



IDENTIFICATION OF PHARMACOGNOSTIC AND PHYTOCHEMICAL BIOMARKERS TO DISTINGUISH BETWEEN *FUMARIA PARVIFLORA* LAM. AND ITS ADULTERANT, *RUNGIA REPENS* (L.) NEES.

Gohil Amit N* & Daniel M

Phytochemistry Laboratory, Department of Botany, Faculty of Science,
The Maharaja Sayajirao University of Baroda, Vadodra-390 002.

*Corresponding Author Email: gohilamit555@yahoo.com

ABSTRACT:

Fumaria parviflora Lam. is a well-known medicinal herb referred to as parpataka or parpat often adulterated with a small herb, *Rungia repens* (L.) Nees., commonly known as Parpataka of Gujarat. Micromorphological, pharmacognostic, phytochemical and HPTLC fingerprint differences between these two herbs were identified for the biomarkers aiding in correct identification of the former plant, as well as to detect adulteration. Different parameters were studied for the plant materials in fresh form, powder form and extract form. The study of stomata in fresh plants showed stomata to be of anomocytic in *F. parviflora* Lam. and diacytic in *R. repens*. Microscopic studies of the powders showed that the trichomes in *R. repens* were found absent in *F. parviflora* whereas the deposition of light brown contents in cells of cortical parenchyma and septate fibers were found specific to *F. parviflora* only. Phytochemical analysis of the plants indicated the presence of two flavonols in *F. parviflora* and three in *R. repens*. Phenolic acid content also varied in both plants. The HPTLC fingerprints of both the plants showed enough variation for identification of adulteration.

KEYWORDS:

Adulteration, *Fumaria parviflora*, HPTLC fingerprinting, pharmacognostic and phytochemical biomarkers, *Rungia repens*.

INTRODUCTION

Adulteration becomes a very serious problem with crude drugs, and often occurs when a drug is not easily available or when its price is comparatively high. Adulteration, in many cases, may not be intentional. In many cases it could occur due to mistaken identity of the plant. The adulterator chooses a suitable material that is cheap and readily available. Since flowers form the key tool for identification of a plant, in their absence, the vegetative parts are considered for identification purpose. Similar looking leaves can mislead a person and thus cause wrong identification of the plant. Collection of the wrong plant erroneously by unskilled collectors also is a major reason contributing to the adulteration of the plant of interest. The therapeutic efficiency of the drugs used in these systems depends greatly on the use of proper and genuine raw materials. Due to this reason, the assurance of safety, quality and

subsequent efficacy of the medicinal plants and herbal products has now become a major and key issue [1]. Checks on adulteration mainly includes biomarkers identified by micromorphological, anatomical and powder studies, though TLC or HPTLC fingerprinting also is an essential feature. Therefore, in the present study, *Fumaria parviflora* Lam. and its adulterant *Rungia repens* (L.) Nees. were subjected to micromorphological studies, powder characteristics, phytochemical analysis and variation in the HPTLC fingerprints to detect the biomarkers which distinguishes the genuine drug from the other.

In spite of wide use of 'Parpataka' in various ayurvedic formulations, there are lots of controversies regarding authentic source of 'Parpata' or 'Parpataka'. Many plants are used and sold under the same name in different parts of the country. Most of the authors and the Ayurvedic pharmacopoeia of India accepted

Fumaria parviflora Lam. of Fumariaceae as the source of drug but Some others equate it with *Rungia repens* Nees of the family Acanthaceae. This is the parpataka of Gujarat [2]. The important preparations available in the local markets of using drug are Arvindaasava ,Amrtaarishta, Chandanasava, Parpatadyarishtam, Mahaatikta Ghrita, Parpataka-Kashaaya , Jatyadi tailam.

Fumaria parviflora Lam. is a small, scandent, branched annual herb distributed throughout India, growing wild in plains and lower hills particularly on the banks of the Ganges and in the Himalayas up to an altitude of 2700 m. It is also found in Europe, Africa and many other Asian countries. Parpataka is an important Ayurvedic drug used in the Indian systems of medicine. The whole plant possesses medicinal properties [3,4]. The drug is diuretic, anthelmintic, digestive and relieves constipation. It is bitter, cooling and constrictor. It is used in the treatment of Rakta pitta (haemorrhage), Trishna (Thirst), Brama (giddiness) and Daaha (burning sensation) [5].

Rungia repens (L.) Nees. is a spreading decumbent herb found throughout India mostly as a weed in moist places [6,7]. The herb is used in the treatment of cough and fever and is also credited with vermifugal and diuretic properties [8]. Fresh, bruised leaves are mixed with castor oil and applied to scalp to cure *Tinea capitis* , a scaly fungoid infection, usually occurring amongst children [9, 10, 11, 12]. The juice of the leaves is considered cooling and aperients, and is given to children suffering from smallpox. Bruised leaves are applied to relieve pain and reduce swelling. In Bihar, the roots are used as a febrifuge by the tribal population [8,9].

The parameters that can aid in rapid identification such as micromorphological studies, powder characteristics, phytochemical analysis and variation in the HPTLC fingerprints were looked into during the present study. Micromorphology can be used to detect adulteration when the plant is obtained in fresh form. In case of dried

plant powder, differences in powder characteristics as well as HPTLC fingerprint profiles can be utilized to ascertain the purity of the given plant powder.

MATERIAL AND METHODS

Fumaria parviflora Lam. collected from Tarikhet, Uttaranchal, India. *Rungia repens* (L.) Nees. was collected from Vadodara, Gujarat, India, identified and authenticated at Department of Botany, M. S. University of Baroda. The plant materials were washed, shade dried for a day and then dried completely in an oven at 38°C. The plants were coarsely powdered using a rotary grinder and stored in airtight plastic containers, and then used for phytochemical analysis and HPTLC fingerprinting. Fresh leaves were used for micromorphological studies.

Micromorphology

Fresh leaves were washed and small fragments of leaves were taken from the middle region of the lamina of mature leaves. Washed leaf fragments were first boiled in 90% alcohol for about 3-5 minutes to remove chlorophyll, then washed 2-3 times with water, then boiled again with 10% KOH solution for 2-3 minutes and washed 4-5 times with water and kept in clean water to remove all traces of the clearing agent [13]. The epidermal layer was peeled off using the help of pointed needle and forceps. The epidermal peels were washed in water, stained with Safranin (0.5%) in water and then mounted in 50% glycerine; the margins of the cover slips were sealed with DPX [14] and the slides were observed under the microscope.

Powder studies

Completely dried plant material was finely powdered and sieved through BSS mesh No. 85. The fine powder obtained was stained using Safranin in water. The stained powder was mounted on a slide and observed under a microscope to locate and identify the characters present.

Phytochemical Analysis

Methanolic Soxhlet's extracts of the two plants were individually analyzed for the various classes of phytoconstituents such as flavonoids, phenolic acids,

quinones, alkaloids, steroids and tannins using standard phytochemical methods [15].

HPTLC analysis:

One gm of coarse powder material was extracted by refluxing in 5ml of methanol at 60°C in a water bath for

30 min. Extracts were filtered, concentrated and re-suspended in 1ml of methanol and used directly for HPTLC analysis (**Table-1**).

Table 1: Optimized chromatographic conditions for HPTLC analysis.

HPTLC Sample applicator	Linomat 5 (CAMAG)
Make of syringe	Hamilton
Capacity of syringe	100µL
Development chamber	CAMAG twin trough chamber (10x10cm)
Stationary phase	Precoated 60F ₂₅₄ silica plates (Merck)
Size of plate used	10 x10 cm
Sample applied	10 µl
Distance between tracks	12 mm
Band length	8 mm
Solvent front	90 mm
Mobile phase	Toluene :Formic acid : Ethyl formate (4 : 1 : 5) (v/v/v)
Mode of visualization	Short wave UV light (UV 254nm) Long wave UV light (UV 366nm)
HPTLC scanner	TLC Scanner 3 (CAMAG)
Radiation source	Deuterium lamp
Software	WinCats

RESULTS AND DISCUSSION

The characters observed in the micromorphology and in the powders of the two plants are shown in **Figures 1 and 2**. Phytochemicals obtained from both the plants are

compared. Similarly the HPTLC chromatograms of methanolic extracts of *F. parviflora* and *R. repens* prepared under similar conditions are presented in **Figures 3 and 4**.

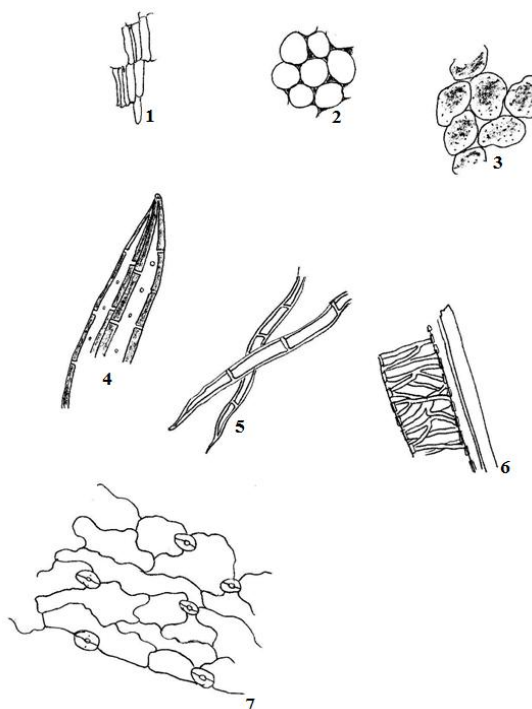


Figure1: Characters observed in powder of *Fumaria parviflora* : 1.Cork, 2.Collenchyma, 3.Parenchyma with light brown deposits, 4.Sclerenchyma, 5. septate fibers, 6.Reticulate vessel, 7.Epidermal fragments with stomata.

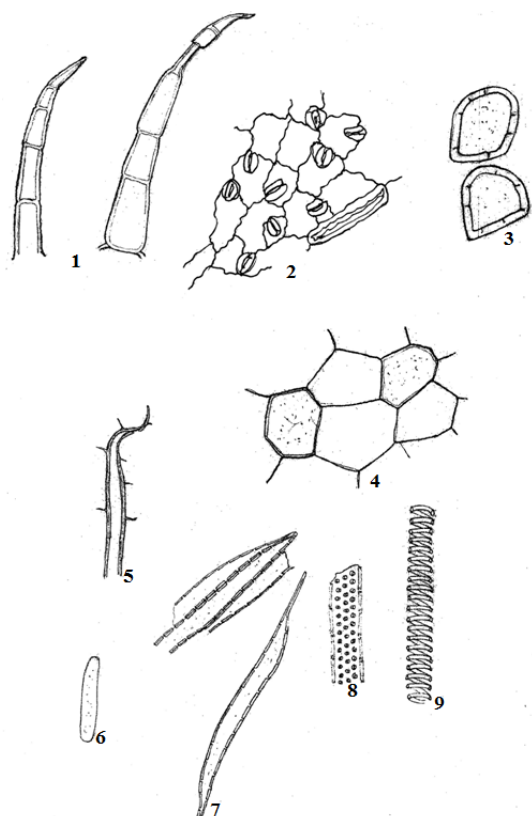
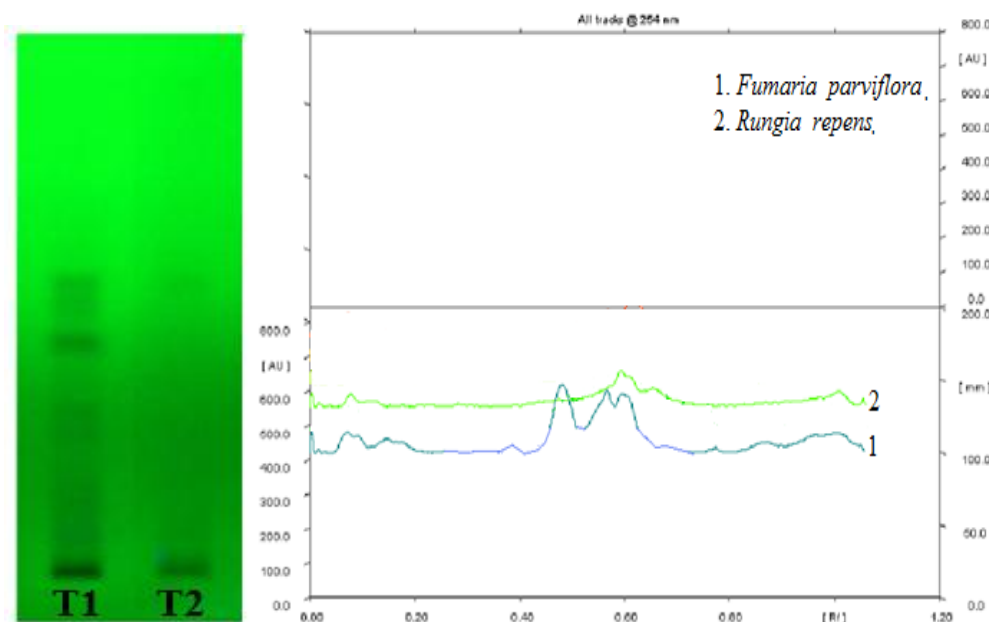


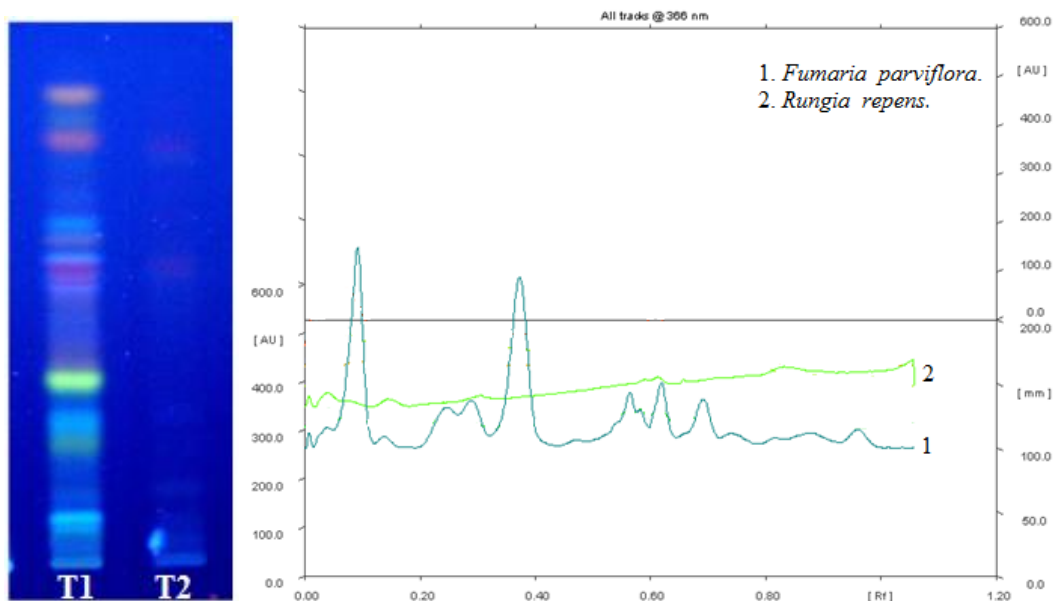
Figure 2: Characters observed in powder of *Rungia repens* : 1.Trichomes, 2. Epidermal fragment with stomata, 3.Sclereids, 4.Cortical parenchyma, 5. Broad lumened fiber,6. Ray parenchyma, 7.Fiber tracheids, 8. Boarded pitted vessel, 9. Spiral vessel.

Figure 3: HPTLC chromatogram of *Fumaria parviflora* and *Rungia repens* (UV 254 nm).



(T1- *Fumaria parviflora*, T2- *Rungia repens*)

Figure 4: HPTLC chromatogram of *Fumaria parviflora* and *Rungia repens* (UV 366 nm).



(T1- *Fumaria parviflora*, T2- *Rungia repens*)

PHARMACOGNOSY

Micromorphology

Fumaria parviflora clearly exhibited the presence of anomocytic stomata, while *Rungia repens* possessed diacytic stomata. Both these stomatal types are very different from each other and can thus help in differentiating between the two plant species.

Powder study

The components present in the powder of *F. parviflora* were cork, fragments of collenchyma, parenchyma with light brown deposits, sclerenchyma, septate fibers, epidermal fragment with stomata and reticulate vessels. Whereas the components present in the powder of *R.*

repens were thick-walled unicellular as well as multicellular, uniseriate trichomes having broad basal cell and warty walls with blunt tip, sclereids, fragments of collenchyma, thin walled cortical parenchyma, spongy parenchyma, broad lumen fibers, ray parenchyma, fiber tracheids, boarded pitted and spiral vessels. The diagnostic characters such as parenchyma with light brown deposits and reticulate vessels of *F. parviflora* were found to be absent in *R. repens*. The whole plant powder of *R. repens* showed the presence of a large number of trichomes which were found absent in case of *F. parviflora*. Another very interesting character observed in the *F. parviflora* powder was the presence of septate fibers, none of which were observed in case of *R. repens*. These characters can help in differentiating between the powders of the two plants.

PHYTOCHEMICAL ANALYSIS

Analysis of the methanolic extracts of *Fumaria parviflora* Lam. and *Rungia repens* (L.) Nees. revealed many chemical differences which could help in differentiating between the extracts of the two plants. Flavonol quercetin was found common in both plants while 3'-OMe quercetin found present only in *F. parviflora* and 7'-Me quercetin, kaempferol and its 4'-OMe derivative in *R. repens*. Vanillic and ferulic (*cis*- and *trans*- isomers) acids were the phenolic acids found common in both plants. However, syringic, gentisic, protocatechuic, *p*-coumaric and *p*-hydroxybenzoic acids were found present only in *R. repens*. However, qualitative tests showed the presence of alkaloids and steroids in both the plants.

H.P.T.L.C. FINGERPRINTING

Though the methods of extraction and chromatographic conditions for both plants were kept identical, the HPTLC chromatograms obtained when the HPTLC plate was scanned at 254 nm and 366 nm showed immense variations.

Fumaria parviflora exhibited the presence of 13 peaks when observed at 254 nm (Figure-3). There were 3

major peaks found at R_f 0.48(20.21 %), R_f 0.56 (18.55%) and R_f 0.59(17.62%) with high concentration while other peaks at R_f 0.07(3.61 %), R_f 0.09(2.42 %), R_f 0.15(5.33 %), R_f 0.29(3.67 %), R_f 0.34(8.73 %), R_f 0.66(3.53 %), R_f 0.77(0.80 %), R_f 0.87(3.60 %), R_f 0.96(4.81 %) and R_f 1.00 (7.22%) were of low concentrations whereas under UV 366 nm (Figure-5) it showed the presence of 15 peaks. There were 3 major peaks found at R_f 0.09(22.12%), R_f 0.37(24.81%) and R_f 0.56(10.41%) having good concentration while peaks at R_f 0.01(0.27%), R_f 0.04(1.89%), R_f 0.14(1.01%), R_f 0.25(6.84%), R_f 0.29(7.01%), R_f 0.47(1.07%), R_f 0.62(6.74%), R_f 0.69(7.19%), R_f 0.74(2.55%), R_f 0.82(1.48%), R_f 0.88(3.95%) and R_f 0.96(2.57%) were found with low concentrations. On the other hand, the extract of *Rungia repens* exhibited the presence of 08 peaks when observed at 254 nm (Figure-3). The major peak was found at R_f 0.59 with the highest concentration 48.54 %. Other peaks at R_f 0.65 (17.80%) and R_f 1.01(12.54%) were of moderate concentrations and the peaks at R_f 0.97(6.92%), R_f 0.08(6.17%), R_f 0.12(3.64%), R_f 0.45(3.64%), and at R_f 0.02(0.60%) were with low concentrations while there were 09 peaks observed at 366 nm (Figure-4) of which the peaks with higher concentration were seen at R_f 0.83(30.22%) and R_f 0.59(28.60%), the peak at R_f 0.78(18.10 %) was of moderate concentrations while other peaks at R_f 0.61(8.38%), R_f 0.30(6.73%), R_f 0.04(3.73%), R_f 0.14(2.15%), R_f 0.08(1.44%) and R_f 0.01(0.64%) were with low concentrations.

Under UV 254 nm the intense peak at R_f 0.59 and under UV 366 the peaks at R_f 0.01, R_f 0.04 and R_f 0.14 are common to both the plants. Since the chromatographic conditions were common for both the plant extracts, the peak should correspond to that of same compound in the plants. However, other intense peaks under UV 254 nm at R_f 0.48 and R_f 0.56 in case of *Fumaria parviflora* extract were absent in case of *Rungia repens*. These peaks could be taken as an important identification factor to differentiate between the two plants. Thus both the chromatograms showed enough variation to differentiate

between the two plant extracts. However, the HPTLC fingerprint for *Rungia repens* needs to develop separately as a quality control parameter for the plant. The chromatogram displayed for the plant in **Figure 4** and 5 is only for the purpose of comparison with *Fumaria parviflora*, using the chromatographic conditions of the latter.

CONCLUSION

A large number of differences were observed between *Fumaria parviflora* and its adulterant *Rungia repens*. Out of 13 peaks seen in *Fumaria parviflora*, only one was common in *Rungia repens* at 254nm i.e. similarity is only 7.7% and at 366 nm 03 peaks were common i.e. similarity is 20% only. The plant *Fumaria parviflora* contained anomocytic stomata, parenchyma with light brown deposits, septate fibers, reticulate vessels and 3'-OMe quercetin. It also showed absence of trichomes. *Rungia repens* showed the presence of diacytic stomata, trichomes, 7'-Me quercetin, kaempferol and its 4'-OMe derivative. These differences can play a key role in proper regulation of collection and authentication of both the plant species, as well as detect adulteration. Irrespective of whether the plant is provided in the form of fresh material, powder or extract, diagnostic characters for each case have been identified for both plants.

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*Corresponding author address:

Email address:

gohilamit555@yahoo.com