iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 **Issue:** I **Month of publication:** January 2018

DOI: http://doi.org/10.22214/ijraset.2018.1111

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor :6.887
Volume 6 Issue I, January 2018- Available at www.ijraset.com

Phytochemical Fingerprinting through Computerized HPTLC System for Quality Control of Herbal Drugs

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Abstract: High performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, etc. enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and bio-molecules. Development of chemical fingerprints using HPTLC is an effective tool for linking the chemical constituent's profile of the plant with botanical identity as well as qualitative and quantitative estimation of chemical and bio-chemical markers. It has the potential to determine authenticity and reliability of chemical constituent of herbal drugs and formulations.
Key words: HPTLC, Herbal Drugs, Phytochemical, Quality control.

I. INTRODUCTION

For thousands of years mankind is using plants as bio-resource to alleviate or cure illness. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contains many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, roots etc. The beneficial medical effects of plant materials typically results from the combination of these bioactive components, which are used for the preparation of herbal drugs. Herbal medicines have a long therapeutic history and are still serving many of the health needs of a large population of the world. But the quality control and quality assurance still remains a challenge because of the high variability of chemical compounds involved. Herbal drugs, singularly and in combinations, contains a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs [1]. In the recent year advancement in of chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines [2]. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and its products and therefore be used for authentication and identification of herbal plant [3,4].Chromatographic fingerprinting is a rational option to meet the need for more effective and powerful quality assessment to traditional medicine. The optimized chromatographic fingerprinting is not only an alternative analytical tool for authentication, but also an approach to express the various pattern of chemical ingredients distribution in the herbal drugs [5,6,7].The objective of this study was to standardize HPTLC fingerprint profile of Barleriaprionitis Linn.(Family- Acanthaceae), a well-known medicinal plant in ayurvadic system of medicine having various pharmacological properties [8,9].The chromate graphic chemical profile will provided adequate information and parameters for comprehensive identification, qualitative and quantitative assessment and comparison of major active constituent in the samples studied. This can be used as a reference for the identification and quality control of the drug and ensure therapeutic efficacy.

II. HPTLC SYSTEM

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is a stationary phase over which the other mobile phase moves in a specific direction. Thin-layer chromatography (TLC) is an important planar chromatographic technique in which the stationary phase is spread on a flat, planar surface[10,11]. High performance thin-layer chromatography (HPTLC) is the most advanced form of TLC. It promotes for higher separation efficiencies, shorter analysis time, lower amounts of mobile phase, and efficient data acquisition and processing. HPTLC is an entire concept that

includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis. Sophisticated instruments, controlled by an integrated software platform ensure to the highest possible degree of the usefulness, reliability and reproducibility of generated data. HPTLC is therefore the term for a method that meets all quality requirements of today's analytical labs even a fully regulated environment[12].

A. Advantages of the modern HPTLC technique

HPTLC system combined with automated sample application and densitometric scanning is sensitive and completely reliable, suitable for use in qualitative and quantitative analysis. HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. To fully take advantage of this unique feature inherent to HPTLC, reproducible results and images must be ensured. Special advantages of HPTLC include high sample throughput and low cost per analysis; multiple samples and standards can be separated simultaneously, and sample preparation requirements are often minimal because the stationary phase is disposable. Other advantages include static, off-line detection of zones using a great variety of complementary post-chromatographic universal and selective detection methods that are often applied sequentially, and storage of the separation, containing all sample components, on the layer for identification and quantification at a later time by in situ or elution methods [4,12].

III. METHODOLOGY

A. Apparatus and Reagents

Soxhlet apparatus, water bath, TLC $60F_{254}$ plates (Merck), Camag HPTLC system (Muttenz, Switzerland) equipped with a sample applicator Linomat V, twin trough chamber, Camag TLC visualiser, HPTLC Scanner 4, win CATs software (version-1.4.6), Hamilton (Reno, Nevada, USA) Syringe (100µL) and Camag TLC plate heater III were the basic apparatus and instruments used for the study. Analytical grade reagents and solvents were obtained from E. Merck.

B. Sample Collection And Preparation

Whole plant samples of Barleriaprionitis Linn. were collected from forest area. The aerial parts of the plant were manually separated, air dried, powdered, sieved, weighed and stored in air tight container. Different parts of the selected plants, are potential drugs of Ayurvedic formulations but inadequate information is available on their quality and purity standard. Therefore proximate (i.e. physicochemical) analysis was carried out for setting standards for crude raw material of plant as per the WHO guidelines[13].

C. Plant Extraction

The powder was extracted with different solvents ranging from non-polar to polar solvents. About 10 g of the crude drug powder was subjected for extraction (Soxhlet extraction) in round bottom flask with different solvents viz. methanol, benzene, chloroform, acetone, ethanol (95%) and water. Extracts were concentrated under vacuum and finally made up to 10 ml with HPLC grade methanol and ready for HPTLC analysis.

D. Chromatographic Conditions

Various concentration of sample (2µl, 5µl and 10 µl) were applied in the form of bands of width 8.0 mm using a Hamilton 100 µL syring on silica gel which was precoated on aluminium plate $60F_{254}$ (E. Merck Ltd) (20cm ×10 cm) with the help of Linomat 5 applicator attached to Camag HPTLC system, which was programmed through winCATS software (version 1.4.6).

After sample application plates were developed in a Camag twin through glass tank pre-saturated with the optimized mobile phase toluene-chloroform-ethanol (4:4:1 v/v) for 20 min. The plate was developed horizontally in Camag horizontal developing chamber (20 cm × 10 cm) at the room temperature. Densitometric scanning of developed plate was then performed with a Camag TLC Scanner 4 equipped with winCATS software version 1.4.6 at λ_{max} 254 nm and 366 nm using Deuterium and Mercury light source respectively. The developed plate was derivatized by dipping the plate in anisaldehydesulphuric acid reagent and heated at 110ºC onto Camag TLC plate heater III for 2 min. After derivatization of plate scanning was performed at λ_{max}540 nm using Tungsten light source.Photo-documentation of chromatograms was carried out with the Camag TLC visual izerat 254nm and 366 nm and under visible light respectively.

IV. RESULTS

The proximate (i.e. physicochemical) analysis was carried out for setting standards for crude raw material of *Barleriaprionitis* Linn. The results obtained from the study are compiled in Table-1 given below.

Table 1- Proximate analysis of selected plants

S. No.	Parameter	Mean % Content*
1	Foreign organic matter	00.029
2	Water soluble extractive	19.400
3	Ethanol soluble extractive	10.985
4	Total ash	04.455
5	Acid insoluble ash	00.347
6	Water soluble ash	03.530
7	Sulphated ash	03.438
8	Loss on drying (Percentage moisture content)	07.773

*All values are mean of three readings

The foreign organic matter indicates the presence of contamination of soil, stone, sand, dust and other organic matter adhere to the plant material. The foreign organic matter was found is under acceptable criteria. The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. Extraction values are useful for determination of crude drugs and it gives an idea about the nature of the chemical constituents present. Higher alcohol extractive value compared to water suggests that plant possess high alcohol soluble chemical compounds. Ash determination furnishes a basis for judging the identity and quality of the drug gives information to its adulteration with inorganic matter. Low ash value indicates less inorganic substances in it. This value varies within limits and is therefore important parameter for purpose of evaluation of crude drug. The loss on drying measures the amount of water and volatile matter in a sample. The HPTLC chromatogram shown in Fig.1, 2 and 3 indicates the separation of sample constituents of leaves, stem, root and whole plant extracts of Barleriaprionitis Linn. appliedat their various concentrations (2µL, 5µL and 10µL) and scanned at 254 nm, 366 nm and under visible light.The separation is more prominent in derivatized plate observed under visible light.

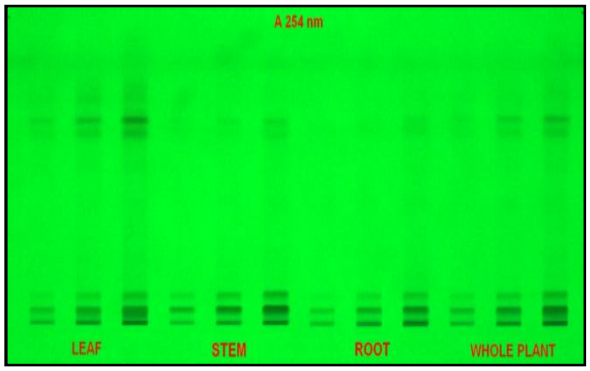


Fig. 1- HPTLC Chromatogram Image at 254 nm

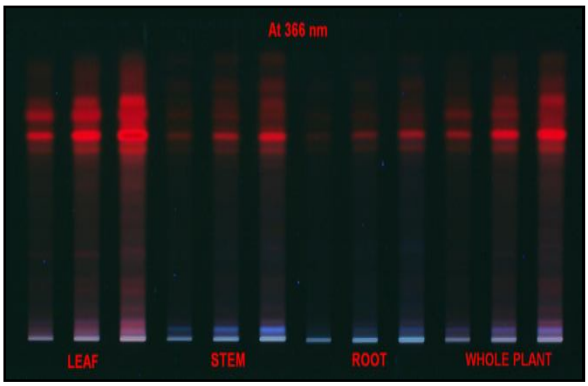


Fig. 2- HPTLC Chromatogram Image at 366 nm

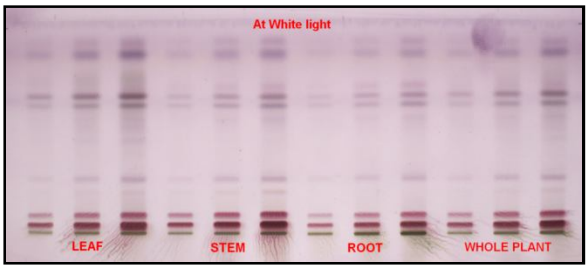


Fig. 3- HPTLC Chromatogram Image at visible light

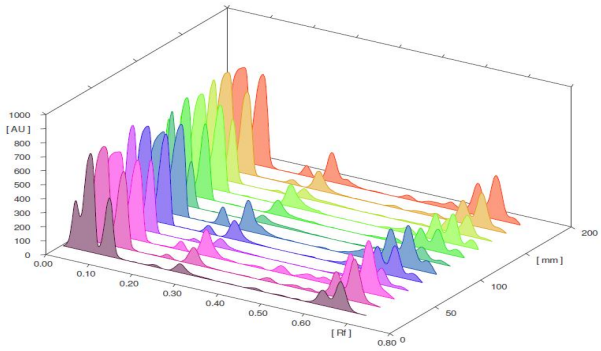
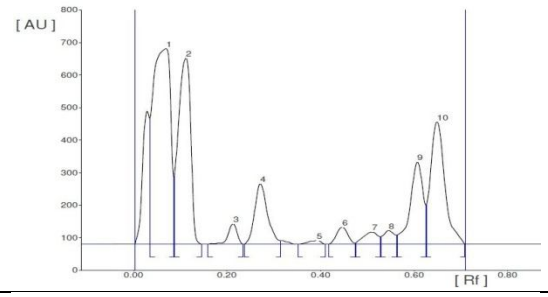


Fig. 4 - Three dimensional plots for all tracks

Fig. 4 shows the three dimensional graph of peaks for all the tracks.

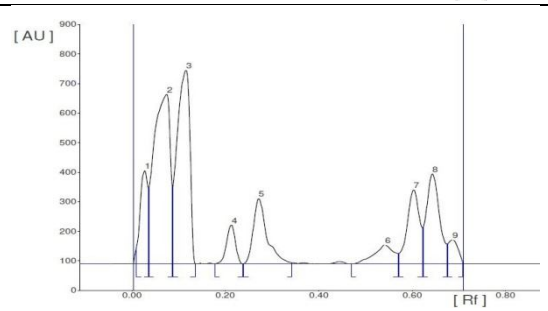
Fig. 5indicates the presence of 10, 9, 9 and 11 different components in the fingerprint of leaves, stem, root and whole plant extracts of Barleriaprionitis Linn. respectively. Their respective peak list and Rf values, peak height, and peak area were also represented. The components having higher percentage area are in the higher concentrations in the sample.

A



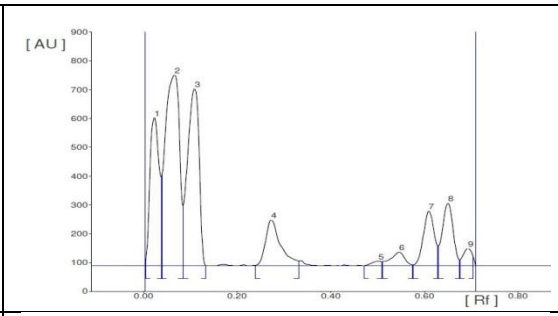
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	387.9	0.07	602.0	27.38	0.09	206.3	19180.6	34.34
2	0.09	217.3	0.11	572.8	26.05	0.15	0.2	12621.0	22.60
3	0.16	0.1	0.21	63.3	2.88	0.23	0.1	1029.1	1.84
4	0.24	0.1	0.27	186.9	8.50	0.32	11.2	4309.5	7.72
5	0.35	0.1	0.39	11.1	0.50	0.41	0.1	269.0	0.48
6	0.42	0.2	0.45	52.6	2.39	0.47	4.3	1077.0	1.93
7	0.48	4.8	0.51	38.1	1.73	0.53	24.0	1010.4	1.81
8	0.53	24.4	0.55	42.5	1.94	0.56	28.4	852.4	1.53
9	0.57	28.8	0.61	253.6	11.53	0.63	121.1	5832.6	10.44
10	0.63	122.1	0.65	376.1	17.10	0.71	1.7	9674.3	17.32

B



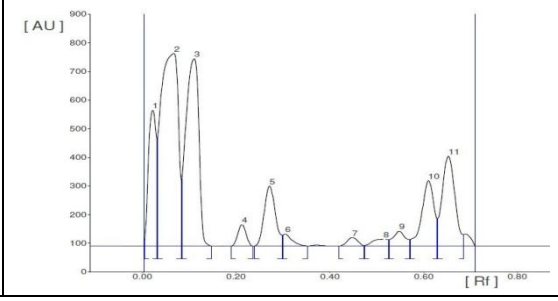
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	55.4	0.03	315.4	12.13	0.04	255.6	4416.0	7.36
2	0.04	259.4	0.08	574.0	22.08	0.09	259.5	16862.8	28.09
3	0.09	264.7	0.12	655.2	25.20	0.14	0.3	14909.1	24.83
4	0.18	0.2	0.21	131.7	5.07	0.24	0.0	2091.9	3.48
5	0.24	0.2	0.27	221.5	8.52	0.34	4.0	5425.1	9.04
6	0.47	0.1	0.54	64.1	2.47	0.57	35.8	2252.6	3.75
7	0.57	36.1	0.60	251.5	9.67	0.62	122.2	5569.8	9.28
8	0.63	124.6	0.64	304.7	11.72	0.68	67.3	7108.0	11.84
9	0.68	68.3	0.69	81.7	3.14	0.71	5.5	1403.7	2.34

C



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	18.5	0.02	513.1	20.74	0.04	308.5	8621.6	16.40
2	0.04	316.6	0.07	661.4	26.74	0.08	209.0	16824.3	32.01
3	0.09	213.5	0.11	613.2	24.79	0.13	0.3	12662.5	24.09
4	0.24	2.0	0.27	158.7	6.41	0.33	16.4	4238.3	8.06
5	0.47	0.1	0.50	15.0	0.61	0.51	14.0	256.3	0.49
6	0.51	14.4	0.55	46.8	1.89	0.58	3.0	1150.8	2.19
7	0.58	3.1	0.61	189.8	7.67	0.63	67.6	3719.0	7.08
8	0.63	68.8	0.65	216.3	8.74	0.68	19.8	4146.8	7.89
9	0.68	20.3	0.69	59.6	2.41	0.71	31.0	935.7	1.78

D



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	20.0	0.02	475.7	17.08	0.03	375.8	6719.0	10.59
2	0.03	383.9	0.07	673.4	24.18	0.08	232.5	20746.0	32.69
3	0.08	235.7	0.11	655.4	23.53	0.15	0.2	15256.9	24.04
4	0.19	0.3	0.21	76.2	2.74	0.24	0.0	1121.5	1.77
5	0.24	0.4	0.27	210.3	7.55	0.30	38.4	4217.7	6.65
6	0.30	38.8	0.31	42.9	1.54	0.35	0.3	720.6	1.14
7	0.42	0.5	0.45	30.5	1.10	0.47	2.3	590.6	0.93
8	0.47	2.6	0.52	24.5	0.88	0.53	23.2	689.8	1.09
9	0.53	23.4	0.55	52.7	1.89	0.57	23.8	1149.3	1.81
10	0.57	24.0	0.61	229.4	8.23	0.63	95.3	5050.4	7.96
11	0.63	96.8	0.65	314.5	11.29	0.69	40.7	7201.3	11.35

Fig. 5 - Fingerprint, Peak list and Rf values of components in
A. Leaves, B. Stem, C. Root and D. Whole plant extract of *Barleriaprionitis* Linn.

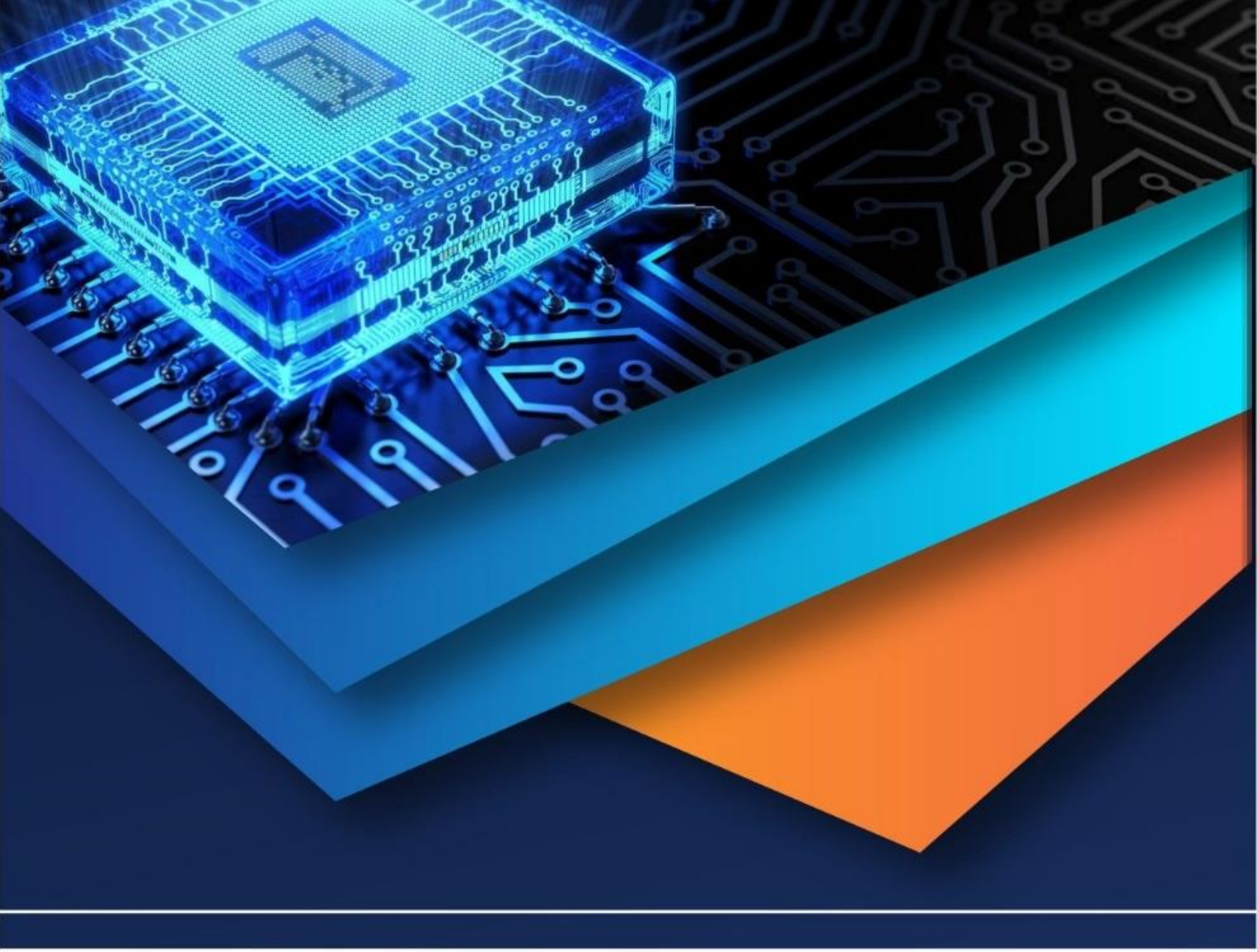
V. CONCLUSION

Authentication of medicinal plants at genetic and chemical level is a critical step on the use of botanical materials for both research purpose and commercial preparations. For many of living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. In plant taxonomy the morphological characters play a vital role in plant systematic study and are used as a tool for the classification of a taxon. In recent times, in addition to morphological markers, anatomical, cytological, biochemical and molecular markers are also being used to classify the organism. HPTLC is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories[14,15]. Thus HPTLC fingerprint profile is useful as phytochemical marker and also a good estimation of genetic variability in plant populations. The pattern recognition can be used to discriminate different kinds of samples of herbal medicines investigated. The variation determination of common peaks/regions in a set of chromatographic fingerprints could provide useful qualitative and quantitative information on the characteristic components of herbal medicines investigated. It not only confirms but also establishes the identity of natural products. It is also an ideal screening tool for detection of adultration[12,16,17].On the other hand, whether the real samples were identified as the herbs with the same quality grade could be determined successfully by way of comparing the chromatographic fingerprints with the similarity index and linear correlation analysis.Thus chromatographic fingerprint analysis serves as a promising tool for identification, authentication, and quality control of herbal drugs, herbal drug preparations, and herbal medicinal products.

REFERENCES

[1] World Health Organization (WHO), WPR/RC52/7: A Draft Regional Strategy for Traditional Medicine in Western Pacific. WHO Regional Committee, 52nd Session Darussalam, 10-14 Sept. 2001.
[2] F. Gong, B.T. Wang andF.T. Chau. Data preprocessing for chromatographic fingerprint of herbal medicine with chemometric approaches. Anal Lett. 2005, 38 : 2475-2492.
[3] Y.Z. Liang, P .Xie andK. Chan. Quality control of herbal medicines. J. Chromatogr. B., 812: 53-70, 2004.
[4] M.M. Shrivastava. (ed.) High-Performance Thin-Layer Chromatography (HPTLC), Springer-Verlag Berlin Heidelberg. 2011.
[5] State Drug Administration of China, Technical requirement of the fingerprint in injection of Chinese MateriaMedica. Chon Tradit Pat Med 2000; 22. 825.
[6] E.S. Ong. Chemicalassay of atropine and Phenobarbital in pharmaceutical preparations containing Valeriana Liquid extract. J Chromatoger 1982; 241- 399.
[7] P.S. Xie. A feasible strategy for applying chromatography fingerprint to assess quality of Chinese herbal medicine. Tradit Chin Drug Res Clin Pharm 2001; 12(3), 141-169.
[8] D. Banerjee, A.K. Maji, S. Mahapatra and P. Banerji. BarleriaprionitisLinn.: A review of its Traditional Uses, Phytochemistry, Pharmacology and Toxicity. Res. J. Phytochem. 6(2): 31-41, 2012.

[9] P. Sharma, B. Shrivastava, G. N. Sharma and H.R. Jadhav. Phytochemical and Ethnomedicinal value of Barleriaprionitis Linn. An overview. J. Harmonized Res. Pharmacy 2(3): 190-199, 2013.
[10] B. Fried and J. Sherma. Thin Layer Chromatography, 4th Edition, published by Marcel Dekker Inc., New York.1999.
[11] P.E. Wall. Thin-layer Chromatography – A Modern Practical Approach, published by The Royal Society of Chemistry, Cambridge, UK. 2005.
[12] E. Reich, A. Schibli. High-Preformance Thin-Layer Chromatography for the Analysis of Medicinal Plants. NewYork; Thieme; 2010.
[13] World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. 2006.
[14] P.K. Jaiswal. High-performance Thin-Layer Chromatography for foodAnalysis. CBS Publication; New Delhi. 2010.
[15] H. Wagner, S. Bladt. Plant Drug Analysis: A Thin LayerChromatography Atlas. 2nd ed. Springer, Berlin. 1995.
[16] S.K. Subramanian, N.Ramakrishnan. Chrmatographic fingerprintanalysis of Naringicrenulataby HPTLC technique. Asian Pec J Trop Biomed. pp.195-198. 2011.
[17] P.D. Sethi.High performance Thin Layer chromatography: Quantitative analysis of pharmaceutical formulations. CBS Publication. NewDelhi. pp. 10-60. 1996.



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