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Standardization of *Ruellia tuberosa* L. with special emphasis on trichome variations

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ABSTRACT

The present study attempts to standardize the pharmacognostic, physic-chemical parameters, UV-Vis and HPTLC fingerprinting of the plant, *Ruellia tuberosa*. Various trichome morphotypes were the unique observation seen in the plant by anatomical as well as powder microscopic studies. Four prominent peaks were detected by UV-Visible spectroscopy and HPTLC fingerprint revealed many peaks with a wide range of R_f values. The present study of botanical and chemical screening will be useful for developing pharmacopeial standards for *R. tuberosa*.

Keywords: *Ruellia*, Pharmacognostic, Trichomes, HPTLC, UV.

INTRODUCTION

Ruellia tuberosa L., (Acanthaceae) also known as minnieroot, is a short-lived perennial plant with funnel-shaped striking violet bracteate flowers on dichotomous cymes. 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 antidotal toxic agents [1, 2]. The plant is reported to contain phytochemicals such as Coumarin, phenolic compounds, Oleic acid, methyl esters, steroids, terpenoids, long-chain aliphatic compounds, and flavonoid etc. [3, 4]. 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.

Trichomes refer to outgrowths seen on the surfaces of leaves and other epidermal surfaces of plants as outgrowth from epidermis. They are either unicellular or multicellular, which act as a first line mode of defence mechanism in plants against pathogens [5]. The morphological and mechanical characteristics like density, size, shape, surface texture, hair orientation of trichomes can influence various biological and non-biological aspects such as temperature, mechanical abrasion, pollinator attraction, increase light reflectance, leaf wetness, allelopathy, decrease water loss through reflection, protection of phylloplane organisms and so on [6]. The present study aims at the pharmacognostic, UV-Vis spectroscopy and HPTLC studies of the whole plant of *R. tuberosa* with a special focus on trichome variations in the plant.

MATERIALS AND METHODS

Collection of plant materials

The plant material is collected from the SRRI campus, Poojappura, Thiruvananthapuram, Kerala and authenticated as *Ruellia tuberosa* by Dr. Ghanthi Kumar, Research Officer (Botany), SRRI, Thiruvananthapuram.

Anatomical studies

Hand sections of various parts of the plant material are taken and stained with saffranin and mounted in Glycerin under 10X as well as 40X objective of microscope.

Powder microscopy

The powdered tissues of whole plant of *R. tuberosa* was mounted in glycerin at room temperature for 24 h and observed under 10X and 40X objective of microscope for diagnostic powder features.

Extraction for HPTLC studies

1g of *R. tuberosa* was refluxed with 10 mL alcohol at a temperature of 60°C for 10 minutes to get

alcohol extract. The extracts were filtered and concentrated to desired volume.

Physico-chemical evaluation

Physico-chemical parameters like acid insoluble ash value, total ash value, alcohol soluble extractive value, water soluble extractive value, loss on drying at 105 °C and volatile oil content were determined as per standard protocol [7].

HPTLC fingerprinting

The alcohol extract of *R. tuberosa* and its powder ingredients were subjected to HPTLC fingerprinting analysis. CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4 with win CATS software version 1.4.4 was the instrument employed. 15 µL and 20 µL volumes of each extract was applied on two tracks. Solvent system, Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.1) in a twin trough chamber was used for developing the plate. The plate was developed up to 7 cm, removed from the chamber and allowed to dry and it was then scanned using TLC Scanner 3 and analyzed with win CATS software version 1.4.4. at λ_{max} 254 nm using deuterium light source, at λ_{max} 366 nm with mercury light source and the slit dimensions were 8.00 mm × 0.40 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 575 nm. TLC chromatograms, R_f values and fingerprint data were recorded by win CATS software.

UV-VIS Spectroscopy

UV-Vis Spectroscopic method is based on electronic absorption caused by the compounds present in the plant extract. The alcohol extract of the whole plant of *R. tuberosa* was subjected to the analysis and the extract was scanned at wave length ranging from 200 to 1100 nm using UV-VIS Spectrophotometer (Analytical Technologies Limited, Model: UV3120) and the characteristic peaks were detected and recorded.

RESULTS AND DISCUSSION

Anatomical studies

Stem

Single layered epidermis made of rectangular cells covered by a thick cuticle. Hypodermis is made of 4 layered collenchyma tissues followed by a wide cortex of parenchyma cells. Xylem forms a continuous ring that surrounds the pith. Phloem elements are seen in small isolated groups around the xylem vessels. Pith is large composed of thin walled polygonal parenchyma cells with intercellular spaces (Fig. 1).

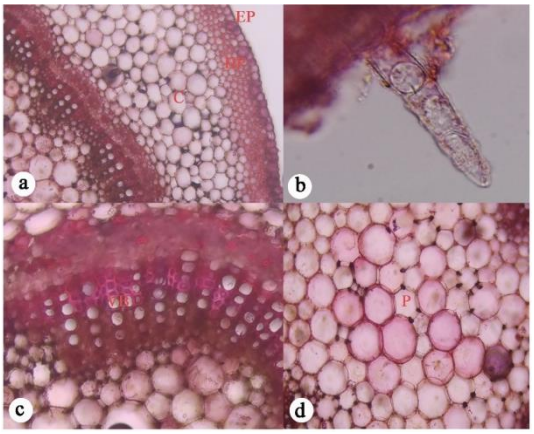

Figure 1: Cross section of stem of *R. tuberosa;* a: Half view, b: trichome, c: vascular bundle, d: pith. EP: epidermis, HP: hypodermis, C: cortex, VB: vascular bundle, P: pith.

Leaf

Dorsal surface of the midrib is having a depression on the centre and its ventral side is flat. 2-3 layered collenchyma tissues were seen below the upper epidermis and above the lower epidermal side. Arc shaped collateral vascular bundle were seen to be embedded in the parenchymatous cortex and two small vascular bundles on both sides (Fig. 2). Lamina is composed of mesophyll tissues which are clearly differentiated into palisade and spongy parenchyma.

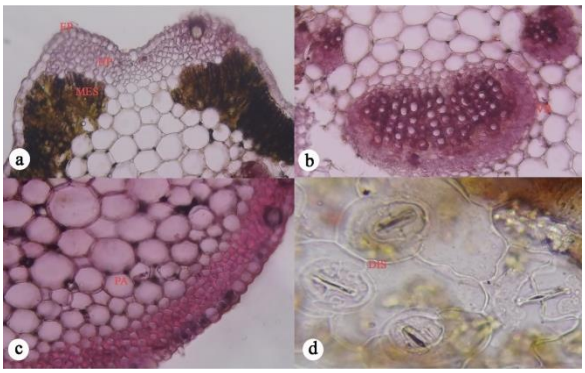

Figure 2: Cross section of leaf of *R. tuberosa*; a: a portion of midrib, b: vascular bundle, c: lower epidermis, d: diacytic stomata and subsidiary cells. EP: epidermis, HP: hypodermis, PA: parenchyma, VB: vascular bundle, DIS: diacytic stomata.

Root

Epidermis is made of thick walled rectangular cells bearing dense root hairs. Hypodermis is single layered and made of thin walled rectangular cells seen below the epidermis. Cortex is wide and homogenous made of thin walled parenchyma cells. Phloem occupies larger area and phloem elements were seen around the xylem vessels. Pith consists of large parenchyma with intercellular spaces (Fig. 3).

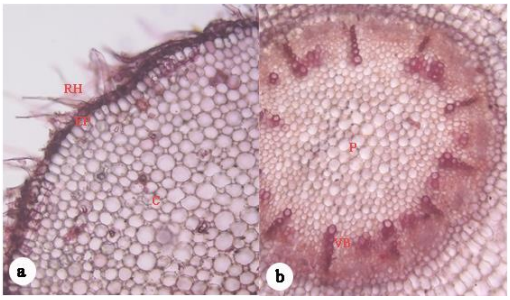

Figure 3: Cross section of root of *R. tuberosa*; a: a portion enlarged, b: vascular bundle ring and pith. RH: root hair, EP: epidermis, C: cortex, VB: vascular bundle, P: pith.

Pedicel

Single layered epidermis is made of rectangular cells which are covered by a thick cuticle. Hypodermis is seen beneath the epidermis in 3-4 layers of collenchyma cells. Cortex is made of thin walled parenchyma cells which are compactly arranged. Xylem and phloem forms a continuous ring surround the pith. Pith consists of large parenchyma with intercellular spaces (Fig. 4).

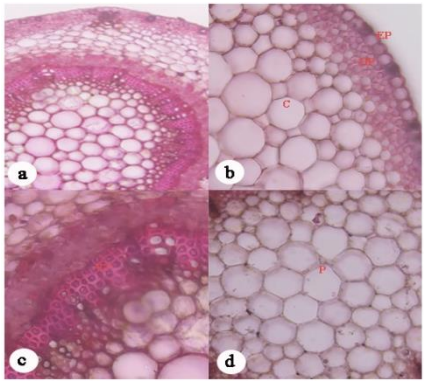

Figure 4: Cross section of pedicel of *R. tuberosa*; a: half view, b: epidermis and cortex, c: vascular ring, d: pith. EP: epidermis, HP: hypodermis, C: cortex, VB: vascular bundle, P: pith.

Powder microscopy

Powder is dark green in colour without any characteristic smell and taste, showed many characters like unicellular, uniseriate smooth trichomes, multicellular trichomes, stone cells, sclereids, xylem vessels with spiral thickening, diacytic stomata etc. (Fig. 5).

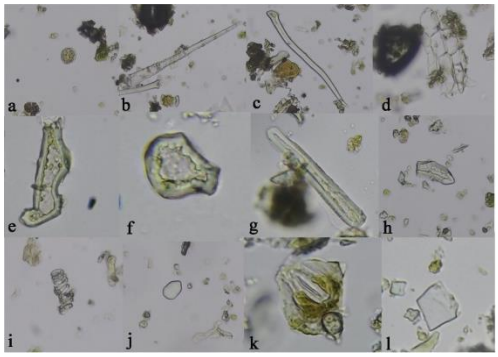

Figure 5: Powder microscopy of whole plant of *R. tuberosa*; a: pollen grain, b: multicellular unbranched trichome, c: smooth uniseriate trichome, d: fragment of parenchyma, e & f: stone cell, g: sclreid, h: stone cell, i: fragment of xylem vessel with spiral thickening, j: starch grain, k: diacytic stomata, l: prismatic calcium oxalate crystal.

Trichomes

To the best of our knowledge, this is the first ever report of different trichome morphotypes in *R. tuberosa* (Fig. 6). Non-glandular trichomes were found to be abundant compared to glandular types. Uniseriate multicellular trichomes were observed in *R.tuberosa* in more number. Uniseriate smooth unicellular trichomes were also observed as a characteristic feature in *R. tuberosa*. All morphotypes of non-glandular trichomes were unbranched in appearance and multicelluar trichomes consist of a stalk and a base. Non-glandular trichomes consists of a multicellular basal pedestal having approx. 10 epidermal cells, depending on the stage of differentiation. These raised cells acts as a support to the trichome and to provide a point of attachment that "anchors" the trichome to the epidermal surface [8]. In a previous study, trichome density in *R. nudiflora* were evaluated and one morphotype of glandular trichome i.e., peltate trichome were observed [9].

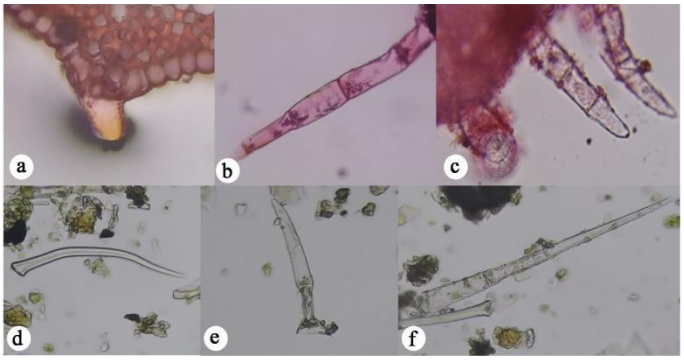

Figure 6: Different morphotypes of trichomes in *R. tuberosa*; a: glandular trichome initial, b: multicellular unbranched trichome, c: glandular and non-glandular trichomes, d: uniseriate smooth unicellular trichome, e: trichome with base and stalk cells, f: fragment of unbranched multicellular trichome.

Physicochemical evaluation

The physico-chemical constants such as extractive values (water soluble extractive and alcohol soluble extractive), loss on drying at 105 ˚C, acid insoluble ash and total ash content were evaluated and results were tabulated (Table 1).

Table 1: Physicochemical constants of *R.tuberosa*

Sl. No.	Physicochemical constants	Values (%)
1.	Alcohol soluble extractive	7.3
2.	Water soluble extractive	20.02
3.	Total ash value	15.78
4.	Acid insoluble ash	0.39
5.	Loss on drying at 105 °C	17.11

Loss on drying at 105°C indicates the amount of volatile substance and moisture present in the drug. The amount of siliceous matter (dust, sand etc.) present in that drug can be determined by acid insoluble ash value [10]. In this study, total ash value was found to be high (15.78%) and acid insoluble ash value was 0.39%. Extractive values represent the amount of phytoconstituents soluble in alcohol and water [11]. In the present study, water soluble extractive value is 20.02% and alcohol soluble extractive is 7.3%. It indicates the ability of water to extract the maximum components of *R. tuberosa* into it.

UV Visible spectroscopy

The UV-VIS spectrum of alcohol extract of the *R. tuberosa* is shown in Fig 7. The qualitative UV- VIS spectrum profile of the *R. tuberosa* extract was scanned at a wavelength range of 200 to 1100 nm. The profile showed sharp peak at 760 nm with a shoulder peak at 710 nm. Another peak shows two λ_{max} values around 480 nm and 420 nm with a shoulder peak 375 nm. One broad peak around 295 nm was also observed at short wavelength. This spectrum can be considered as unique for the alcohol extract of whole plant of *R. tuberosa*. The UV-VIS spectroscopy thus offers a simple, technique to identify the main phytochemicals, discriminating between the lipophilic and hydrophilic molecules based on polarity [12].

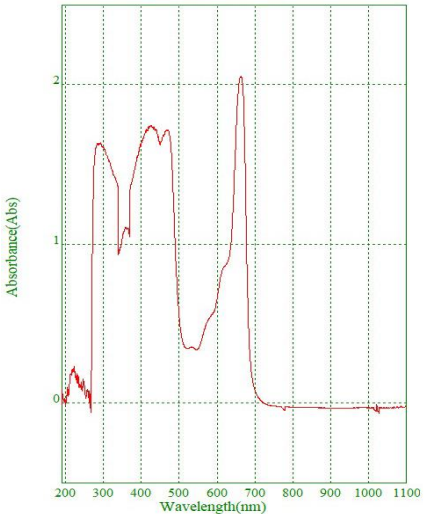

Figure 7: UV-VIS spectrum of alcohol extract of the *R. tuberosa*

HPTLC fingerprinting

HPTLC analysis produces fingerprints which consist of sequence of zones that have specific R_f values, colours and intensity. In the present study, the various patterns of phytochemical constituents were identified based on the colour zones in the chromatogram obtained during the HPTLC analysis under various wavelengths of light. HPTLC chromatogram of *R.tuberosa* is shown in Fig. 8. HPTLC fingerprinting profile, R_f values and their corresponding densitograms are given in Fig. 9. The *HPTLC fingerprinting* results showed several peaks with different *R_f values*. Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.1) was the suitable solvent system which resolved various bands on the chromatogram and it indicates various phytochemicals present in the plant.

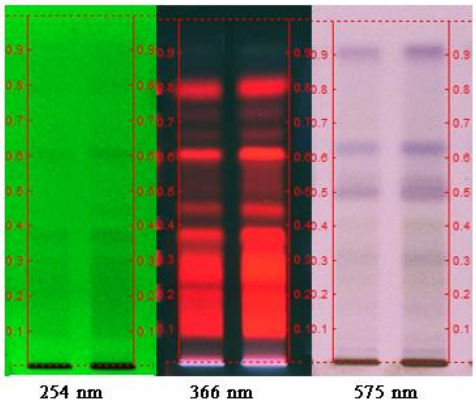

Figure 8: Chromatogram of alcohol extract of *R. tuberosa* viewed in UV short, long wavelength and after derivatization

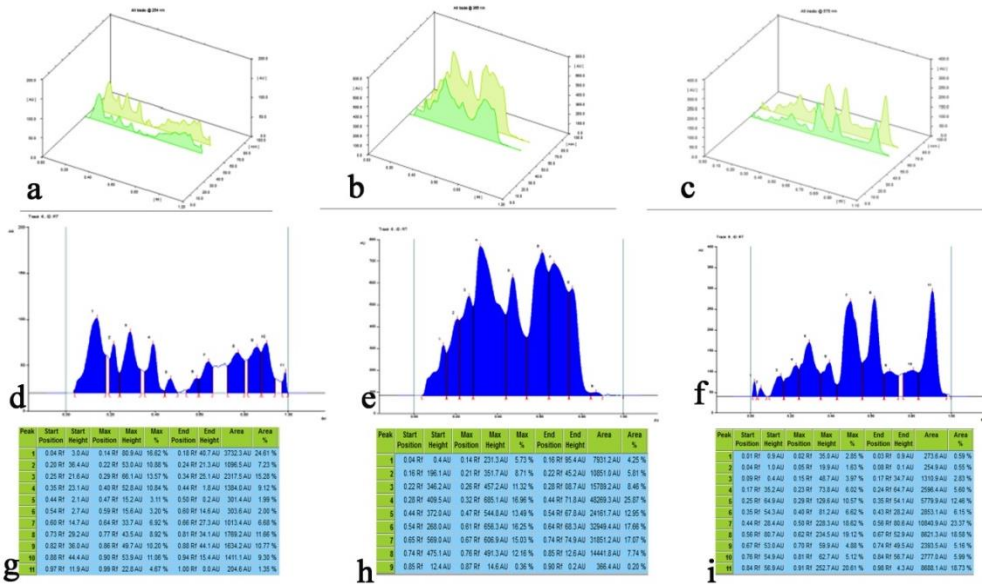

Figure 9: HPTLC finger print profile of 2-10 µL of alcohol extract of *R. tuberosa* at 254 nm(a, d, g), 366 nm (b, e, h) and 575 nm (c, f, i).

At 254 nm, R_f value of 0.38 and 0.6 showed a remarkable band of light green colour with maximum area percentage for the plant *R. tuberosa*. At 366 nm, maximum concentration of phytochemical constituents is observed at the R_f positions 0.32, 0.61 and 0.67 in the form of thick band. In a derivatized plate of 575 nm, maximum area percentage corresponds to the R_f value of 0.29, 0.50, 0.62 and 0.90 were seen. In an earlier study, HPTLC fingerprinting of *R. tuberosa* was done for various extracts of leaf, stem and root [13]. Intense band at the R_f value of 0.56 was observed at 366 nm and 575 nm and it was selected as a marker compound. In the present study also, prominent band at 0.5 R_f was observed in 366 nm and 575 nm. In addition to the prominent bands observed, the other colour bands also indicate the presence of various compounds which could attribute the bioactivity of the plant in a synergetic manner [14].

CONCLUSION

The macro- microscopical characters and HPTLC fingerprint profile combined with the physicochemical parameters can be used as an important diagnostic method to identify and to determine the quality and purity of the herbal drug, *R. tuberosa.* Hence, can be considered as pharmacopoeial standards and will help us to determine the genuiness of the plant, *R. tuberosa.*

Conflict of interest

No conflicts declared.

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