

Research Article

HPTLC Fingerprint Profile and Isolation of Marker Compound of *Ruellia tuberosa*

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The present study was aimed to identification, isolation, and quantification of marker in *R. tuberosa* (Acanthaceae). HPTLC fingerprinting was carried out for various extract of root, stem, and leaf of *R. tuberosa*. From the HPTLC fingerprint the florescent band (under 366 nm) at R_f : 0.56 (mobile phase chloroform : toluene : ethyl acetate (6 : 3 : 1, v/v)) was found in leaf, root, and stem of *R. tuberosa*. So, the florescent band (under 366 nm) at R_f : 0.56 was isolated as marker compound RT-F2 from root of *R. tuberosa*. The marker compound RT-F2 was quantified by using HPTLC technique. The percentage (W/W) amount of RT-F2 was found to 40.0% and 44.6% in petroleum ether and ethyl acetate extract of *R. tuberosa* roots, respectively. Further study is suggested to characterization and biological nature of marker compound.

1. Introduction

Marker compound means chemical constituents within a medicinal that can be used to verify its potency or identity. For sometimes, the marker compounds may be described as active ingredients or chemicals that confirm the correct botanical identity of the starting material. It is very difficult to identify correct marker compounds for all traditional medicinals, because some medicinals have unknown active constituents and others have multiple active constituents. A chromatographic fingerprint of a herbal medicine is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and/or chemical characteristics. By using chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents is not exactly the same for different samples of drug. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic component of the herbal drug [1–5].

Ruellia tuberosa is an erect, suberect, or diffuse perennial herb up to 60–70 cm tall herb and belongs to family Acanthaceae, a native of Central America, introduced into Indian garden as ornament. It is used medicinally in West Indies, Central America, Guiana, and Peru. *R. tuberosa* is commonly known as “Cracker plant” [6–8]. In Siddha system of medicine, leaves are given with liquid copal as remedy for gonorrhea and ear diseases [9], used in stomach cancer [10]. Dried and ground roots in dose of two ounces cause abortion and also used in sore eyes [11]. The herb also exhibits emetic activity and employed substitute of ipecac, also used in bladder stones and decoction of leaves used in treatment of Bronchitis [12]. In Suriname’s traditional medicine system, it is used as anthelmintic and also in management of joint pain and strained muscles. In folk medicine, it has been used as diuretic, antipyretic, antidiabetic, antidotal, thirst-quenching agent and analgesic and anti-hypertensive activity [13, 14]. *Ruellia tuberosa* is used as cooling in urinary problem, uterine fibroids [15, 16]. It has recently been incorporated as a component in a herbal drink in Taiwan [17]. It has been experimentally proved to possess antioxidant [18], antimicrobial

[19], anticancer [20], gastroprotective activity [21], antinociceptive, and anti-inflammatory activity [22]. It is reported that it contains flavonoids, steroids, and triterpenoids and alkaloid [23–26]. But there is no any identified marker reported; so the present study is aimed to identification, isolation, and quantification of marker in *R. tuberosa*.

2. Materials and Methods

2.1. Plant Material. Fresh plant of *Ruellia tuberosa* was collected from the campus of The M. S. University of Baroda in the month of August 2008. Plant was authenticated at Botany Department of The M. S. University. Voucher specimen (PHR/HDT/DC-RT-08) was stored in herbarium of our laboratory. Roots were separated and sun dried separately. Dried plant material was powdered.

2.2. Chemicals. All other reagents were analytical grade, purchased from Merck (Darmstadt, Germany). All UV-Vis measurements were recorded on a Shimadzu UV-1800.

2.3. Preparation of Extracts [27]. Powdered air dried drug, weighing about 50 g, was extracted successively in soxhlet apparatus with the series of solvents of increasing polarity as follows: petroleum ether, toluene, chloroform, ethyl acetate, and methanol. Each time before extracting with the next solvent, the material was dried. All the extracts were filtered through Whatman filter paper and concentrated. Concentrated extracts were applied on the TLC plate as sample solution.

2.4. HPTLC Finger Print Profiles for Various Extracts [28, 29]

2.4.1. TLC Conditions. TLC plate consists of 20 × 10 cm, pre-coated with silica gel 60 F254 TLC plates (E. Merck) (0.2 mm thickness) with aluminum sheet support. The spotting device was a CAMAG Linomat V Automatic Sample Spotter (Camag Muttentz, Switzerland); the syringe, 100 µL (from Hamilton); the developing chamber was a CAMAG glass twin trough chamber (20 × 10 cm); the densitometer consisted of a CAMAG TLC scanner 3 linked to WINCATS software. Mobile phase was chloroform:toluene:ethyl acetate (6:3:1, v/v). Saturation time for mobile phase was 2 hours.

2.4.2. Procedure. Various extracts of roots, leaf, and stem of *R. tuberosa* were applied on TLC plate and the plate was developed in chloroform:toluene:ethyl acetate (6:3:1, v/v) solvent system to a distance of 8 cm. The plates were dried at room temperature in air. The plate was scanned at 254 nm (Figure 1) and 366 nm (Figure 2) before spraying and at 600 nm (Figure 3) after spraying with detection reagent (Anisaldehyde sulfuric acid reagent and plate was heated at 110°C for 5 minutes). The R_f values and color of the resolved bands were noted.

2.5. Isolation and Characterization of Chemical Marker

2.5.1. Isolation of Compound RT-F2 from Petroleum Ether and Ethyl Acetate Extract of Root. The dried powder of root

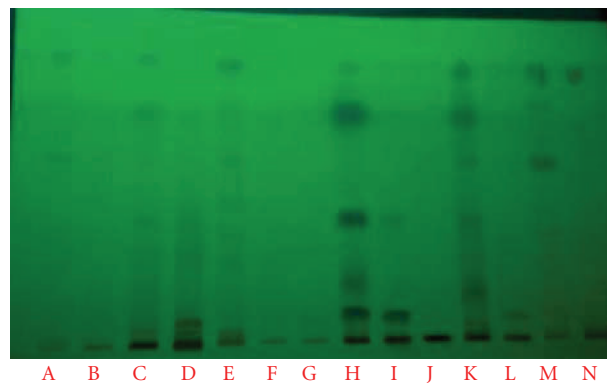


FIGURE 1: HPTLC fingerprint of various extracts of *R. tuberosa* at 254 nm.

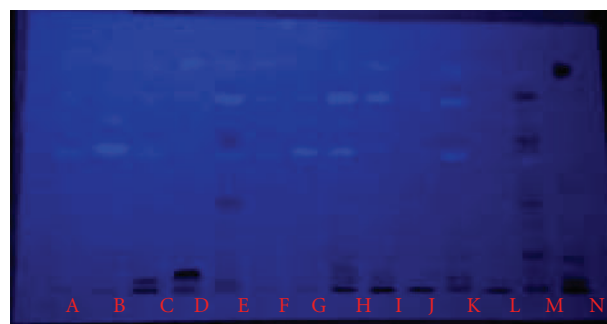


FIGURE 2: HPTLC fingerprint of various extracts *R. tuberosa* at 366 nm.

(200 g) was extracted with petroleum ether and ethyl acetate (500 mL) separately in soxhlet apparatus for 2 days. Then the extracts were concentrated by distilling the solvent and concentrated extracts were subjected to repetitive preparative thin layer chromatography using Silica Gel G as stationary phase (20 × 20 cm glass plates) and chloroform:toluene:ethyl acetate (6:3:1 v/v) as mobile phase. Fluorescent bands under 366 nm at R_f value 0.56 were identified RT-F2 compound. RT-F2 bands were scraped. RT-F2 was separated from silica Gel G by treating with methanol and Chloroform mixture (1:1), filtered through Whatman filter paper, and filtrates were combined, concentrated, and dried. Isolated compounds were subjected to TLC and HPTLC, UV spectroscopy (Figure 4), IR spectroscopy (Figure 5), and Mass spectroscopy (Figure 6).

2.5.2. Quantification of RT-F2 in Petroleum Ether and Ethyl Acetate Extract Using HPTLC Method

Standard Stock Solution. A solution of F2 compound (500 µg/mL) was prepared in chloroform.

Sample Preparation

Ethyl Acetate Extract. Stock solution of sample 2 mg/mL of extract was prepared in chloroform.

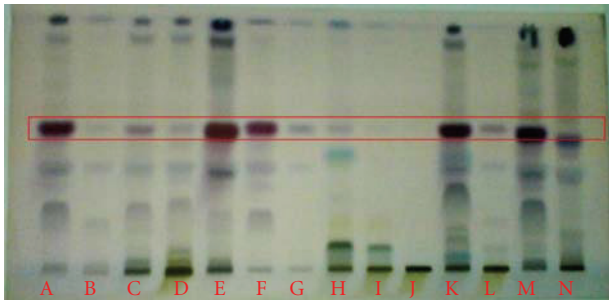


FIGURE 3: HPTLC fingerprint of various extracts of *R. tuberosa* at 600 nm. (A) Petroleum Ether extract (R). (B) Toluene extract (R). (C) Chloroform extract (R). (D) EtOH extract (R). (E) Petroleum Ether Fraction (R). (F) Hexane Fraction (R). (G) Toluene Fract (R). (H) Chloroform Fract (R). (I) EtOH Fract (R). (J) Methanol Fract (R). (K) EtOH extract (R). (L) methanol extract (R). (M) Petroleum Ether extract (S). (N) petroleum ether (L). R: root; Fract: hydroalcoholic fraction; L: Leaf; S: stem.

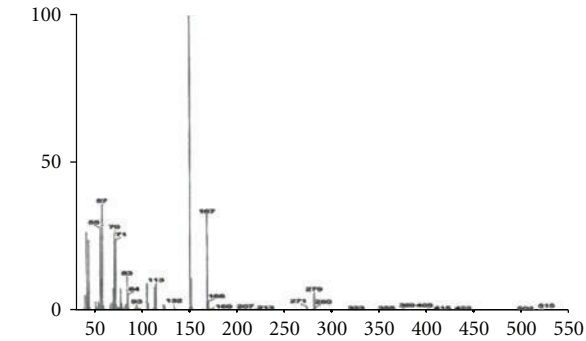


FIGURE 6: Mass spectra of RT-F2 compound.

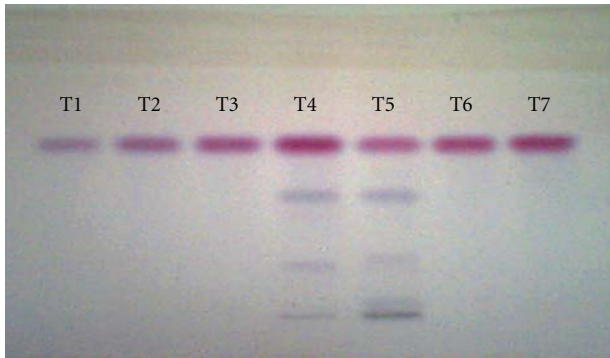


FIGURE 7: Densitogram of RT-F2 compound at 600 nm, where T1, T2, T3, T6, and T7 are 1.25, 2.5, 3.75, 5.0, and 6.25 concentration of standard RT-F2 ($\mu\text{g/mL}$), respectively. T4 and T5 are petroleum ether extract and ethyl acetate extract ($\mu\text{g/mL}$), respectively.

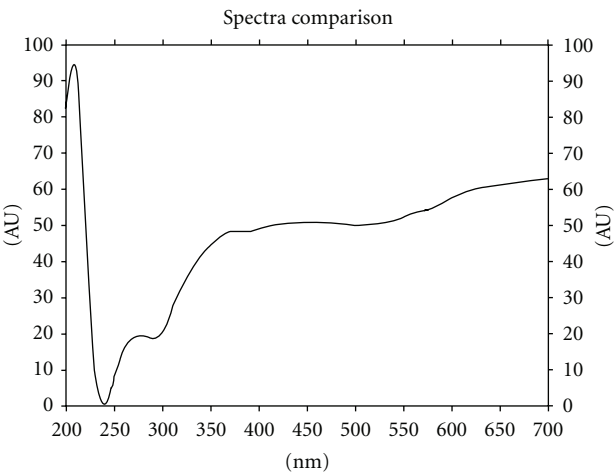


FIGURE 4: UV spectra of RT-F2 compound (λ_{max} 208 nm, 272 nm).

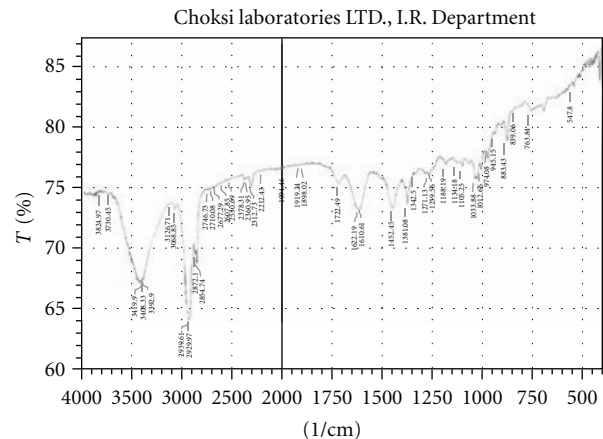


FIGURE 5: IR spectra of RT-F2 compound.

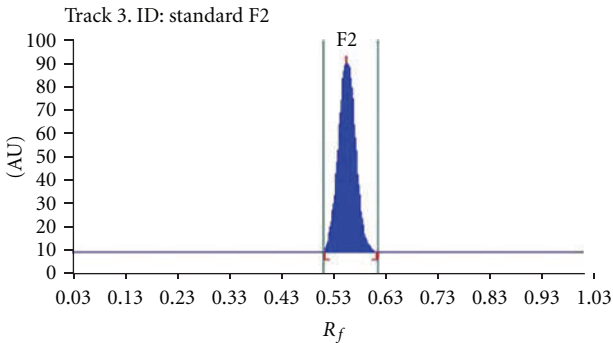


FIGURE 8: Chromatogram of RT-F2 standard compound at 600 nm.

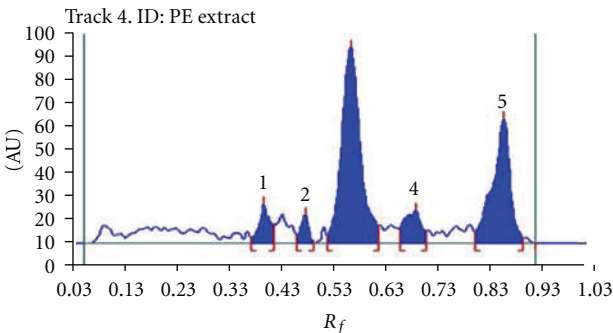


FIGURE 9: Chromatogram of petroleum ether extract at 600 nm.

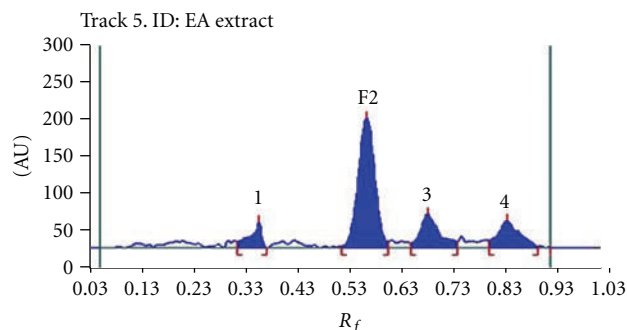


FIGURE 10: Chromatogram of ethyl acetate extract at 600 nm.

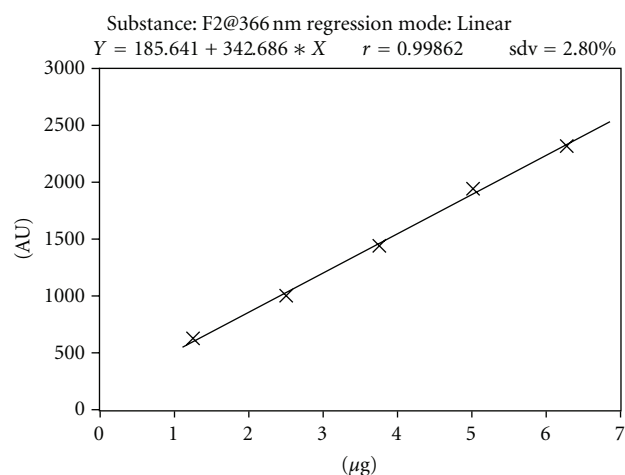


FIGURE 11: Calibration curve of RT-F2 compound.

Petroleum Ether Extract. Stock solution of sample 1 mg/mL of extract was prepared in chloroform.

Calibration Curve. From the standard stock solution 2.5–12.5 μ L solutions were applied on precoated plate of Silica Gel G, to produce the range of 1.25–6.25 μ g of RT-F2 per spot, respectively (Figure 7). Calibration curve is given in Figure 11.

Sample. A 10 μ L of each extract was applied.

Mobile Phase. The mobile phase was chloroform:toluene:ethyl acetate (6:3:1).

Stationary Phase. The stationary phase was Precoated plate, Silica Gel G 60 F254.

Applicator. The applicator phase was CAMAG LINOMAT 5.

Development. Plate was developed in a twin trough chamber.

Detection. We spray with Anisaldehyde sulfuric acid reagent and heat at 110°C for 5 minutes.

The plate was scanned at 366 nm under fluorescent mode before spraying and at 600 nm (Figure 7) after spraying. The Chromatograph of RT-F2 standard compound (Figure 8),

petroleum ether extracts (Figure 9), and ethyl acetate extract (Figure 10) were reported.

3. Results and Discussion

3.1. HPTLC Fingerprint Profile. HPTLC fingerprint showed that purple colored band (after derivatisation) (Figure 3) at R_f : 0.56 was found in leaf, root, and stem of *R. tuberosa*. The florescent band (under 366 nm) at R_f : 0.56 was selected as marker compound and identified as RT-F2.

3.2. Isolation and Characterization of Marker RT-F2 Compound. Data from fingerprinting results provide information about presence of major terpenoid in petroleum ether extract and ethyl acetate extract of root, targeted for isolation.

3.3. Compound RT-F2. Isolated compound F2 has sticky type of nature. It gives violet purple color with Anisaldehyde sulfuric acid reagent and Liebermann-Burchard reagent.

Analysis

TLC. R_f : 0.56, Solvent system-toluene:chloroform:ethyl acetate (3:6:1).

Detection. Anisaldehyde sulfuric acid reagent (heat at 105°C for 5 minutes).

IR (KBr, cm^{-1}). 3409, 1622.

MS. (m/z) 279, 167, 149, 113, 83, 55.

3.4. Quantification of RT-F2 in Petroleum Ether and Ethyl Acetate Extract Using HPTLC. Figure 7 shows the HPTLC chromatogram of standard RT-F2 compound, petroleum ether extract and ethyl acetate extract.

The percentage (W/W) amount of RT-F2 was found to 40.0% and 44.6% in petroleum ether and ethyl acetate extract of *R. tuberosa* roots, respectively (Tables 1 and 2).

4. Conclusion

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. A TLC densitometric method for the quantification of isolated marker compound RT-F2 was established in petroleum ether and ethyl acetate extract of roots of *R. tuberosa*. The present HPTLC fingerprinting profile can be used as a diagnostic tool to identity and to determine the quality and purity of the *R. tuberosa* in future studies.

TABLE 1: Calibration curve data for RT- F2 compound of HPTLC method.

Track	R_f	Concentration of RT-F2	Height of peak	Calculated RT-F2	Area of peak	Calculated RT-F2
1	0.57	1.250 μg	51.72		641.96	
2	0.56	2.500 μg	48.37		1005.73	
3	0.56	3.750 μg	71.26		1446.38	
4	0.56	Unknown*	73.75	3.758 μg	1558.45	4.006 μg
5	0.57	Unknown**	158.38	>6.875 μg	3246.91	>6.875 μg
6	0.57	5.000 μg	86.79		1945.75	
7	0.58	6.250 μg	110.10		2313.74	

* Petroleum ether extract, ** Ethyl acetate extract

Regression equation (Height) $Y = 27.094 + 12.415X$, $r = 0.9574$

Regression equation (Area) $Y = 185.641 + 342.686X$, $r = 0.9986$.

TABLE 2: Quantification of RT-F2 compound.

Stationary phase	Precoated Silica Gel 60 GF ₂₅₄
Mobile phase	Chloroform : toluene : ethyl acetate (6 : 3 : 1)
Calibration range of F2	2.5–12.5 $\mu\text{g}/\text{spot}$
Detection	Anisaldehyde sulphuric acid reagent heated at 110°C for 5 min and detected at 600 nm.
Regression equation	$Y = 185.6 + 342.686X$ (area wise)
R value	0.99862 (area wise)

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