLC system, HPTLC aluminium sheet silica gel (E-Merck), CamagLinomat V, Camag TLC scanner, Camag Visualizer, Win cats software.

2.2. Materials and Reagents

HPLC grade ethanol, chloroform, methanol were obtained from E-Merk India.

2.3. Plant Materials

Ormocarpum Sennoides were collected from the forest in Kanchipuram District, Tamil Nadu, India and authenticated by Botanical Survey of India (BSI)/Ministry of environment and forest-Coimbatore, India (BSI/SRC/5/23/2013-14Tech/550, shade dried leaves were ground to get course powder that was stored in air tight container.

2.4. Preparation of Ethanolic Extract

The course powder weighed accurately 600gms was soaked in n-hexane for defatting for 48 hours and then successively extracted in 80% ethanol at room temperature, the solvent was then removed by filtration and fresh solvent was added to the plant materials, the extraction process was twice repeated the combined filtrate were then evaporated under reduced pressure to give a dark green Viscous Mass the extract was stored at 0-4^{o}C, 20% yield was acquired[5].

2.5. Chromatography

10µ Aliquots of the extract separately applied on silica gel 60 F254 pre-coated HPTLC plates (5x10 cms) and desiccator used to stockpile the plates Hamilton micro syringe usedfor the purpose of application and the same was mounted.Everything tested using Camag Linomat-V applicator included in the Camag HPTLC systemprogram of the Wincats Software [9].

Spotting was done on the TLC. Ascending development of the plate, the migration distance 85mm (distance to the lower edge was 5mm) was performed at 25 ± 20^{o}Cwith Chloroform: Methanal (6:4) as a mobile phase in a Camag chamber previously saturated for 30 minutes. 10µl concentration of the samples was applied in three Tracks as 8mm bands at spraying rate of 150nl/s. After development the plate was Air-dried.

Densitometry scanning was then performed with a Camag TLC scanner equipped with Win-Cats software version 1.3.0 at λ_{max}=254nm and 366nm the slit dimensions were 6.00 x 0.03 mm micro and the chromatogram were recorded [6]

III. RESULTS

The Ethanolic extracts of Ormocarpum Sennoides were subjected to HPTLC analysis by specific solvent system chloroform: methanol (6:4) and detected under UV at 254nm(Fig.1) and 366nm(Fig.2).

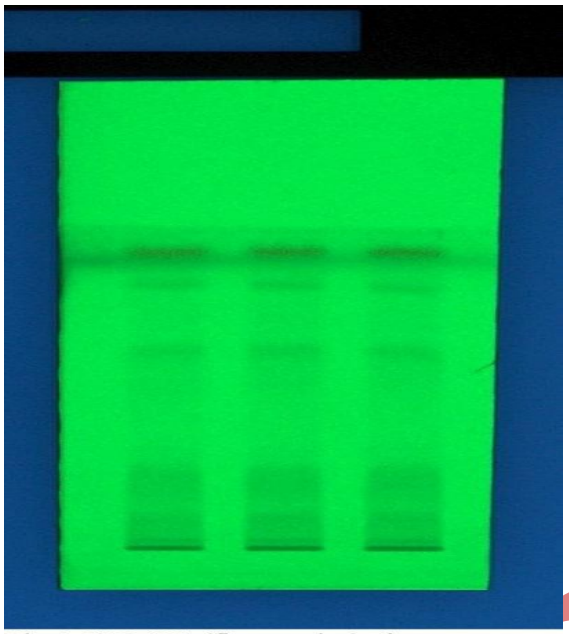

Fig.1. HPTLC (fingerprint) chromatogram of ethanol extract of Ormocarpum Sennoides at 254nm wavelength

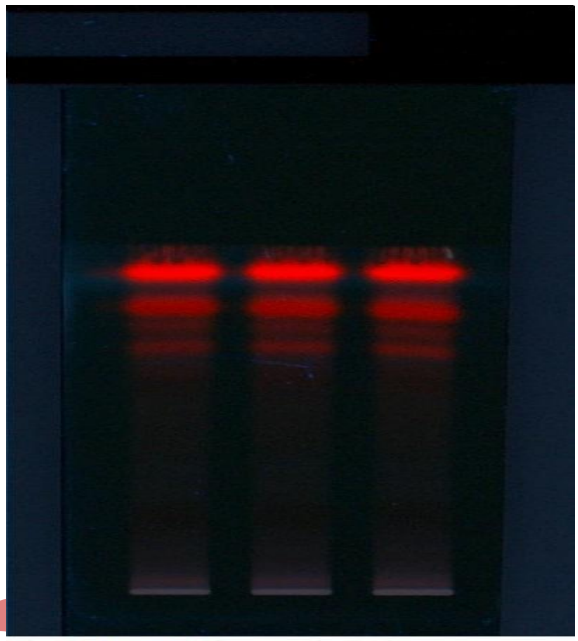

Fig.2.HPTLC (fingerprint) chromatogram of ethanol extract of Ormocarpum Sennoides at 366nm wavelength

The HPTLC images shown in Fig.3 andFig.4 indicate all the sample constituents were clearly without any tailing and diffuseness.

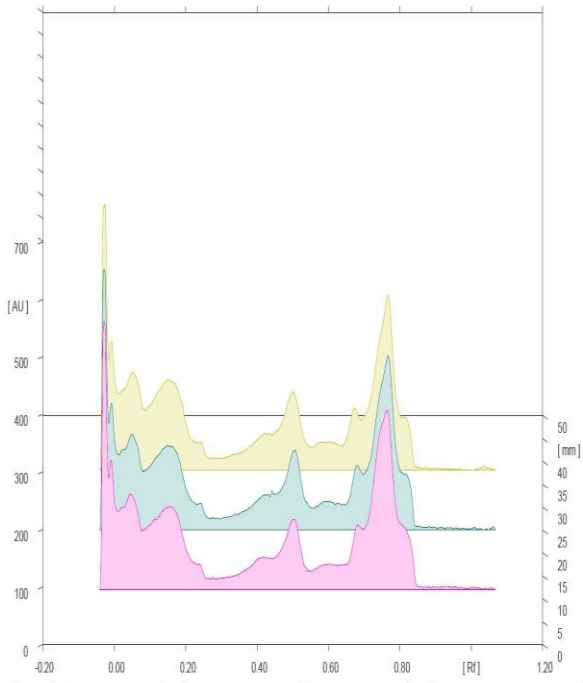

Fig.3. All chromatogram tracks of Ormocarpum Sennoides at 254nm wavelength

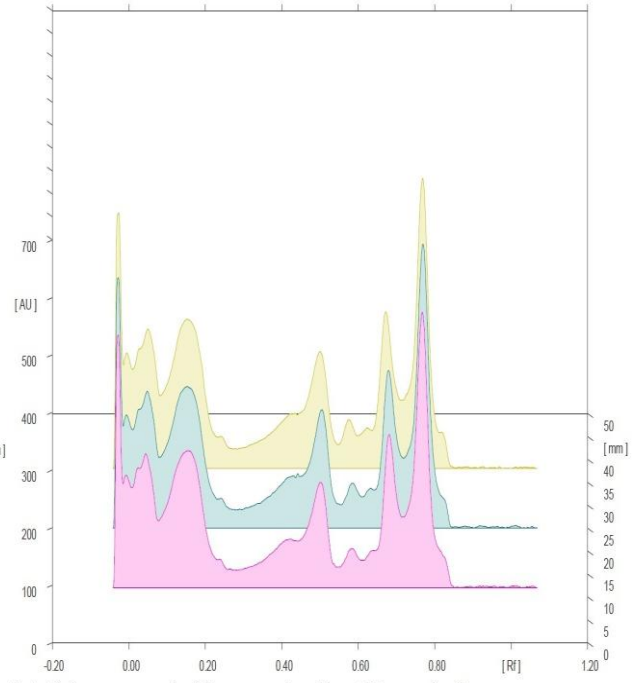

Fig.4. All chromatogram tracks of Ormocarpum Sennoides at 366nm wavelength

It is evident from TABLE1i.e., 10µl of ethanol extract of Ormocarpum Sennoides, at application position 12mm, 25mm, 38mm at 254nm express 9,10,9 spots respectively.

TABLE1. Peak list and Rf values of the chromatogram of 10 µl of methanol extract of Ormocarpum Sennoides at 12mm application position

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	0.4	-0.03	466.5	28.34	-0.02	193.4	5162.5	9.03
2	-0.02	196.9	-0.01	224.5	13.64	0.01	135.7	3507.0	6.13
3	0.03	142.4	0.04	165.3	10.05	0.08	101.9	5488.8	9.60
4	0.08	101.9	0.16	144.2	8.76	0.23	42.9	12011.5	21.01
5	0.35	26.3	0.42	56.1	3.41	0.43	54.3	2748.4	4.81
6	0.44	53.0	0.50	122.1	7.42	0.54	32.0	5710.1	9.99
7	0.57	37.1	0.60	44.1	2.68	0.62	41.9	1515.5	2.65
8	0.64	42.4	0.68	111.8	6.80	0.70	104.3	3129.0	5.47
9	0.70	104.6	0.77	311.2	18.91	0.85	4.6	17903.9	31.31

The following Max Rf values -0.03, -0.01, 0.04, 0.16,0.42, 0.50, 0.60, 0.68, 0.77 (Fig.5) indicating Rf values 0.04, 0.16, 0.50, 0.68, 0.77 were found predominantly at percentage area, 9.6%, 21.01%,9.99%, 5.47%, 31.31% and remaining spots were found to less in quantity, as the percentage area for all the spots were less than 5.6%.

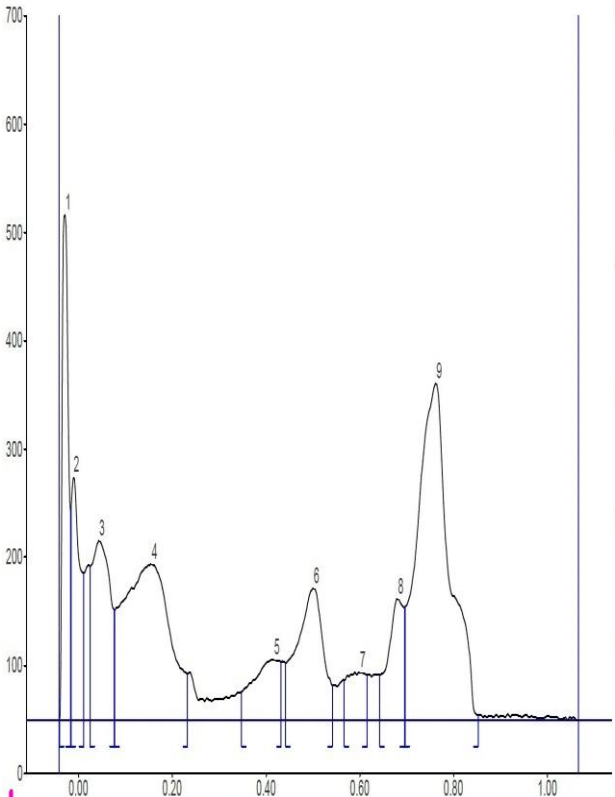

Fig.5. Track1: Ormocarpum Sennoides at application position of 12mm(254nm wavelength)

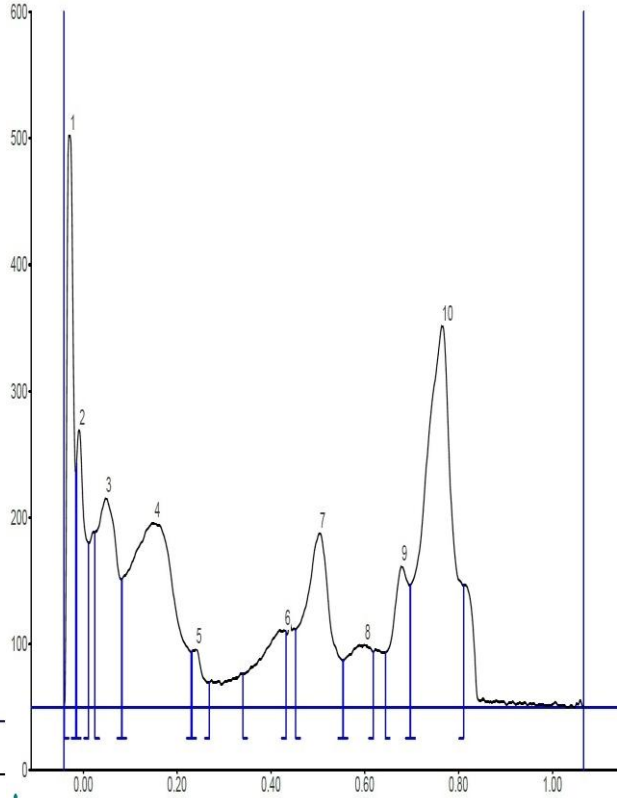

Fig.6. Track2: Ormocarpum Sennoides at application position of 25mm(254nm wavelength)

TABLE2 shows 10 spots at max Rf values -0.03, -0.01, 0.05, 0.15, 0.24, 0.43, 0.51, 0.60, 0.68, 0.77(Fig.6). The components with Rf values -0.03, 0.05, 0.15, 0.51, 0.77 were found predominant as the percentage area was more with 9.38%, 10.54%,20.67%,10.94%,26.34%, and remaining spots were found to less in quantity, as the percentage area for all the spots were less than 4.5%.

TABLE2. Peak list and Rf values of the chromatogram of 10 µl of methanol extract of Ormocarpum Sennoides at 25mm application position

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	1.1	-0.03	453.0	26.74	-0.02	185.5	5299.6	9.38
2	-0.01	192.5	-0.01	220.1	12.99	0.01	130.4	3272.9	5.79
3	0.03	139.1	0.05	166.0	9.79	0.08	101.2	5956.2	10.54
4	0.08	101.6	0.15	146.2	8.63	0.23	43.9	11682.2	20.67
5	0.23	44.2	0.24	46.2	2.73	0.27	19.6	919.7	1.63
6	0.34	26.4	0.43	61.2	3.61	0.43	60.1	2961.6	5.24
7	0.45	61.8	0.51	138.1	8.15	0.55	37.4	6181.3	10.94
8	0.56	37.6	0.60	49.6	2.93	0.62	44.0	2130.5	3.77
9	0.65	43.5	0.68	111.7	6.59	0.70	96.8	3221.2	5.70
10	0.70	97.3	0.77	302.3	17.84	0.81	97.0	14882.3	26.34

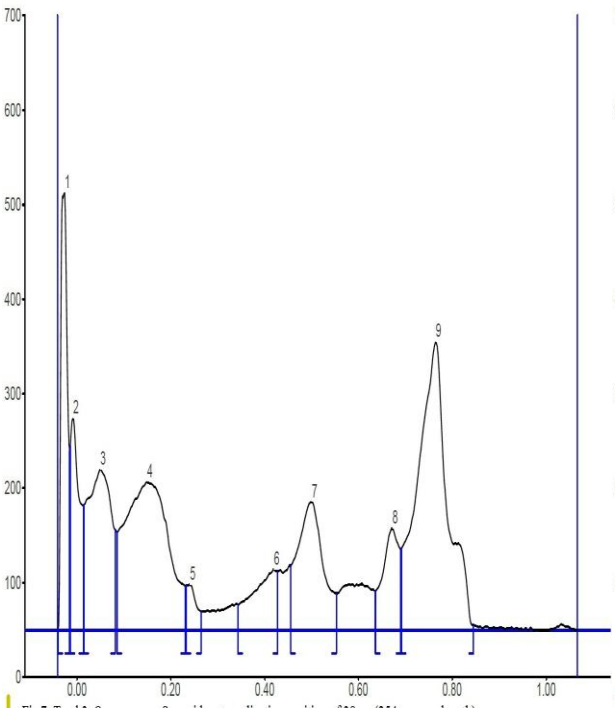

Fig.7. Track3: Ormocarpum Sennoides at application position of 38mm(254nm wavelength)

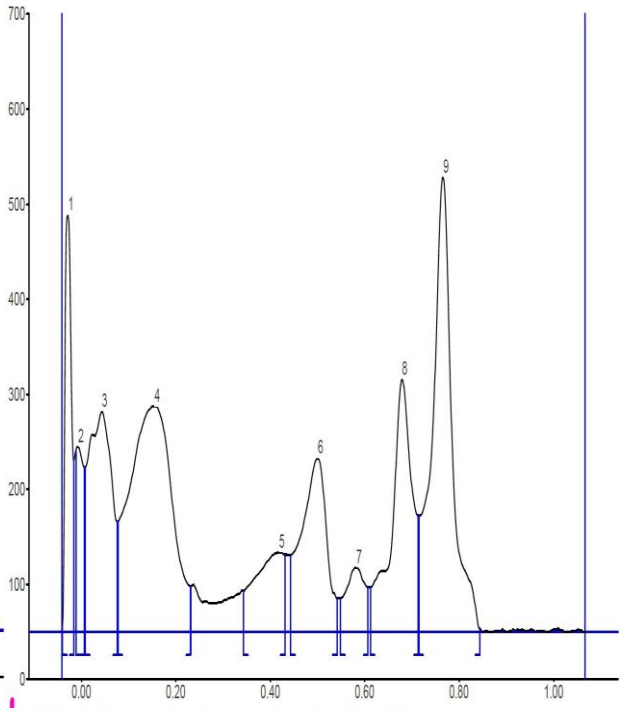

Fig.8. Track1: Ormocarpum Sennoides at application position of 12mm(366nm wavelength)

TABLE3. Peak list and Rf values of the chromatogram of 10 µl of methanol extract of Ormocarpum Sennoides at 38mm application position

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	1.3	-0.03	462.8	27.59	-0.02	190.3	5480.1	9.48
2	-0.01	195.3	-0.01	224.8	13.40	0.01	132.5	3623.2	6.27
3	0.02	132.8	0.05	170.5	10.17	0.08	106.9	7167.0	12.40
4	0.09	104.3	0.15	157.3	9.38	0.23	47.7	12273.5	21.24
5	0.23	47.9	0.24	48.4	2.89	0.27	20.1	870.9	1.51
6	0.34	27.8	0.42	64.4	3.84	0.43	62.8	2847.2	4.93
7	0.46	68.7	0.50	136.1	8.12	0.55	39.6	6207.1	10.74
8	0.64	41.9	0.67	108.0	6.44	0.69	86.4	3157.2	5.46
9	0.69	87.0	0.77	305.1	18.19	0.85	5.5	16168.3	27.98

TABLE3 shown 9 spots at max Rf values -0.03, -0.01, 0.05, 0.15, 0.24, 0.42, 0.50, 0.67, 0.77 (Fig.7) components with Rf values -0.03, 0.05, 0.15, 0.50, 0.77 has more percentage area 9.48%, 12.40%, 21.24%, 10.74%, 27.98% respectively, and others are less than 4.5%.

Fig.8, 10µl of ethanol extract Ormocarpum Sennoides at application position 12mm,25mm, 38mm at 366nm express 9, 11, 11 spots respectively.

TABLE4. Peak list and Rf values of the chromatogram of 10 µl of methanol extract of Ormocarpum Sennoides at 12mm application position

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	4.3	-0.03	439.1	20.10	-0.02	180.4	5136.5	6.70
2	-0.01	191.9	-0.01	195.3	8.94	0.01	173.4	2608.7	3.40
3	0.01	173.6	0.04	232.3	10.63	0.08	116.0	9588.2	12.51
4	0.08	116.2	0.16	237.8	10.88	0.23	48.7	18060.1	23.56
5	0.34	43.6	0.42	84.1	3.85	0.43	81.7	4344.5	5.67
6	0.44	80.9	0.50	182.8	8.37	0.54	35.5	8190.9	10.69
7	0.55	35.9	0.58	68.2	3.12	0.61	47.3	2306.5	3.01
8	0.61	47.3	0.68	266.2	12.18	0.72	122.4	9664.2	2.61
9	0.72	122.4	0.77	479.0	21.93	0.85	3.6	16753.6	21.86

TABLE4 revealed that 9 components are present, the component with Rf values 0.04, 0.16, 0.50, 0.68, 0.77(Fig.9) were fond predominantly with percentage area 12.51%, 23.56%, 10.69%, 12.61%, 21.86% and the remaining area were found to be less in quantity as the percentage area for all the spots were less than 4.7%.

TABLE5 shows the presents of 11 compounds the components with Rf values -0.03,0.15,0.50,0.68,0.77(Fig.9) were found predominantly with percentage area 6.40%, 22.63%, 10.94%, 11.47%, 20.50% and the remaining area were found to be less in quantity as the percentage area for all the spots were less than 4.6%.

TABLE5. Peak list and Rf values of the chromatogram of 10 µl of methanol extract of Ormocarpum Sennoides at 25mm application position

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	4.2	-0.03	435.3	18.30	-0.02	177.1	5188.0	6.40
2	-0.01	179.1	-0.01	196.4	8.26	0.01	171.0	3326.1	4.10
3	0.01	171.6	0.05	237.4	9.98	0.08	122.6	9802.9	12.09
4	0.08	122.9	0.15	246.0	10.34	0.23	50.3	18342.3	22.63
5	0.23	50.4	0.24	51.5	2.17	0.27	32.5	1136.5	1.40
6	0.34	42.6	0.43	90.9	3.82	0.43	89.5	4623.8	5.70
7	0.45	90.1	0.50	205.6	8.64	0.55	41.8	8867.4	10.94
8	0.56	40.1	0.58	78.0	3.28	0.61	54.2	2466.8	3.04
9	0.61	54.2	0.63	69.3	2.91	0.64	66.2	1389.5	1.71
10	0.64	66.3	0.68	274.0	11.52	0.72	121.5	9296.0	11.47
11	0.72	122.0	0.77	493.9	20.77	0.85	1.5	16619.9	20.50

TABLE6 express 11 components, the Rf values 0.05,0.15,0.50,0.67,0.77(Fig.10) were found predominantly with percentage are 12.01%, 22.04%, 11.25%, 10.53%, 20.22% respectively, the remaining spots were found to be less in quantity and percentage area for all the spots were less than 4%.

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TABLE6. Peak list and Rf values of the chromatogram of 10 µl of methanol extract of Ormocarpum Sennoides at 38mm application position

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	4.7	-0.03	445.1	18.27	-0.02	180.2	5364.4	6.21
2	-0.01	180.3	-0.01	201.1	8.26	0.01	172.3	3383.2	3.92
3	0.01	172.8	0.05	242.4	9.95	0.08	126.3	10380.6	12.01
4	0.08	126.6	0.15	259.9	10.67	0.23	54.4	19046.2	22.04
5	0.23	54.5	0.24	55.9	2.30	0.27	34.0	1277.1	1.48
6	0.30	36.4	0.43	95.6	3.92	0.44	93.4	6497.0	7.52
7	0.44	93.7	0.50	203.4	8.35	0.55	41.1	9718.5	11.25
8	0.55	41.2	0.58	84.9	3.48	0.60	56.8	2483.2	2.87
9	0.60	56.9	0.62	70.4	2.89	0.64	65.7	1690.4	1.96
10	0.64	66.2	0.67	272.7	11.20	0.71	118.3	9100.7	10.53
11	0.71	118.3	0.77	504.7	20.72	0.86	1.1	17472.3	20.22

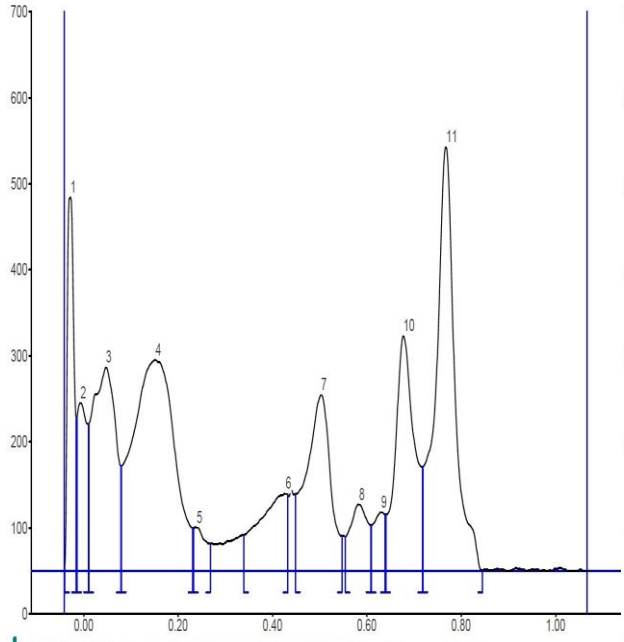

Fig.9. Track2: Ormocarpum Sennoides at application position of 25mm(366nm wavelength)

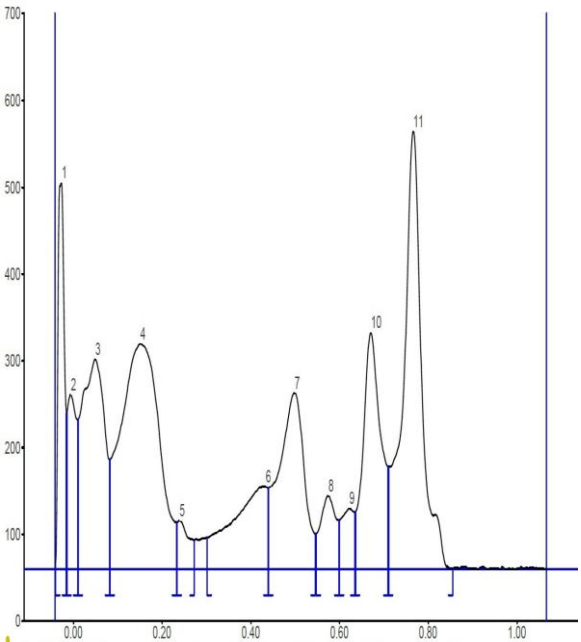

Fig.10. Track3: Ormocarpum Sennoides at application position of 38mm(366nm wavelength)

IV. DISCUSSION

Thus the HPTLC chromatogram developed for Ormocarpum Sennoides ethanol extract with specific Rf values serve as a better tool for standardization of the plant drug. Characteristic HPTLC finger printing of particular plant species will help in the identification and quality control of a particular species, and provide basic information for isolation, purification of various compounds and drug development for various disease condition.

V. CONCLUSION

HPTLC is the most reliable method for development of chromatographic fingerprints to determine major active constituents of medicinal plants, HPTLC analysis of Ormocarpum Sennoidesleaves can provide standard fingerprint and can be used as a reference for the identification and quality control of the drug.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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