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HPTLC FINGERPRINTING ANALYSIS OF THE ETHANOLIC EXTRACT OF BAUHINIA RACEMOSA LEAVES

Dr. P. Anitha[1]*, E. Ravichandhiran[2] and Rubathi Krishnan P.[3]

*[1]Assistant Professor, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore, Tamilnadu.

[2]PG Biochemistry, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore, Tamilnadu.

[3]UG Biochemistry, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore, Tamilnadu.

***Corresponding Author: Dr. P. Anitha**

Assistant Professor, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore, Tamilnadu.

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ABSTRACT

Objective: To establish the fingerprint profile of *Bauhinia racemosa* leaves using high performance thin layer chromatography (HPTLC) technique. **Methods:** Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS1.3.4 software was used. **Results:** HPTLC finger printing of ethanol extract of leaf revealed 14 peaks with Rf values in the range of 0.02 to 0.85 with flavonoid compound compared to Rf value of standard quercetin **Conclusions**: It can be concluded that HPTLC fingerprint analysis of leaf extract of *Bauhinia racemosa* are used in quality assessment and identification of Phyto marker compounds. By isolating and distinguishing the biomarker compounds, novel drugs can be produced to treat various diseases.

KEYWORDS: *Bauhinia racemosa*, HPTLC fingerprinting, flavonoids.

INTRODUCTION

The presence of bioactive phytochemicals in medicinal plants, plays a very significant role in human life for the prevention and treatment of various diseases. There are thousands of medicinal plants known to have a long history of usage for their curative properties against various diseases and ailments related to oxidative stress and free radicals.[1] Herbal drugs, mainly their secondary metabolites, have proved a vital contribution to modern therapeutics.

India is the Botanical Garden of the World as it is the largest producer of medicinal plants, as the medicinal value lies in the bioactive phytochemical constituents that produce definite physiological effects on the human body These natural compounds formed the base of modern drugs we use today.[2]

HPTLC is rational for the expansion of chromatographic fingerprints to determine major active constituents of medicinal plants. The separation and determination are good and the results are more consistent and reproducible than TLC. Combined with digital scanning profiling, it has the main advantage of *in situ* qualitative and quantitative measurements by scanning densitometry. Besides, the colorful graphic HPTLC image provides extra, innate visible color and/or fluorescence parameters for parallel assessment on the same plate. It also revealed a better separation of individual phytochemicals.[3]

*Bauhinia racemosa*_belongs to *Caesalpiniaceae* Family. It occurs in India, Ceylon, and China. *Bauhinia racemosa* is a small, crooked, bushy, deciduous tree that can grow under very difficult climatic environments with drooping branches. Many uses of different plant parts are used to treat diuretics, dysentery, inflammation of the liver, snake bites, and scorpion string. Seeds are tonic and aphrodisiac.[4,5] The plant has been studied by many researchers with special reference to its pharmacological activity but no isolation of phytochemicals has been reported.[6] Hence phytochemical investigation of the leaves of *Bauhinia racemosa* was undertaken.

MATERIALS AND METHODS

Preparation of the *Bauhinia racemosa* extracts

Healthy, fresh and matured parts leaves of *Bauhinia racemosa* were pulverized into powdered form. For HPTLC 10 gm of the dried powdered sample was taken in a Soxhlet and extracted using ethanol solvent for 8h (hot extraction). The extract was then concentrated using a rotary vacuum evaporator. For cold extraction, 1gm powder was mixed with 20 ml of the ethanol solvent and kept in a sonicator for 20 min.

HPTLC fingerprinting profile

HPTLC finger printing studies were carried out according to the method of Wagner and Baldt[7] and Harbone.[8]

HPTLC fingerprint profile of the leaf extract of *Bauhinia racemosa* was carried out. The given *Bauhinia racemosa* sample, 20mg was weighed accurately in an electronic balance (Afcoset), dissolved in 250µl of the respective solvent, and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis. 2 µl of the test solution and 2 µl of standard solution were loaded as 5mm band length in a 3 x 10 Silica gel 60F254 TLC plate using a Hamilton syringe and CAMAG LINOMAT 5 instrument. The resulting plate is dried with hot air to evaporate the solvent from the plate. Place the plate on a camera (CAMAG REPROSTAR 3) and capture images under visible light, UV 254nm and UV 366nm. The formed plate is sprayed with a spray and dried in a hot oven at 100°C. Photo recording of the plate is made using a video recorder (CAMAG REPROSTAR 3) chamber in visible and UV 366nm mode. After derivatization, the plates were mounted on a level scanner (CAMAG TLC SCANNER 3) and scanned at UV 366nm. Record the peak, peak display, and peak intensity graph. The software used is win CATS version 1.3.4.

The sonicated leaf extract (for the general account) of *Bauhinia racemosa* Lam. were spotted on HPTLC plate and developed using Toluene: Methanol: Diethyl amine (30:04:04) as a mobile phase. The methanolic sulphuric acid was used as derivatizing (universal) agent. The plate was scanned at the wavelength of 254nm and 366nm (Photo plate - 1 and 2). The spectra obtained for the studied sample was correlated with the standard compound used.

Preparation of standard solution and linearity

The standard stock solution for Quercetin was prepared by dissolving 5mg standard compound powder each in 5ml of ethanol and sonicated for 5 minutes. From this stock (1mg/ml), seven different concentrations (100-700µg/ml) of each standard were prepared. The linearity of standard compound was determined by applying the standard solution of different concentrations ranging from 0.5 -3.0µg/spot. All the solvents used in the analysis were HPLC grade.

Plates were scanned at 254 nm which was selected experimentally on the basis of distinctive absorption spectra of the compounds between 200 and 400 nm. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light and UV 366 nm and 254 nm. The peak numbers with its height and area, peak display, and peak densitogram were identified.

RESULT AND DISCUSSION

HPTLC Fingerprint for Flavonoids

The HPTLC fingerprint of ethanolic extract of *Bauhinia racemosa* leaves for flavonoids was analyzed using Quercetin as standard (Table 1 and Figure 1&2). The peak integration of the data with Rf values is shown in Table 1. The ethanolic extract of *Bauhinia racemos*a leaves envisioned the presence of 14 different types of Flavonoids and different Rf values ranging from 0.02 to 0.85. Among those peaks, peaks 12 showing Rf value of 0.81 respectively were identified to be flavonoid compounds as it was close to Rf value of standard Quercetin (0.80).

Table 1: The Chromatogram of Ethanolic leaf Extract of *Bauhinia racemosa Lam.*

Track	Peak	Start Rf	Max Rf	Max Height AU	Max %	End Height AU	Area M AU	Area %	Assigned Substance
Sample (*Bauhinia racemosa* Extract)	1	0.03	-0.00	696.6	23.64	466.7	8042.3	10.77	AutoGenerated2
	2	0.01	0.03	693.9	23.55	64.0	31156.8	41.72	AutoGenerated1
	3	0.22	0.26	109.0	3.70	66.3	5524.7	7.40	AutoGenerated6
	4	0.32	0.35	80.0	2.72	76.3	1937.9	2.60	AutoGenerated16
	5	0.37	0.40	142.7	4.84	46.4	5126.9	6.87	AutoGenerated10
	6	0.51	0.53	60.3	2.05	56.2	984.7	1.32	AutoGenerated27
	7	0.54	0.55	63.5	2.15	35.3	1492.0	2.00	AutoGenerated23
	8	0.59	0.62	48.5	1.64	47.1	948.2	1.27	AutoGenerated25
	9	0.63	0.64	58.2	1.98	51.0	845.9	1.13	AutoGenerated13
	10	0.69	0.71	76.1	2.58	57.6	1697.1	2.27	AutoGenerated15
	11	0.76	0.75	59.1	2.01	43.1	766.5	1.03	AutoGenerated9
	12	0.81	0.85	192.3	6.53	42.1	5846.7	1.72	Flavonoid 1
	13	0.85	0.93	89.3	3.03	69.3	2116.8	2.83	Flavonoid 2
	14	0.94	0.95	83.2	2.83	75.2	701.1	0.94	AutoGenerated11

The Chromatogram of Flavonoid standard Quercetin

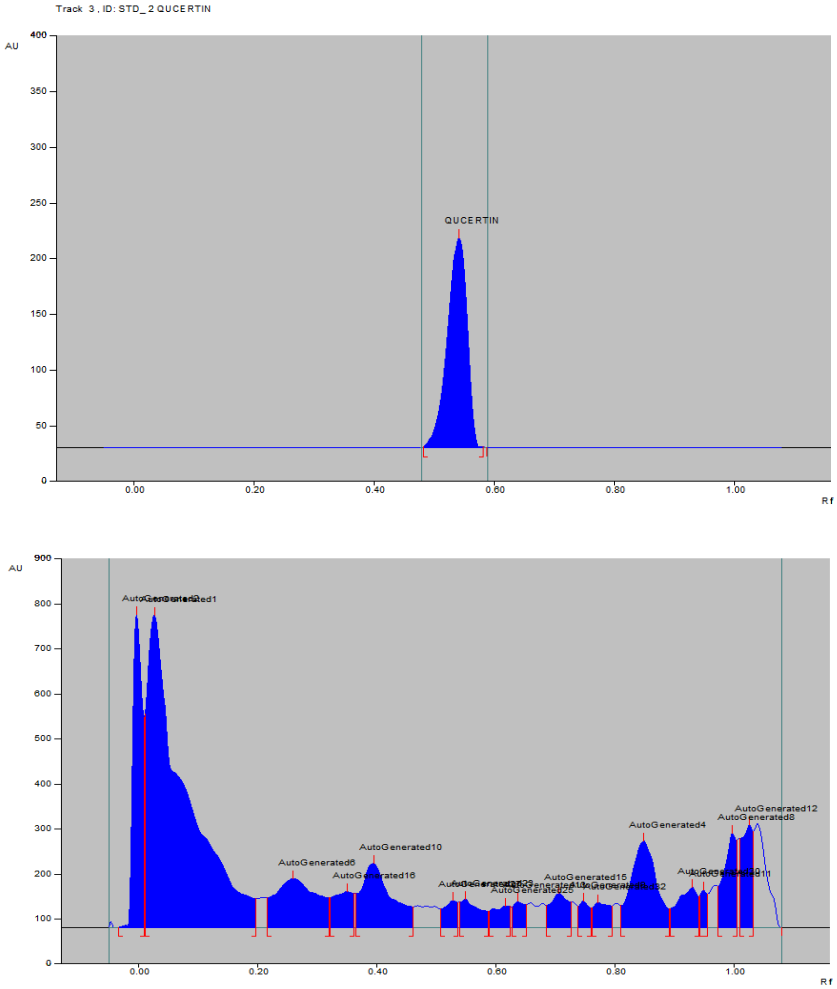

Figure 2: The Chromatogram of Ethanolic leaf Extract of *Bauhinia racemosa Lam*.

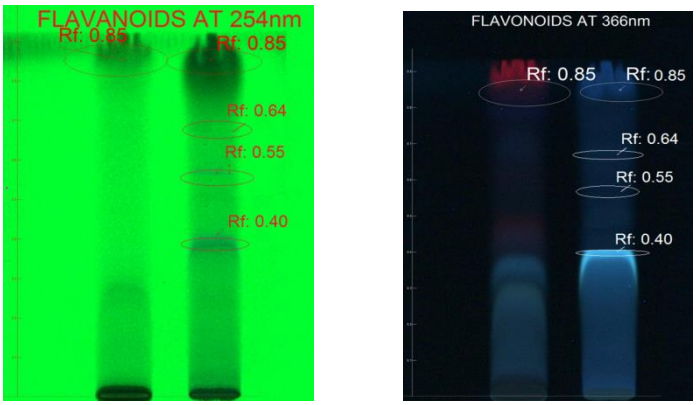

Plate 1& 2: HPTLC Photo documentation of Flavonoid standard and Ethanolic leaf Extract of *Bauhinia racemosa Lam*. at 254nm and 366nm.

The leaf extract samples were scanned at 254nm and 366nm wavelength to obtain the spectral peak along with the standard Quercetin compounds. The densitogram shown in Plate 1 and 2 *Bauhinia racemosa* at 254 nm and 366 nm indicate that all sample constituents were separated. The following Max Rf 0.85 which was shown in the plates. It is indicating Rf values 0.40, 0.55,0.64 and 0.85 were found to be more frequent as the percentage area was more with 4.84%, 2.00% 1.13% and 2.83% respectively.

The HPTLC fingerprinting profile were employed to analyze medicinal plants to illustrate their application in qualitative (fingerprint) and quantitative determination, demonstrating their feasibility in the quality control of phytoconstituents from herbal drugs and formulations.[9] This will help for the standardization of the plant

products, and utilized in herbal medicine treatment. From the HPTLC studies, it has been found that the ethanol leaves extract of *Bauhinia racemosa* contains a mixture of compounds and so it is established that the pharmacological activity shown by them are due to the presence of bioactive secondary metabolites.

CONCLUSION

HPTLC fingerprint investigation can be used as a diagnostic tool for the accurate identification of the phytoconstituents in the plant. The results of the present study provided a valuable Phytochemical marker for the determination and descript the selected plants. Thus, the selected plants shown as a potential source of useful drugs. The present study may lead to practical implementation for isolation, characterization and interpretation of the bioactive compounds in future. In addition to their medicinal use some secondary metabolites from these plants can also serve as powerful pharmacological tool to help as therapeutic agent for human in management of various ailments.

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Conflict of Interest

None declared.

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