CHAPTER-22

HPTLC FINGERPRINTING OF *RUELLIA TUBEROSA* L., A MEDICINAL HERB

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Summary

Ruellia is a genus of flowering plants commonly known as Wild Petunias which belongs to the family Acanthaceae. It contains about 250 genera and 2500 species. Most of these are shrubs or twining vines; some are epiphytes. Some of these are used as medicinal plants. The phytochemicals constituents: glycosides, alkaloids, flavonoids and terpenoids are present. The genus has been traditionally claimed to be used for the treatment of flu, asthma, fever, bronchitis, high blood pressure, eczema, and diabetes.

Present study is an attempt to identify some phytochemical markers in *R. tuberosa*. HPTLC fingerprinting was carried out for various extract of leaf, stem and root of *R. tuberosa*. HPTLC fingerprint revealed 6 types of 12 phenolic compounds, 4 types of 8 flavonoids and 4 alkaloids. The identified compounds are possibly imparting medicinal value to the plant and this chemical screening will be helpful for developing pharmacopeial standards for *R. tuberosa*.

Keyword: *Ruellia tuberosa*, Acanthaceae, HPTLC, Pharmacopeial.

Introduction

Ruellia tuberosa L., is belong to family Acanthaceae also known as minniroot, dragonroot or popping pod is a short-lived perennial herb with funnel-shaped striking violet bracteate flowers. Fruit is subcylindrical puberulent capsule having more or less 20 seeds per locule, thick fusiform tuberous roots in cluster. In traditional medicine, it has been used as anti-diabetic, anti-inflammatory, antinociceptive, antipyretic, analgesic, antihypertensive, antioxidant, insecticidal, anticancer, and toxic agent. The plant contains phytochemicals such as, phenolic compounds, alkaloids, steroids, terpenoids, tannins, glycosides, and flavonoid etc.

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In Siddha literature the plant is mentioned as Kiranthinayagam. It has germicide activity, indicated for skin diseases and eye diseases. The grinded leaves can be externally applied for herpes and other dermatological lesions, wounds. And also, decoction of root with cow milk is taken for curing bone fracture, leaves are chewed on snake bite as antidote. The present study aims at the pharmacognostic, and HPTLC fingerprinting studied of the leaf, stem and root of *R. tuberosa*.

Material and Methods

Collection and Identification of Plant

The plant was collected from various places of Western Vidarbha region and authenticated in Department of Botany using flora of Marathwada (Naik, 1998) and flora of Maharashtra (Singh and Karthikeyan, 2000). The collected plants after identification, was dried under shade for about 7- 10 days and then ground into fine powdered with the help of a blender.

Preparation of Extract

The fresh powder material of leaf, stem and root were used for extraction 10gm of coarsely powder of leaf, stem and root were extracted with 100ml of methanol in Soxhlet apparatus separately. Procedure was carried out for 10 cycles for each sample extract and temperature was adjust just below the melting point of solvent. The collected extract was used for HPTLC fingerprinting.

HPTLC Fingerprinting

The methanolic extract of leaf, stem, and root of *R. tuberosa* were subjected to HPTLC fingerprinting analysis. CAMAG HPTLC system equipped with Linomat 5 sample applicator TLC autosampler 4 with win CATS software, was the instrument employed. 10µl, 10µl and 20µl volume of each extract was applied on three tracks. Solvent system, Toluene: ethyl acetate: methanol: ammonia25% (30:30:15:1) in a twig through chamber was used for foe developing the plate (20 × 10 cm). The plate was developed up to 7 cm, removed from the chamber and allowed to dry and it was then scanned using CAMAG TLC Scanner and analysed with win CATS software version at λmax 254 nm using deuterium light source, at λmax 366 nm with mercury light source and the slit dimensions were 4.00 × 0.30 mm. After densitometric documentation, the plate was observed under 254 nm and 366 nm and TLC chromatograms were recorded. Then the plate was derivatized in vanillin-sulfuric acid reagent and dried at 105 °C on a hot plate till the bands appears. The plate was visualized under white light and scanned at 254 nm and 366 nm TLC chromatograms, Rf values and fingerprint data were recorded by win CATS software.

Result and Discussion

HPTLC analysis produces fingerprints which consist of sequence of zones that have specific Rf values, colours and intensity. In the present study, the various patterns of phytochemical constituents were identified based on the colour

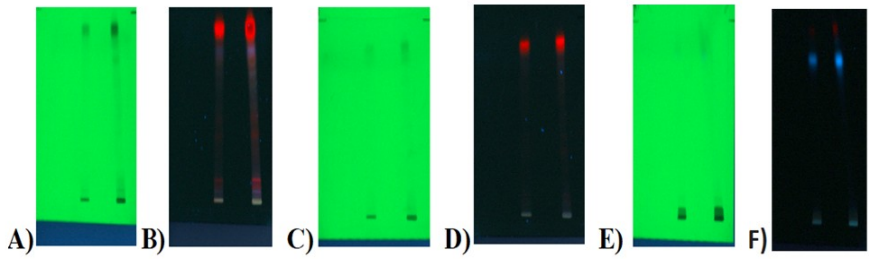

Fig. 1. HPTLC profile of Methanolic Extract of Leaf A &B, Stem C&D, and Root E&F of R. tuberosa viewed in UVshort and long wavelengths.

zones in the chromatogram and R_f values obtained during the HPTLC analysis under 254nm and 366nm wavelengths of light. HPTLC chromatogram of *R.tuberosa* leaf **A) &B)**, stem **C)&D)** and root **E)&F)** are shown in **Fig.1**.

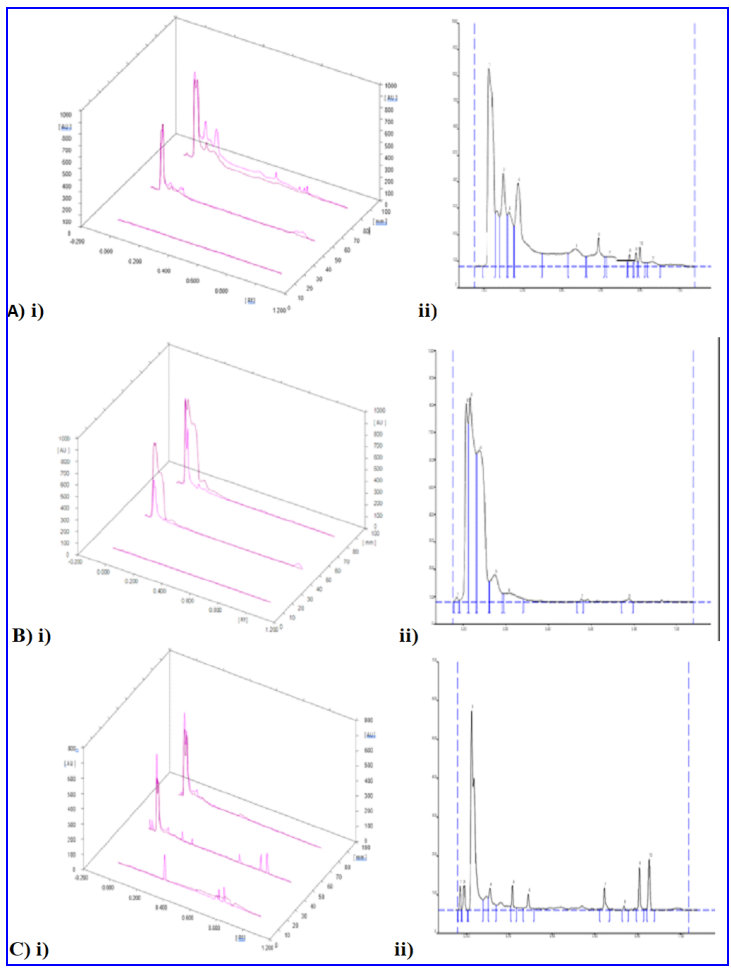

Figure 2: Showing densitogram of leaf, **A) i)** & **ii)**, stem **B) i)** & **ii)** And root **C) i)** & **ii)** of **R**. tuberosa.

HPTLC fingerprinting profile, Rf values and their corresponding densitograms of leaf **A) i & ii**, stem **B) i & ii** and root **C) i & ii** are given in **Fig.2**. The *HPTLC* fingerprinting results showed several peaks with different *Rf values*. Toluene: ethyl acetate: methanol: ammonia25% (30:30:15:1) was the suitable solvent system which resolved various bands on the chromatogram and it indicates various phytochemicals present in the plants which are given in **Table.1** below.

Table 1. Showing R_f values and Identified compounds at different peak of *R. tuberosa* leaf, stem and root

	Sr.No.	R_f values	Max Height	Area	Identified Compounds
Leaves					
	1	0.93	12.9	335.6	Caffeic acid (Flavonoids)
	2	0.46	13.1	262.6	Alkaloids
	3	0.80	15.9	475.1	Vitexin (Flavonoids)
	4	0.55	13.0	334.8	P-coumaric (Phenolics)
	5	1.00	42.6	1968.0	Luteolin (Flavonoids)
	6	0.47	67.4	3197.4	Alkaloids
	7	0.59	111.1	2966.8	Chlorogenic acid (Flavonoids)
	8	0.64	39.4	2123.4	Alkaloids
	9	0.75	47.6	466.3	Hydroxybenzoic (Phenolics)
	10	0.78	53.4	392.7	Vitexin (Flavonoids)
	11	0.86	19.4	593.0	γ - Resorcillic (Phenolics)
Stem	**1**	0.56	14.5	107.9	P-coumaric (Phenolics)
	2	0.78	14.2	222.4	Vitexin (Flavonoids)
Root	**1**	0.59	21.5	573.8	Chlorogenic acid (Flavonoids)
	2	0.77	43.	1210.4	Vitexin (Flavonoids)
	3	0.84	24.5	32.72	γ - Resorcillic (Phenolics)
	4	0.43	25.3	504.4	Alkaloids
	5	0.55	14.4	146.0	P-coumaric (Phenolics)
	6	0.70	29.3	299.8	Isovanillic (Phenolics)
	7	0.72	74.9	339.4	Isovanillic (Phenolics)
	8	0.76	100.2	502.0	Hydroxybenzoic (Phenolics)
	9	0.65	58.5	403.7	β - Resorcillic (Phenolics)
	10	0.74	11.2	58.6	Vanillic (Phenolics)
	11	0.86	131.7	819.5	γ - Resorcillic (Phenolics)

The HPTLC analysis of R. tuberosa leaf identified 11 compounds with different Rf values i.e., 0.93, 0.46, 0.80, 0.55, 1.0, 0.47,0.59, 0.64, 0.75, 0.78, 0.86 and two for stem i.e., 0.56 and 0.78 were identified and 11 for root these are 0.59, 0.77, 0.84, 0.43, 0.55, 0.70, 0.72, 0.76, 0.65, 0.74 and 0.86 which recognised 6 types of 12 phenolic compounds, 4 types of 8 flavonoids and 4 alkaloids.

Conclusion

The present study enhanced pharmacognostic characters of *R. tuberosa* leaf, stem and root. HPTLC fingerprint profile can be used as an important diagnostic method to identify the intensity / availability of the herbal drug, *R. tuberosa*. These data can serve as diagnostic tool for the authentication, and identification of medicinally important properties of leaf, stem and roots of *R. tuberosa*. Hence can be appraise as pharmacopeial standards and will help us to determine the legitimacy of the plant, *R. tuberosa* in future.

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