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High-Performance Thin Layer (HPTLC) analysis of anti-diabetic activity spectra of a polyherbal formulation

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Abstract

Since there is a very huge development in the use of traditional medicine, assurance and potency as well as quality control of herbal medicines and traditional therapies are becoming more important. Hence, to determine the active principal compounds and its possible biological and pharmacological activities of a polyherbal formulation (PHF) was made up of 11 plants namely *Moringa oleifera* Lam, *Aegle marmelos* (L.) Correa, *Murraya koenigii* (L. Spreng), *Annona reticulata* L., *Piper betle* L., *Psidium guajava* L., *Momordica charantia* L., *Syzygium cumini* (L.) Skeels, *Gymnema sylvestre* R.Br, *Nelumbo nucifera* Gaertn, *Pterocarpus marsupium* Roxb. HPTLC method used and got the HPTLC results.

Key words : HPTLC analysis, Antidiabetic activity, Polyherbal formulation.

Diabetes mellitus is one of the most common metabolic disorders affecting millions of people worldwide. It occurs when the body is unable to properly regulate blood sugar (glucose) levels due to problems with insulin production, insulin function, or both. Insulin is a hormone produced by the pancreas that helps glucose move from the bloodstream into the body's cells, where it is used for energy. When this process is disrupted, glucose accumulates in the blood, leading to a condition known as hyperglycaemia.

Diabetes is generally classified into three main types: Type 1 diabetes, Type 2 diabetes, and gestational diabetes mellitus[11]. Type 1 diabetes develops when the body's immune system mistakenly attacks the insulin-producing cells of the pancreas, resulting in little or no insulin production. Type 2 diabetes, the most common form, occurs when the body becomes resistant to insulin or does not produce enough insulin to maintain normal blood glucose levels. Gestational diabetes develops during pregnancy and may increase the risk of developing Type 2 diabetes later in life.

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The disease has been recognized since ancient times and was often identified by the presence of "sweet urine," a result of excess glucose being excreted from the body through urine. Under normal conditions, insulin helps maintain blood glucose levels within a healthy range. However, in people with diabetes, either insufficient insulin production or the body's inability to respond effectively to insulin causes blood glucose levels to remain elevated.

Over the past few decades, the prevalence of diabetes has increased significantly, making it a major global health concern. It is estimated that around 250 million people worldwide are affected by diabetes, with nearly 50 million cases reported in India alone. The number of people living with diabetes is expected to continue rising, placing a substantial burden on healthcare systems and society.

Although considerable progress has been made in diabetes management, controlling the disease remains a challenge. Modern treatments, including insulin produced through recombinant DNA technology, have improved the quality of life for many patients. However, these treatments may not completely eliminate the complications associated with diabetes. Therefore, researchers continue to explore natural and alternative approaches that may offer safer, more effective, and long-term solutions for the management of both Type 1 and Type 2 diabetes mellitus[1]. For the preparation of the manuscript relevant literature[1-12] has been consulted.

Types of Diabetes :

1. Diabetes insipidus (DI)
2. Diabetes mellitus
3. Gestational

Diabetes mellitus :

Diabetes mellitus refers to a collection of metabolic disorders characterized by elevated blood sugar levels over an extended duration. Symptoms associated with high blood sugar include frequent urination, increased thirst, and heightened hunger. If diabetes mellitus is not treated, it may lead to numerous complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Significant long-term complications involve cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes. The condition arises from either the pancreas failing to produce sufficient insulin or the body's cells not responding effectively to the insulin produced.

Impact of Diabetes :

Over time, diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage result from injury to small blood vessels, known as microvascular disease. Additionally, diabetes plays a significant role in hastening the hardening and narrowing of the arteries (atherosclerosis), which can result in strokes, heart disease, and other forms of large blood vessel disease. This condition is referred to as macrovascular disease. Approximately 30 million individuals in the United States are affected by diabetes, while 85 million have prediabetes (characterized by blood glucose levels that are elevated but not high enough for a diabetes diagnosis, indicating that the body's cells have developed resistance to insulin). It is estimated that around 10 million people in the United States have diabetes

without being aware of it. From an economic standpoint, the total annual cost of diabetes in 2019 was projected to be 300 billion dollars in the United States. This figure includes 220 billion in direct medical expenses (healthcare costs) for individuals with diabetes, along with an additional 165 billion in other costs associated with disability, premature death, or loss of work. Medical costs for individuals with diabetes are more than twice as high as those for individuals without diabetes.

When analysing substances, HPTLC has several benefits over other methods such as HPLC, spectrophotometry, titrimetry, etc. The following are some benefits of HPTLC:

• The choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step.
• The separation process is easy to follow, especially with colored compounds.
• Ability to analyse crude samples containing multi-components.
• Two-dimensional separations are easy to perform. Stability during chromatography should be tested using two-dimensional development.
• Several samples can be separated parallel to each other on the same plate resulting in high output, time-saving, and rapid low-cost analysis.
• Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed.
• HPTLC can combine and consequently be used for different modes of evaluation, allowing the identification of compounds having different light-absorption characteristics or different colors.
• Specific and sensitive color reagents can be used to detect separated spots (Dragendroff reagent).
• HPTLC method may help to minimize the exposure risk of toxic organic effluents and significantly reduces its disposal problems, consequently, reducing the environmental pollution.

Polyherbal formulation (PHF) :

Polyherbal formulation (PHF) is made up of 11 plants namely *Moringa oleifera, Aegle marmelos, Murraya koenigii, Annona reticulata, Piper betle, Psidium guajava, Momordica charantia, Syzygium cumini, Gymnema sylvestre, Nelumbo nucifera, Pterocarpus marsupium.*

One of the most important steps for qualitative and quantitative analysis is method development in thin-layer (planar) chromatography. A thorough literature review is always the first step when developing a new analytical process, providing essential details regarding the physicochemical properties of the sample and its nature (structure, polarity, volatility, stability, and solubility). There are a lot of trial-and-error processes involved.

Selection of the Stationary Phase :

During method development, stationary phase selections should be based on the type of compounds to be separated. HPTLC uses smaller plates (10*10 or 10*20 cm) with significantly decreased development distance (typically 6cm) and analysis time (7–20 min). HPTLC plates provide improved resolution, higher detection sensitivity, and improved in situ quantification and are used for industrial pharmaceutical densitometric quantitative analysis.

Mobile Phase Selection and Optimization :

The selection of the mobile phase is based on the adsorbent material used as the stationary phase and the physical and chemical properties of the analyte.

Sample Preparation and Application :

A good solvent system moves all components of the mixture off the baseline but does not put anything on the solvent front. The peaks of interest should be resolved between Rf 0.15 and 0.85. The elution power of the mobile phase depends on a property called eluent strength which is related to the polarity of the mobile phase components. The more nonpolar the compound, the faster it will elute (the less time it will remain on the stationary phase) and the more polar the compound the slower it will elute (or more time on the stationary phase). The following chart helps predict the order of elution. Pharmaceutical preparation with a sufficiently high concentration of analyte is simply dissolved in a suitable solvent that will completely solubilize the analyte and leave excipients undissolved to yield a test solution that can be directly applied on an HPTLC plate. It is a fact that the application of the sample is the most critical step to obtaining a good resolution for quantification in HPTLC.

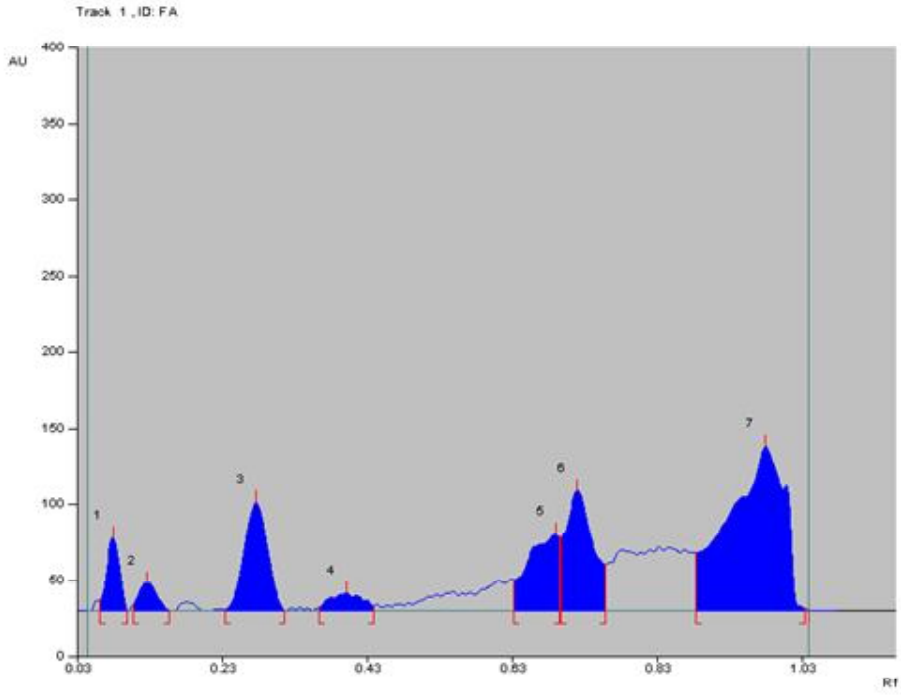

Fig. 1. HPTLC Densitometric 3D Chromatogram Fingerprint profile Recorded at 254 nm for Track 1
(Sample FA)

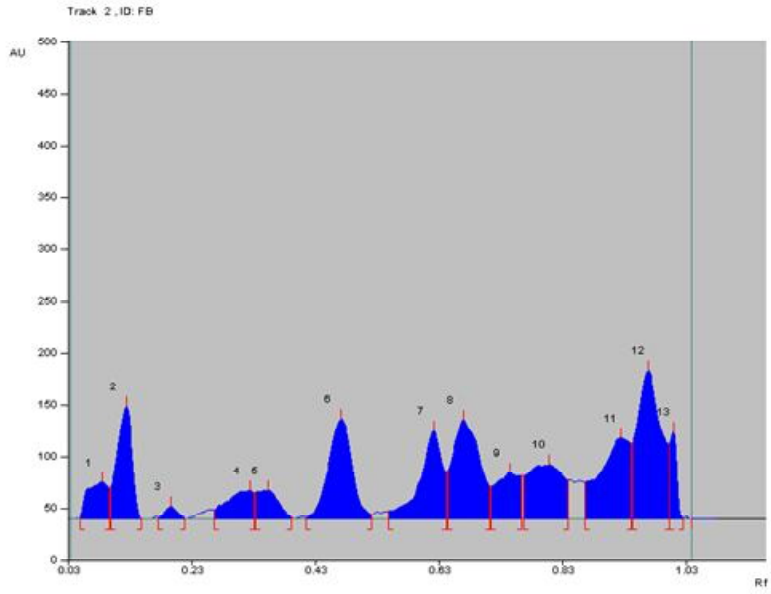

Fig. 2. HPTLC Densitometric 3D Chromatogram Fingerprint profile Recorded at 254 nm for Track 2
(Sample FB)

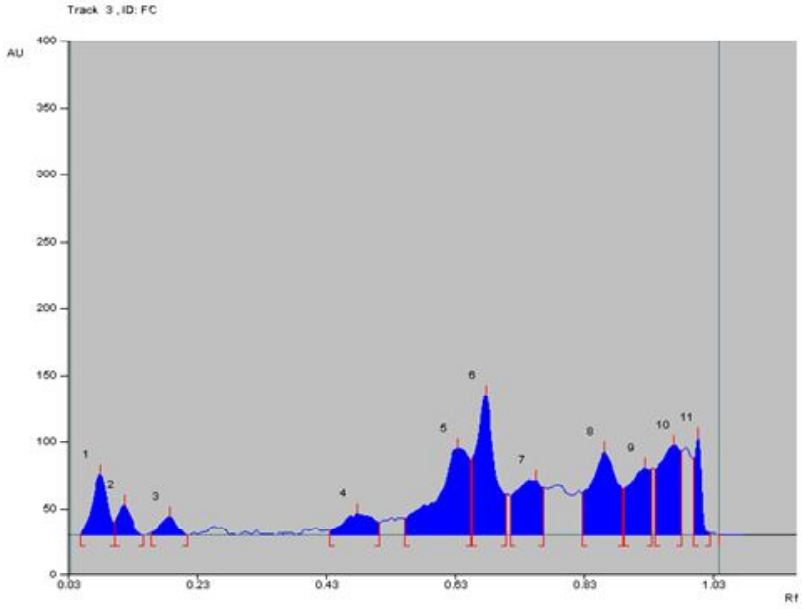

Fig. 3. HPTLC Densitometric 3D Chromatogram Fingerprint Profile Recorded at 254 nm for Track 3
(Sample FC)

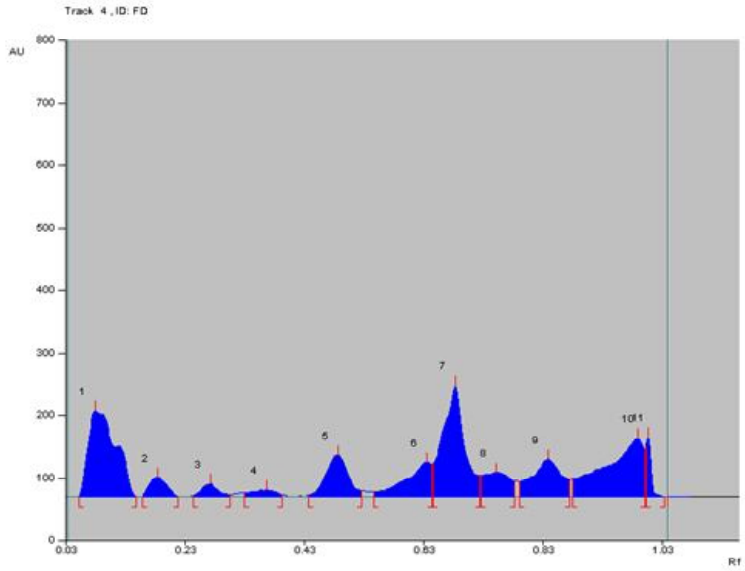

Fig. 4. HPTLC Densitometric 3D Chromatogram Fingerprint Profile Recorded at 254 nm for Track 4
(Sample FD)

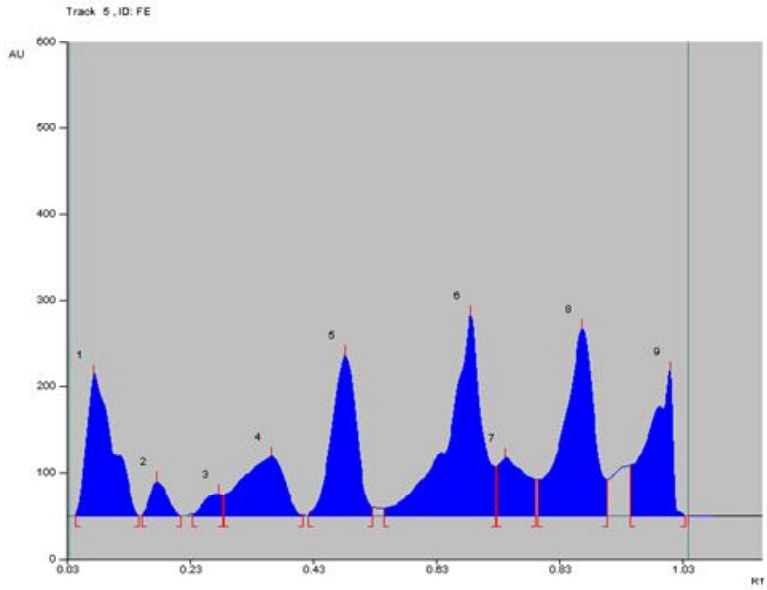

Fig. 5. HPTLC Densitometric 3D Chromatogram Fingerprint Profile Recorded at 254 nm for Track 5
(Sample FE)

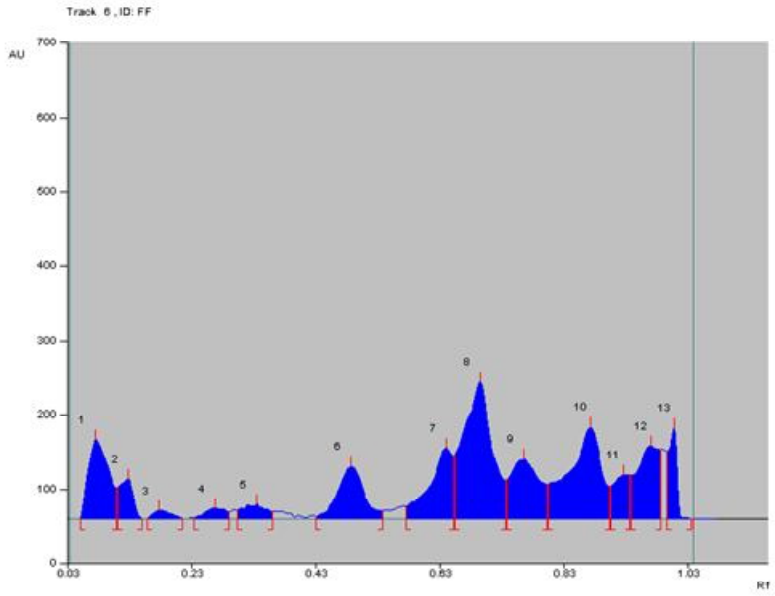

Fig. 6. HPTLC Densitometric 3D Chromatogram Fingerprint Profile Recorded at 254 nm for Track 6
(Sample FF)

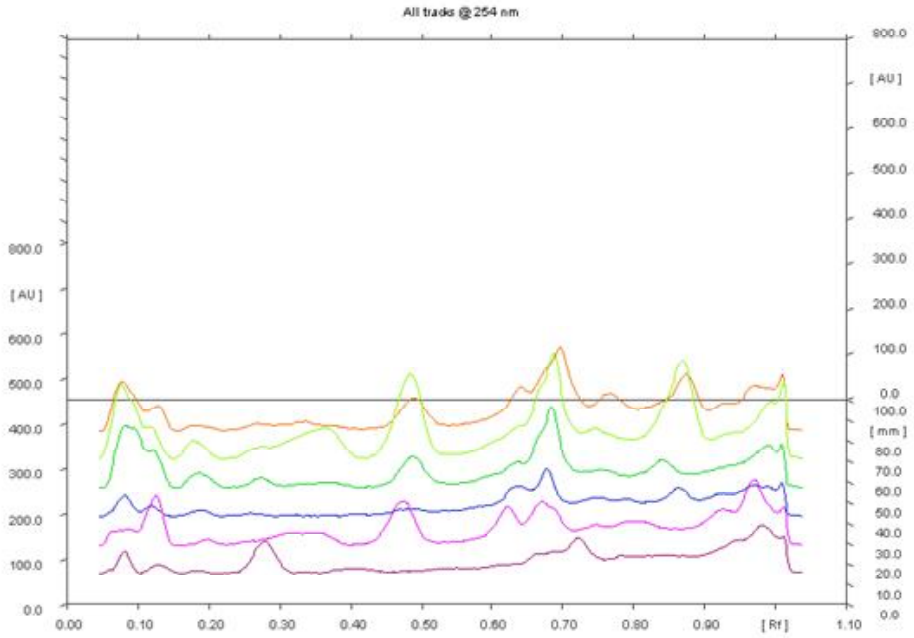

Fig. 7. Comparative HPTLC Chromatographic Fingerprint of Different Track

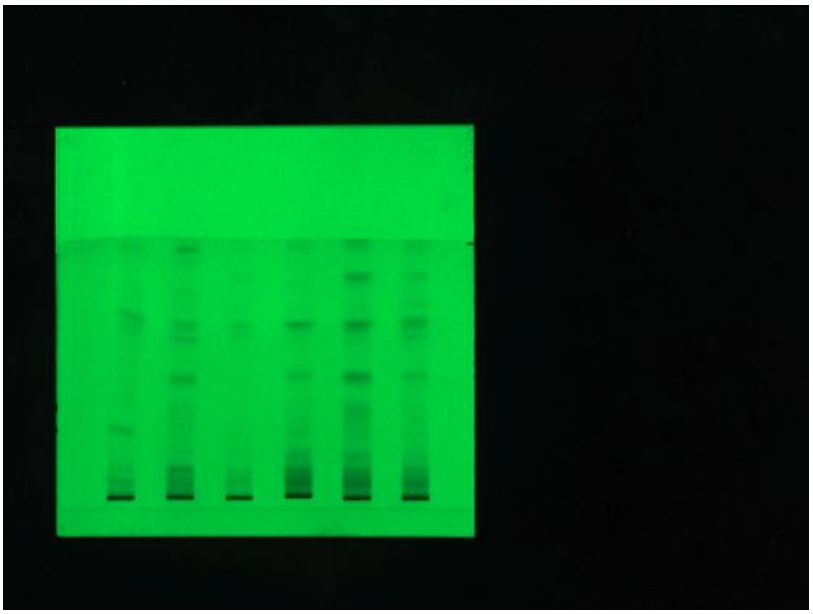

Fig. 8. Chromatographic Fingerprint of Samples by HPTLC under Short-Wave UV (254 nm)

Table-1. Comparative HPTLC Densitometric Data of Tracks 1–6.

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Hei-ght	Area	Area %	Assig-ned subs-tance
1	1	0.06 Rf	6.7 AU	0.08 Rf	48.1 AU	12.67%	0.10 Rf	0.7 AU	606.6 AU	4.99%	1
1	2	0.11 Rf	4.2 AU	0.13 Rf	18.2 AU	4.79%	0.16 Rf	0.2 AU	321.9 AU	2.65%	2
1	3	0.24 Rf	0.7 AU	0.28 Rf	71.3 AU	18.79%	0.32 Rf	0.2 AU	1634.2 AU	13.45%	4
1	4	0.36 Rf	2.2 AU	0.40 Rf	11.0 AU	2.90%	0.44 Rf	3.0 AU	361.4 AU	2.98%	5
1	5	0.63 Rf	18.7 AU	0.69 Rf	48.6 AU	12.81%	0.70 Rf	47.0 AU	1474.7 AU	12.14%	8
1	6	0.70 Rf	47.0 AU	0.72 Rf	77.4 AU	20.39%	0.76 Rf	28.5 AU	2068.9 AU	17.03%	9
1	7	0.88 Rf	36.0 AU	0.98 Rf	105.0 AU	27.66%	1.03 Rf	0.1 AU	5677.9 AU	46.75%	12
2	1	0.05 Rf	0.9 AU	0.09 Rf	35.9 AU	4.05%	0.10 Rf	29.6 AU	843.7 AU	3.71%	1

2	2	0.10 Rf	30.4 AU	0.13 Rf	109.1 AU	12.29%	0.15 Rf	0.1 AU	1877.5 AU	8.25%	2
2	3	0.18 Rf	1.4 AU	0.20 Rf	12.2 AU	1.37%	0.22 Rf	0.8 AU	181.3 AU	0.80%	3
2	4	0.27 Rf	8.7 AU	0.33 Rf	27.3 AU	3.07%	0.33 Rf	25.1 AU	843.6 AU	3.71%	14
2	5	0.33 Rf	25.5 AU	0.35 Rf	27.5 AU	3.09%	0.39 Rf	1.6 AU	717.7 AU	3.15%	5
2	6	0.42 Rf	1.7 AU	0.47 Rf	96.8 AU	10.90%	0.52 Rf	4.3 AU	2639.9 AU	11.60%	6
2	7	0.55 Rf	7.4 AU	0.62 Rf	85.0 AU	9.57%	0.64 Rf	45.2 AU	2191.4 AU	9.62%	7
2	8	0.65 Rf	45.8 AU	0.67 Rf	95.5 AU	10.76%	0.71 Rf	31.0 AU	2948.8 AU	12.95%	8
2	9	0.72 Rf	31.1 AU	0.75 Rf	44.3 AU	4.99%	0.76 Rf	41.1 AU	1206.8 AU	5.30%	9
2	10	0.77 Rf	40.7 AU	0.81 Rf	51.7 AU	5.82%	0.84 Rf	36.6 AU	2135.3 AU	9.38%	10
2	11	0.87 Rf	34.6 AU	0.93 Rf	77.3 AU	8.70%	0.94 Rf	71.7 AU	2656.6 AU	11.67%	11
2	12	0.94 Rf	72.0 AU	0.97 Rf	142.1 AU	16.00%	1.00 Rf	70.4 AU	3905.7 AU	17.15%	12
2	13	1.00 Rf	71.4 AU	1.01 Rf	83.3 AU	9.39%	1.02 Rf	0.5 AU	619.5 AU	2.72%	13
3	1	0.05 Rf	0.4 AU	0.08 Rf	45.5 AU	8.20%	0.10 Rf	9.9 AU	743.2 AU	5.90%	1
3	2	0.10 Rf	10.2 AU	0.12 Rf	22.7 AU	4.10%	0.15 Rf	0.0 AU	325.0 AU	2.58%	2
3	3	0.16 Rf	1.9 AU	0.19 Rf	13.8 AU	2.48%	0.22 Rf	0.1 AU	254.5 AU	2.02%	3
3	4	0.44 Rf	3.1 AU	0.48 Rf	16.0 AU	2.88%	0.51 Rf	9.3 AU	539.4 AU	4.28%	6
3	5	0.55 Rf	12.3 AU	0.64 Rf	64.7 AU	11.67%	0.66 Rf	55.6 AU	2338.0 AU	18.57%	7
3	6	0.66 Rf	56.2 AU	0.68 Rf	104.2 AU	18.81%	0.71 Rf	29.7 AU	2112.7 AU	16.78%	8
3	7	0.72 Rf	28.5 AU	0.76 Rf	40.3 AU	7.26%	0.77 Rf	34.8 AU	1153.6 AU	9.16%	9
3	8	0.83 Rf	30.3 AU	0.86 Rf	61.0 AU	11.00%	0.89 Rf	33.6 AU	1801.1 AU	14.30%	10
3	9	0.89 Rf	33.7 AU	0.93 Rf	48.8 AU	8.80%	0.94 Rf	48.5 AU	1162.9 AU	9.24%	11

3	10	0.94 Rf	48.2 AU	0.97 Rf	66.2 AU	11.94%	0.98 Rf	62.0 AU	1588.7 AU	12.62%	12
3	11	1.00 Rf	55.8 AU	1.01 Rf	71.2 AU	12.85%	1.02 Rf	0.2 AU	573.0 AU	4.55%	13
4	1	0.05 Rf	0.7 AU	0.08 Rf	137.6 AU	17.57%	0.15 Rf	0.3 AU	4636.3 AU	21.12%	1
4	2	0.16 Rf	0.5 AU	0.19 Rf	31.6 AU	4.03%	0.22 Rf	0.1 AU	654.1 AU	2.98%	3
4	3	0.25 Rf	2.2 AU	0.27 Rf	21.0 AU	2.68%	0.31 Rf	3.0 AU	416.5 AU	1.90%	4
4	4	0.33 Rf	6.2 AU	0.37 Rf	11.0 AU	1.41%	0.39 Rf	3.5 AU	333.8 AU	1.52%	5
4	5	0.44 Rf	3.3 AU	0.49 Rf	67.3 AU	8.59%	0.53 Rf	8.9 AU	1778.2 AU	8.10%	6
4	6	0.55 Rf	8.0 AU	0.64 Rf	55.3 AU	7.06%	0.65 Rf	51.6 AU	1680.2 AU	7.65%	7
4	7	0.65 Rf	52.6 AU	0.68 Rf	177.3 AU	22.64%	0.73 Rf	32.5 AU	4579.0 AU	20.86%	8
4	8	0.73 Rf	32.6 AU	0.75 Rf	38.1 AU	4.87%	0.78 Rf	24.6 AU	1202.4 AU	5.48%	9
4	9	0.79 Rf	23.6 AU	0.84 Rf	58.7 AU	7.50%	0.88 Rf	26.7 AU	2030.3 AU	9.25%	10
4	10	0.88 Rf	27.1 AU	0.99 Rf	91.9 AU	11.73%	1.00 Rf	74.9 AU	4052.3 AU	18.46%	12
4	11	1.00 Rf AU	76.0	1.01 Rf AU	93.3	11.91%	1.02 Rf AU	2.2 AU	587.9	2.68%	unkn-own *
5	1	0.05 Rf	1.5 AU	0.08 Rf	164.3 AU	14.14%	0.15 Rf	0.3 AU	4912.7 AU	13.09%	1
5	2	0.15 Rf	0.7 AU	0.18 Rf	39.7 AU	3.42%	0.22 Rf	0.0 AU	785.2 AU	2.09%	3
5	3	0.24 Rf	1.9 AU	0.28 Rf	25.8 AU	2.22%	0.29 Rf	24.4 AU	552.4 AU	1.47%	4
5	4	0.29 Rf	24.8 AU	0.36 Rf	69.1 AU	5.95%	0.41 Rf	1.1 AU	3359.4 AU	8.95%	5
5	5	0.42 Rf	0.3 AU	0.48 Rf	186.5 AU	16.05%	0.53 Rf	8.8 AU	5221.4 AU	13.91%	6
5	6	0.55 Rf	7.8 AU	0.69 Rf	231.7 AU	19.95%	0.73 Rf	55.3 AU	8722.2 AU	23.24%	8
5	7	0.73 Rf	55.6 AU	0.74 Rf	65.8 AU	5.66%	0.80 Rf	39.6 AU	2262.5 AU	6.03%	9

5	8	0.80 Rf	39.8 AU	0.87 Rf	214.1 AU	18.43%	0.91 Rf	39.3 AU	7290.9 AU	19.43%	10
5	9	0.95 Rf	55.1 AU	1.01 Rf	164.8 AU	14.18%	1.03 Rf	0.2 AU	4417.9 AU	11.77%	13
6	1	0.05 Rf	1.8 AU	0.08 Rf	107.4 AU	10.36%	0.11 Rf	42.6 AU	2529.2 AU	9.31%	1
6	2	0.11 Rf	42.8 AU	0.13 Rf	54.1 AU	5.22%	0.15 Rf	0.2 AU	866.1 AU	3.19%	2
6	3	0.16 Rf	0.2 AU	0.18 Rf	10.8 AU	1.05%	0.22 Rf	0.3 AU	229.8 AU	0.85%	3
6	4	0.24 Rf	1.5 AU	0.27 Rf	14.6 AU	1.41%	0.29 Rf	8.8 AU	346.9 AU	1.28%	4
6	5	0.30 Rf	11.6 AU	0.34 Rf	20.2 AU	1.95%	0.36 Rf	9.3 AU	532.8 AU	1.96%	5
6	6	0.43 Rf	3.2 AU	0.49 Rf	70.4 AU	6.79%	0.54 Rf	9.9 AU	2129.7 AU	7.84%	6
6	7	0.58 Rf	17.0 AU	0.64 Rf	95.3 AU	9.19%	0.65 Rf	83.9 AU	2506.1 AU	9.22%	7
6	8	0.65 Rf	84.4 AU	0.70 Rf	183.5 AU	17.71%	0.74 Rf	52.4 AU	6017.0 AU	22.14%	8
6	9	0.74 Rf	52.8 AU	0.77 Rf	79.7 AU	7.69%	0.80 Rf	46.1 AU	2676.4 AU	9.85%	9
6	10	0.81 Rf	46.3 AU	0.87 Rf	122.3 AU	11.80%	0.90 Rf	43.5 AU	4538.9 AU	16.70%	10
6	11	0.91 Rf	44.0 AU	0.93 Rf	59.1 AU	5.70%	0.94 Rf	58.1 AU	1076.1 AU	3.96%	11
6	12	0.94 Rf	58.1 AU	0.97 Rf	97.7 AU	9.43%	0.99 Rf	91.9 AU	2522.4 AU	9.28%	12
6	13	1.00 Rf	89.7 AU	1.01 Rf	121.4 AU	11.71%	1.02 Rf	0.6 AU	1200.4 AU	4.42%	13

The chromatograms showed prominent peaks in the Rf regions of **0.64–0.70**, **0.86–0.87**, and **0.97–1.01**, which were consistently detected across most tracks. These peaks exhibited com-paratively higher area percentages and can therefore be considered as characteristic constituents of the samples. Among all tracks, **Track 1 displayed the highest individual peak area (46.75% at Rf 0.98)**, indicating the predominance of a major constituent.

A comparison of the six tracks revealed that the peaks occurring at approximately **Rf 0.68–0.70** and **Rf 0.97–1.01** were common to most samples, indicating the presence of chemically related compounds or marker constituents. The recurrence of these peaks suggests a degree of chemical similarity among the analysed samples and may serve as characteristic markers for identification and quality assessment.

Track 1 was distinguished by a highly intense peak at **Rf 0.98**, accounting for nearly half of the total chromatographic area, which indicates the dominance of a single constituent. In contrast, Tracks 2–6 exhibited a more balanced distribution of peak areas, suggesting the presence of multiple compounds in appreciable quantities.

The HPTLC fingerprint analysis of Tracks 1–6 revealed a reproducible chromatographic pattern with several common peaks, particularly in the Rf regions of **0.64–0.70** and **0.97–1.01**. The dominance of specific peaks and the presence of shared chromatographic markers indicate a characteristic phytochemical composition, supporting the use of HPTLC for reliable identification and quality evaluation of the samples.

References :

1. Bhutkar, M.A. and S.B. Bhise, (2010). *International Journal of Therapeutics*, *2*(1): 17–22.
2. Dey, L., S.A. Anoja, and C.S. Yuan,(2002). *Alternative Medicine Review*, *7:* 45–58.
3. Jinu John, A. Raghuvanshi, U.K. Aravind, and C.T. Aravind, (2015). *Journal of Food and Drug Analysis*, *23:* 219–224.
4. Kirbis, A., J. Marinsek, and V.C. Flajs, (2005). *Biomedical Chromatography*, *19*(4): 239–265.
5. Kotake, C.K., Purohit and S.P. Gokhale, (2000). *Pharmacognosy*. Pune: Nirali Prakashan; pp. 145–190.
6. Mukherjee, P.K. (2008). *Quality Control of Herbal Drugs*. New Delhi: Business Horizons Pharmaceutical Publishers; p. 186.
7. Nyiredy, S. (2002). *Journal of Chromato graphic Science*, *40*: 553–563.
8. Puskuri, D., M. Sathishkumar, andN. Jyothi, (2021). *EPRA International Journal of Research and Development (IJRD)*, *6*(10).
9. Rajani, M. and N.S. Kanaki, (2008). Phytochemical Standardization of Herbal Drugs and Polyherbal Formulations. In: *Bioactive Molecules and Medicinal Plants*. Berlin, Heidelberg: Springer; pp. 349–369.
10. Tripathi, R. (2000). *The Ayurvedic Pharmacopoeia of India*. Part II, Vol. 1, 2nd ed. Government of India, Department of Indian Systems of Medicine and Homeopathy; p. 191.
11. Velho, G. and P. Froguel,(1997). *Diabetes & Metabolism*, **23**(1), 3–14.
12. Verma, S. (2002). *Indian Herbal Pharmacopoeia*. Revised ed. Indian Drug Manufacturers' Association; pp. 493–494.