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HPTLC Fingerprinting Analysis of Herbal Formulation *Actp* .

S.Selvakumar* and R. Valliammai

Department of Industrial Bio Technology, Bharath University, Chennai-6000073, India.

Abstract: It is well documented that most natural products are enriched with bioactive components that have protective action. There is currently a growing body of evidence that that supplementing the human diet with natural products is of major benefit for human health and well being. Nowadays, the use of complementary and or alternative medicine, functional food and especially the consumption of natural products have been increasing rapidly worldwide, mostly because of the supposedly less frequent side effects. Both in conventional and traditional medicines, natural products continue to provide valuable therapeutic agents. The issues regarding the efficacy and safety of currently available modern medicine agents have prompted the search for safer and more effective alternatives. Therefore, it is of interest to investigate the HPTLC analysis of a herbal formulation ACTP. Our results clearly indicate that the presence of seven various polyvalent phyto constituents which may be therapeutically active.
Keywords : HPTLC, ACTP, Side effects, Natural Products, Traditional medicine**.**

Introduction

Medicinal plants have a long history of use in therapy throughout the world and still make an important part of traditional medicine. Importantly, medicinal plants and herbal products must be safe for the patient. Natural products had served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. Interest in natural products research is attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents, and advances in solving the demand for supply of complex natural products. Opportunities for multidisciplinary research that joins the forces of natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry, and pharmacology to exploit the vast diversity of chemical structures and biological activities of natural products is of tremendous importance [1] .Traditional medicine is an important part of human health care in many developing countries and also in developed countries, increasing their commercial value and the demand for herbal medicines has grown dramatically in recent years with the world market for such medicines has reached US $60 billion with annual growth rates of between 5 and 15%. Thus, the patent applications in the field of natural products, traditional herbal medicine and herbal medicinal products and related plant products are important targets of patent claims. Advances in molecular biology have led to discovery of potential cancer targets and a rich pipeline of anti cancer drugs [2] . A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy [3].

2. Materials and Methods

2.1. Plant Materials

The herbal formulation *ACTP* was prepared in the department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Bharath University, Chennai, India. The equal volume of shade dried leaves of *Acalypha indica, Couropita guinensis, Tritax brogumbenz and Plectra amdoilicus* were taken in to marter and pistle,The plant material was coarsely powdered, then filtered by muslin cloth and the filtrate was used for further extraction.

2.2. Preparation of Plant extract

500 g of the herbal powder *ACTP* were charged in an aspiration bottle and allowed to soak in chloroform for 96 hrs at room temperature. The extract was filtered and concentrated on a water bath. The inorganic material was precipitated and filtered off. The filtrate were again concentrated in a China dish and dried in vacuum. The yield of the extract were weighed and will be stored in refrigerator for further use.

2.3. Chemicals and Reagents

All chemicals were used for this project were purchased from M/s. Sigma Chemicals, USA.

2.4. HPTLC Profile (High Performance Thin Layer Chromatography) analysis of *ACTP*.

HPTLC studies were carried out following the methods such as,

2.4.1. Sample Preparation

Ethyl acetate extract obtained were evaporated under reduced pressure using rotovac evaporator. Each extract residue was re-dissolved in 1ml of chromatographic grade chloroform, ethyl acetate, which was used for sample application on pre-coated silica gel 60F254 aluminium sheets.

2.4.2. Developing Solvent System

A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent n Hexane: chloroform (3.5:1.5).

2.4.3. Sample Application

Application of bands of each extract was carried out (4mm in length and 1ul in concentration for leaf) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.4.4. Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with solvent n-Hexane: ethyl acetate (3.5:1.5) for 15 minutes.

2.4.5. Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light (Figure 1). The chromatograms were scanned by densitometer at 420 nm after spraying with anisaldehydesulphuric acid. The Rf values and finger print data were recorded by WIN CATS software [4] .

3. Results and Discussion

HPTLC Fingerprinting of Herbal Formulation ACTP

Chromatographic condition

Sample	: ACTP
Sample prepared in	: Methanol
Stationary phase	: Silica gel GF_{254}
Mobile phase	: n – hexane: ethyl acetate: formic acid: acetic acid (6:4:0.25:0.25)
Scanning wavelength	: 254 nm
Applied volume	: Track 1 – 7 (2µl – 14µl)
Development mode	: Ascending mode

Photodocument

254nm **366nm**

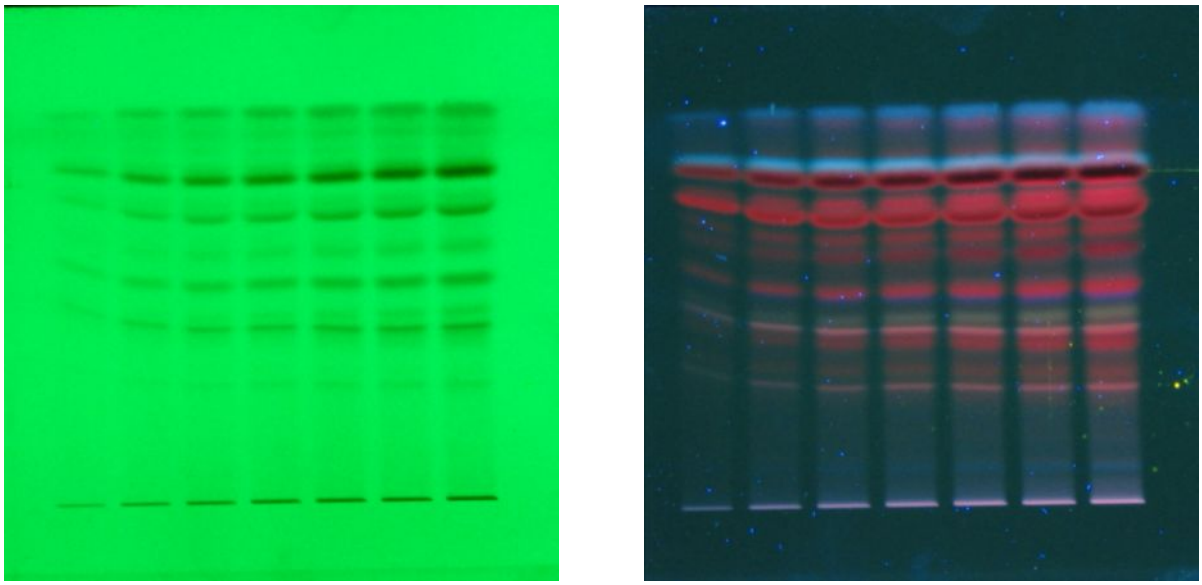
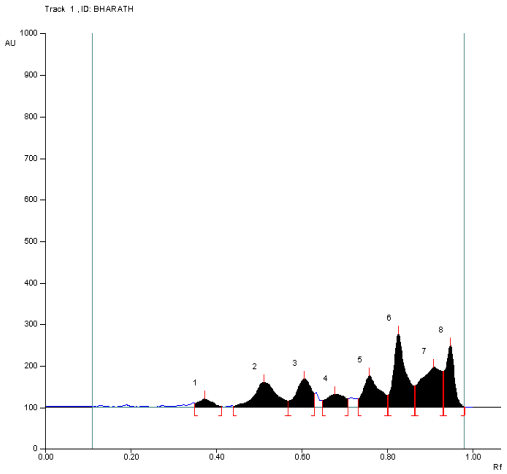
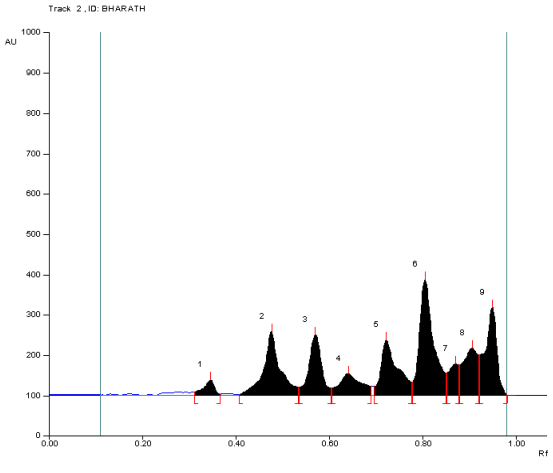


Figure :1 shows that the photo documentation of HPTLC finger print of the herbal formulation ACTP.

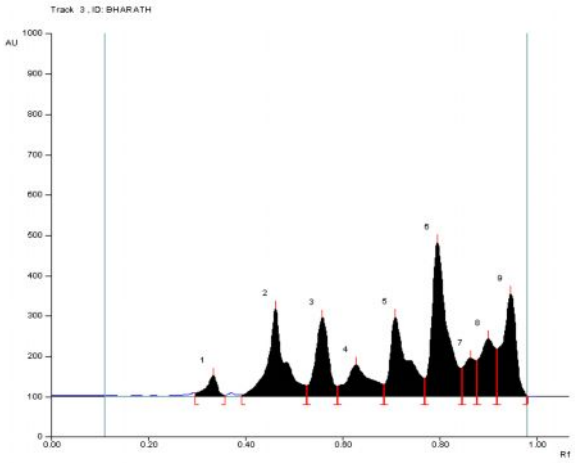
Peak Area

Track 1 – 2µl **Track 2 – 4µl**

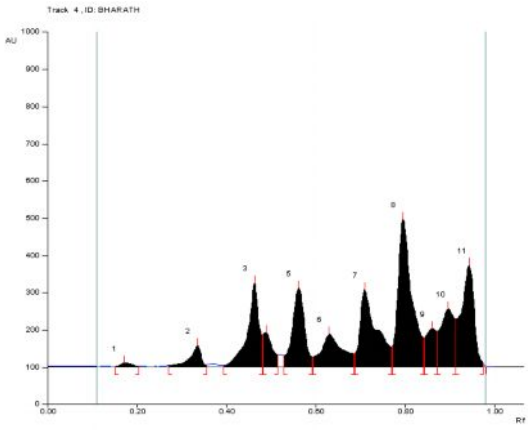




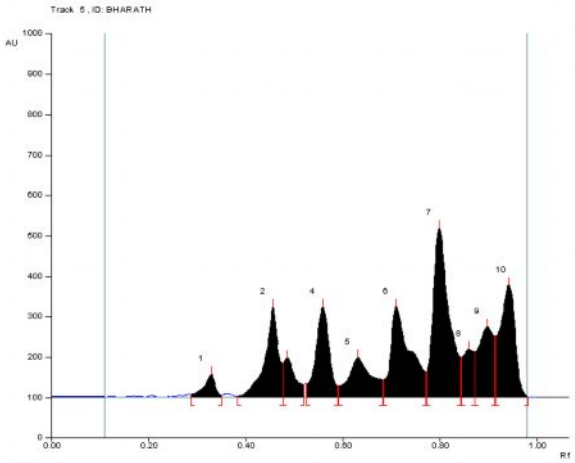
Track 3 – 6µl



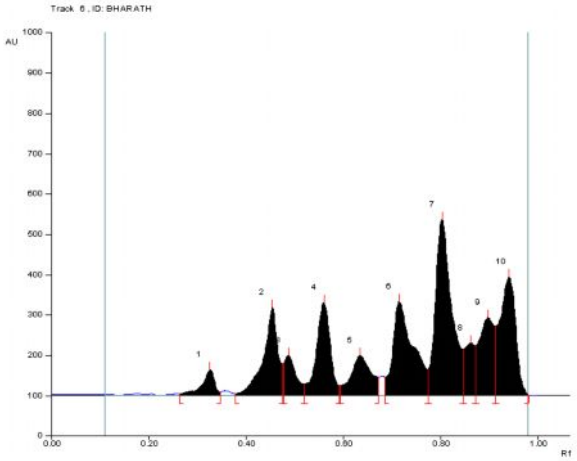
Track 4 – 8µl



Track 5 – 10µl



Track 6 – 12µl



Track 7 – 14µl

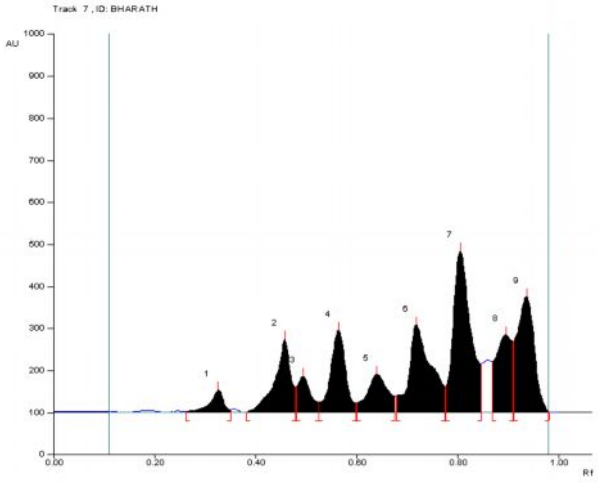


Figure 2- 8: shows that the HPTLC analysis of ACTP.

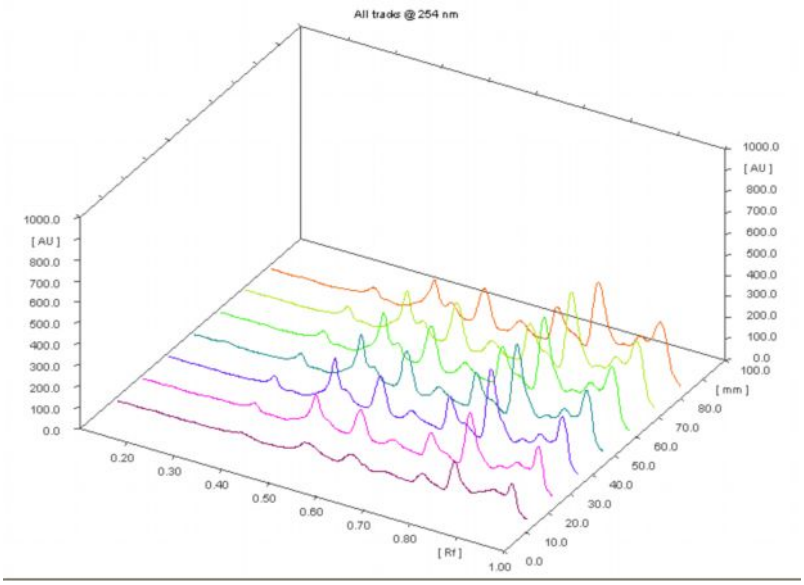


Figure 9: shows that the 3D Display of the Fingerprint of ACTP.

Table : 1 shows that the Peak Table of ACTP .

Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	1	0.35	9.1	0.37	20.2	3	0.41	1.4	549.7	2.78	unknown *
1	2	0.44	2.4	0.51	60	8.89	0.57	15.1	2708.3	13.71	unknown *
1	3	0.57	15.1	0.61	67.9	10.06	0.63	32.8	2026.2	10.26	unknown *
1	4	0.65	16.8	0.68	31	4.59	0.71	19.7	1157.9	5.86	unknown *
1	5	0.73	20.5	0.76	75.3	11.16	0.8	28.3	2445.5	12.38	unknown *
1	6	0.8	28.6	0.83	175.7	26.02	0.86	50.9	4320.1	21.87	unknown *
1	7	0.86	51.1	0.91	96.3	14.26	0.93	86	3942.5	19.96	unknown *
1	8	0.93	86	0.95	148.6	22.02	0.98	0.4	2601.9	13.17	unknown *
2	1	0.31	8.3	0.35	38.3	3.09	0.37	3	793.1	2.44	unknown *
2	2	0.41	2.1	0.48	158	12.76	0.53	20.2	4861.7	14.98	unknown *
2	3	0.53	20.3	0.57	149.9	12.11	0.6	18.2	3525.3	10.86	unknown *
2	4	0.61	18.2	0.64	54.1	4.37	0.69	23.2	2171.3	6.69	unknown *
2	5	0.7	22.6	0.72	137.6	11.11	0.78	33	4253.7	13.1	unknown *
2	6	0.78	33.6	0.8	287	23.19	0.85	54.9	7516.6	23.15	unknown *
2	7	0.85	55	0.87	77.6	6.27	0.88	76.3	1463.1	4.51	unknown *
2	8	0.88	76.3	0.91	116.8	9.43	0.92	100.2	3175	9.78	unknown *
2	9	0.92	100.5	0.95	218.5	17.65	0.98	1.9	4705.1	14.49	unknown *
3	1	0.3	8.3	0.33	52.4	3.24	0.36	2.3	1001.5	2.32	unknown *
3	2	0.39	3.2	0.46	218.3	13.52	0.53	27.1	6492.6	15.02	unknown *
3	3	0.53	27.4	0.56	195.9	12.13	0.59	25	4507.5	10.43	unknown *
3	4	0.59	25.2	0.63	77.6	4.81	0.68	29.6	3311	7.66	unknown *
3	5	0.69	30	0.71	196.7	12.18	0.77	44.2	6044.2	13.99	unknown *
3	6	0.77	44.6	0.79	381.3	23.61	0.84	68.9	10127.1	23.43	unknown *
3	7	0.85	69.6	0.86	95.2	5.9	0.87	88.4	1998	4.62	unknown *
3	8	0.88	88.7	0.9	143.3	8.87	0.92	117.9	3738.1	8.65	unknown *
3	9	0.92	118	0.95	254.1	15.74	0.98	4.5	5994	13.87	unknown *
4	1	0.15	0.9	0.17	10.3	0.56	0.2	1.4	233.2	0.5	unknown *
4	2	0.27	3	0.33	57.6	3.17	0.35	5	1252	2.66	unknown *
4	3	0.39	3.1	0.46	226.3	12.43	0.48	85.5	5019.8	10.67	unknown *
4	4	0.48	86	0.49	92.7	5.09	0.51	32.4	1677.5	3.56	unknown *
4	5	0.53	30	0.56	212.3	11.66	0.59	26.4	4962.6	10.55	unknown *
4	6	0.59	26.5	0.63	87.6	4.81	0.69	35.6	3622.9	7.7	unknown *

4	7	0.69	36	0.71	206.5	11.34	0.77	53	6730.6	14.3	unknown *
4	8	0.77	54.2	0.79	395.9	21.74	0.84	78.4	10620	22.57	unknown *
4	9	0.84	78.9	0.86	103	5.66	0.87	96.3	2082.5	4.43	unknown *
4	10	0.87	96.7	0.9	155.8	8.56	0.91	128.9	4051.2	8.61	unknown *
4	11	0.91	128.9	0.94	272.6	14.97	0.97	9	6804.9	14.46	unknown *
5	1	0.29	6.9	0.33	56.5	2.95	0.35	4.9	1169.6	2.23	unknown *
5	2	0.38	2.7	0.46	222.8	11.64	0.48	84.1	5326.7	10.17	unknown *
5	3	0.48	85.1	0.49	97.5	5.09	0.52	31.5	2018.5	3.85	unknown *
5	4	0.52	31.8	0.56	223.5	11.68	0.59	28.6	5376.7	10.26	unknown *
5	5	0.59	28.6	0.63	98	5.12	0.68	44.1	4021.8	7.68	unknown *
5	6	0.68	44	0.71	224.8	11.75	0.77	63.9	7963.9	15.2	unknown *
5	7	0.77	64.6	0.8	419.6	21.92	0.84	99.4	11963.5	22.83	unknown *
5	8	0.84	99.4	0.86	118.1	6.17	0.87	112.3	2340.4	4.47	unknown *
5	9	0.87	112.5	0.9	175.3	9.16	0.91	151.4	4795	9.15	unknown *
5	10	0.91	151.7	0.94	277.8	14.52	0.98	0	7419.8	14.16	unknown *
6	1	0.26	3.9	0.33	63.5	3.19	0.35	8.6	1456	2.63	unknown *
6	2	0.38	4.2	0.45	218.1	10.94	0.47	79	5638.4	10.19	unknown *
6	3	0.48	79.5	0.49	98.9	4.96	0.52	28.5	2127.4	3.84	unknown *
6	4	0.52	28.6	0.56	229.8	11.53	0.59	24	5709.6	10.32	unknown *
6	5	0.59	24	0.63	98.9	4.96	0.67	45.4	3577.3	6.46	unknown *
6	6	0.69	45.4	0.71	232.2	11.65	0.77	63.6	8450.4	15.27	unknown *
6	7	0.77	64.4	0.8	436	21.88	0.85	113.9	12655.5	22.87	unknown *
6	8	0.85	114.4	0.86	129.2	6.48	0.87	123.6	2351.5	4.25	unknown *
6	9	0.87	124.2	0.9	192.7	9.67	0.91	171.8	5153.8	9.31	unknown *
6	10	0.91	172.2	0.94	293.4	14.72	0.98	2	8219	14.85	unknown *
7	1	0.26	1.9	0.33	53	3.21	0.35	6.2	1242.6	2.56	unknown *
7	2	0.38	0.4	0.46	173.5	10.52	0.48	60.4	4668.2	9.61	unknown *
7	3	0.48	61	0.49	85.7	5.2	0.52	24.6	1914.5	3.94	unknown *
7	4	0.53	24.6	0.56	195.3	11.84	0.6	22.1	4990.8	10.28	unknown *
7	5	0.6	22.5	0.64	90.3	5.48	0.67	39.5	3186.7	6.56	unknown *
7	6	0.68	40	0.72	208	12.61	0.77	61.2	7894.1	16.25	unknown *
7	7	0.78	61.5	0.8	383	23.23	0.85	115.3	11490.8	23.66	unknown *
7	8	0.87	120.7	0.89	184.7	11.2	0.91	168.6	5137.4	10.58	unknown *
7	9	0.91	169.4	0.94	275.4	16.7	0.98	1.5	8039.5	16.55	unknown *

The HPTLC analysis was performed using a HPTLC-CAMAG ,Automatic TLC sampler 4 and scanner-3was used for the chromatographic seperations. The injection volume was 2, 4, 8, 12 and 16 µl, and UV detection was effected at 298 nm. In HPTLC Techniques, the result from the ACTP was determined by using solvent system n-Hexane: chloroform :Formic acid :Acetic acid (60:40:2.5.0:2.5) as mobile phase[5.] The results from HPTLC finger print scanned at wavelength 420 nm for chloroformic extract of ACTP. There are 7 polyvalent phyto constituents ascending order of R_f values start from to in which highest concentration of the phytoconstituents was found to be and its corresponding Rf value was found to be respectively and was recorded in table .The corresponding HPTLC is presenting in fig.. HPTLC is a sophisticated and automated form of TLC. HPTLC is the fastest of all chromatographic methods. HPTLC precoated, silica gel G 60 F_{254} (Merck, Germany) plates were used for the application of sample. A small quantity of extracts was dissolved in respective solvents. Solvent system optimized for TLC study was chosen for HPTLC study. The details of HPTLC were as follows:-

Plate : Aluminium plate precoated with silica gel $GF_{254.}$
Thickness : 250µm
Plate size : 5 × 10 cm
Sample application: 10 µl
Solvent system: n-Hexane : Ethyl acetate :Formic acid : Acetic acid (60:40:2.5.0:2.5)
Detection : U.V. (Visible light, 298 nm)
Instrument : CAMAG TLC Scanner 3 and automatic sampler 4.

The importance of natural products produced by living organisms from the nature for health and disease treatment has been immense throughout the human evolution. Several natural products derived from plants for thousands of years have been traditionally used to treat various types of human illnesses including general injuries, wound healing, and pain. Natural products have been shown to possess significant pharmacological activities that regulate various vital cell signaling pathways that cause mitogenic, cytotoxic, and genotoxic reactions leading to various disease pathologies. Modern synthetic and combinatorial chemistry associated with the new technological tools such as genomics, proteomics, and metabolomics paved wider use of natural products. Nowadays, most of the natural products are processed and developed as potential pharmacological agents with effective antioxidative, antimitotic, anti-infective, anti-inflammatory, antiangiogenic, and anticarcinogenic properties. In fact, some natural products have been employed as lead compounds to obtain highly biologically relevant semisynthetic pharmacological derivatives with increased efficiency and efficacy for the therapeutic use. The prosperity of knowledge we are assembling from the past several decades will significantly inspire us to develop these natural products as novel potential drugs for future therapeutic strategies.

The results are given in Figure, the HPTLC analysis of ACTP shows that the separation of components present in the chloroformic extract of the herbal formulation ACTP. The method may be applied to identify the plant materials of the herbal formulation ACTP from other species. HPTLC fingerprint enables a particular plant or plant materials to be identified and distinguished from closely related species. There are seven polyvalent phytoconstituents and corresponding ascending order of Rf values start from 1.4 to 171.8 in which highest concentration of the phytoconstituents was found to be 3% and its corresponding Rf value was found to be 1.4. The Rf values for chlorofomic extract were given in Table: 1 respectively. The chromatogram of chloroformic extract of ACTP were given in Fig 1-9 and Secondary metabolites are present and they are responsible for therapeutic effects. HPTLC analysis as recommended, in this study provides a chromatographic fingerprint of phytochemicals and is suitable for confirming the identity and purity of medicinal plant raw materials. HPTLC pre-coated plates with the mobile phase n– hexane: chloroform :Formic acid :Acetic acid developed chromatograms which showed distinct phytochemical variations in chloroformic extract of *ACTP* [6] .

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