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Research Article

# HPTLC FINGERPRINT PROFILE OF RUMEX VESICARIUS L.

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**ABSTRACT**

A simple high performance thin layer chromatographic fingerprint method was used for the simultaneous determination of active phytoconstituents present in n-Hexane and aqueous extracts of *Rumex vesicarius* L. (Polygonaceae). *Rumex vesicarius* L. is a well known edible green used in daily diet and cultivated in many parts of India. The plant is well known for its medicinal and curative purposes. Analytical separation technique HPTLC fingerprint is the most poplar method used for quality control of raw material and finished herbal product. The fingerprint analysis of n-Hexane extract showed eight peaks in 5µl and 10 peaks in 10 µl of the sample. The aqueous extract showed 12 peaks in 5 µl and 13 peaks in 10 µl of the sample analysed.

**Key words**: *Rumex vesicarius* L., HPTLC finger printing, n-Hexane extract, aqueous extract.

## INTRODUCTION

India has a cultural rich heritage of traditional medicine comprised of two widely flourishing systems of Ayuvedic and Unani systems, since ancient times. The multiple therapeutic actions and uses of crude herbal drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books and pharmacopoeias[1]. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards[2]. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters, hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations[3,4]. HPTLC fingerprinting technique offers better resolution and estimation of active constituents which can be done with reasonable accuracy in a shorter time[5].

Analytical separation techniques, like High performance liquid Chromatography (HPLC), Gas Chromatography (GC) and Mass Spectrometry (MS) were among the most popular methods of choice used for quality control of raw material and finished herbal product. Finger print analysis approach using high-performance thin-layer chromatography (HPTLC) has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drugs[6].

*Rumex vesicarius* L. belongs to the family Polygonaceae. It grows in several parts of India and cultivated as edible green leafy vegetable (GLV). It occupies a special nutritional and medicinal significance, the plant leaves are used raw as a salad vegetable by Bedowins is well known. The plant leaves is added during the preparation of iqt (dried milk shards) to increase its acidity[7]. The plant is used medicinally as a laxative or tonic, the plant juice is used to diminish toothache, checks nausea, promotes appetite and improves digestion[8]. The plant juice is also used as cooling, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic, and anit bacterial agents. The medicinal importance of this plant is a reflection to its chemical composition since the plant contains may bioactive substance[9].

The present study was aimed to perform HPTLC fingerprint analysis of n-Hexane and aqueous extract of *Rumex vesicarius* L. the data obtained in the present work could be useful in proper identification and authentication of this plant which is a primary pre requisite for standardization and recognition of herbal medicines.

### Materials and Methods

Plant Material-The fresh plant material (*Rumex vesicarius* L.) were collected from the plains of Tiruvannamalai, Tiruvannamalai district, Tamilnadu, South India. The collected specimens were well preserved, botanically identified and authenticated by Dr.G.V.S.Murthy, Scientist "F", BSI. South regional centre, Coimbatore, India. The voucher specimen was deposited at Botany Department, Government Arts College (Autonomous) Kumbakonam, Tamilnadu, South India. The collected plant materials were shade dried and powdered. The powder was well preserved in air tight container for further use.

Preparation of Plant material Extract - About 50g of the shade dried powder was macerated with 100ml of respective solvents (n-Hexane and water) in a closed flask for twenty four hours with frequent shaking at every six hours. The extract was filtered and the filtrate was used for further analysis.

### HPTLC profile (High performance Thin layer Chromatography)

HPTLC finger print profile studies of n-Hexane extract and aqueous extract were carried out separately, following standard methodology of Harborne[10] and Wagner et al[11].

Sample preparation- The n-Hexane extract and aqueous extracts were dissolved separately with HPTLC grade ethanol, which was used for sample application on pre-coated silica gel GF254 aluminium sheets.

Developing solvent system- A number of solvents were tried, but satisfactory resolution was obtained in the developing solvent Toluene: Ethyl acetate: n-Hexane (2:1:2) for n-Hexane extract and developing solvent with Chloroform:Methanol:Water (7.5:2:0.5) for aqueous extract.

Sample application- The samples (5µl at 10 µl) were spotted in the form of bands of width 6mm with a 100 microlitre sample using a Hamilton syringe on silica gel which was precoated aluminum plate GF-254 plates (20cm x 10cm ) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of chromatogram- The mobile phase consisted of Toluene-Ethyl acetate-n-Hexane (2:1:2) for n-Hexane extract and the mobile phase of aqueous extract consisted of Chloroform-Methanol-Water (7.5:2:0.5) and 15ml of mobile phase was used per chromatography run. The linear ascending development was carried out in a 20cm x 10cm twin through glass chamber saturated with the mobile phase.

Detection of spots- The developed chromatogram was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with anisaldehyde sulphuric acid reagent as spray reagent and dried at $100^0$c in hot air oven for 3 min. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured under UV light at 254nm and 366nm. The Rf

values and finger print data was recorded by WIN CATS software. The scanning wavelength of n-Hexane extract chromatograph was scanned at 410nm and the aqueous extract chromatograph was scanned at 262 nm respectively.

Peak development of different extracts- Two separate concentration of 5µl and 10µl of each extract was performed separately, and separate track was maintained for each concentration with separate peak development was performed for each extract with two concentrations separately.

**Results and Discussion**

The HPTLC fingerprint study of n-Hexane extract of *Rumex vesicarius* L. showed 8 peaks at 5µl of the sample and 10 peaks at 10 µl of the sample (Fig. 1a, Fig.1b). The aqueous extract of *Rumex vesicarius* L. showed 12 peaks in 5µl and 13 peak in 10µl of the sample respectively (Fig. 2a, Fig. 2b) peak at Rf value 0.1 is omitted. The photo documentation of n-Hexane extract observed 366nm and 254nm is given (Fig.3) and the photo documentation of aqueous extract is also given (Fig.4). The Rf values of the spots and purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band. The Rf value of n-Hexane extract was given in Table 1and 2, and the aqueous extract is given in Table 3 and 4.

HPTLC finger printing is an valuable quality assessment tool for the evaluation of botanical materials, it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods as the spots are well resolved. The HPTLC method is simple, rapid, accurate, reproducible, selective and economic, can be used for quality control analysis[12] and for quantitative determination of the plant material

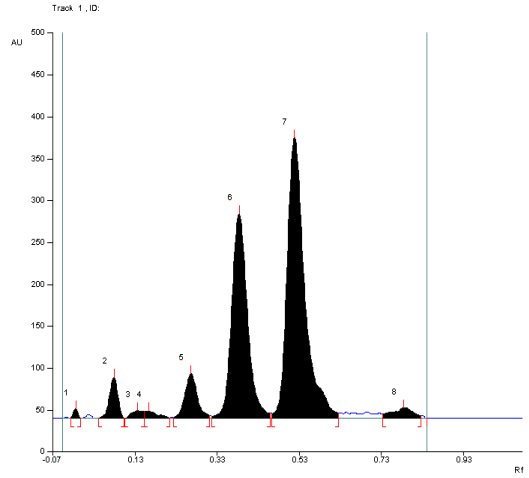


**Fig 1a: HPTLC chromatogram of n-Hexane extract (5µl)**

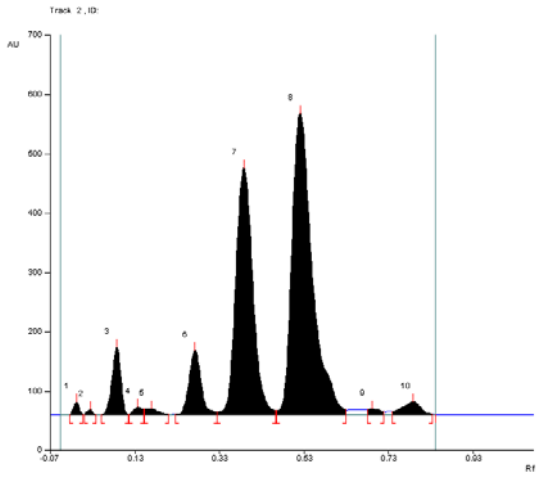


**Fig 1b: HPTLC chromatogram of n-Hexane extract (10µl)**

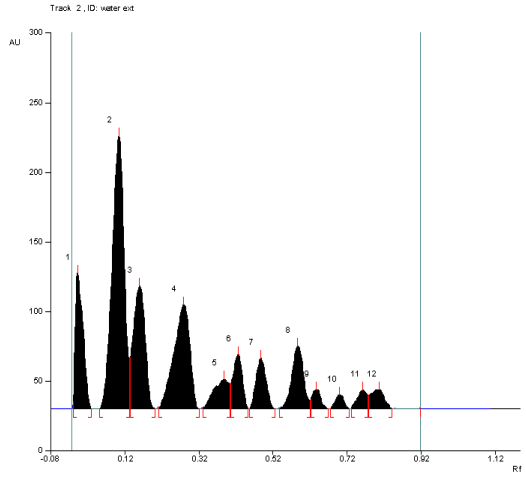


**Fig 2a: HPTLC chromatogram of aqueous extract (5µl)**

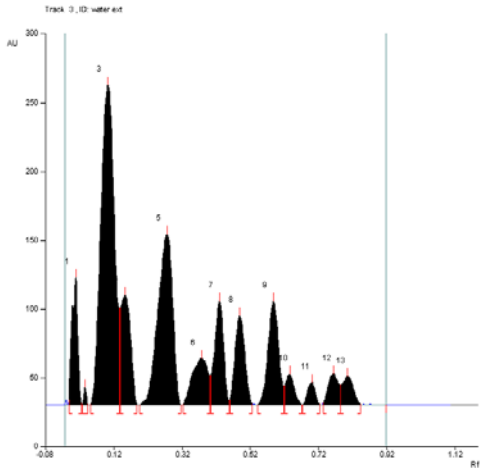


**Fig 2b: HPTLC chromatogram of aqueous extract (10µl)**

**Fig 3: Photo Documentation of n-Hexane solvent extract of *Rumex vesicarius* L.at 366nm and 254nm**

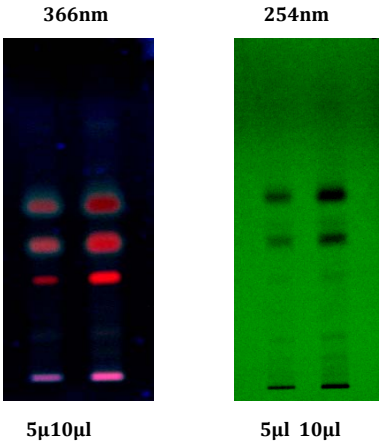


**Fig 4: Photo Documentation of Water solvent extract of *Rumex vesicarius* L. at 366nm and 254nm.**

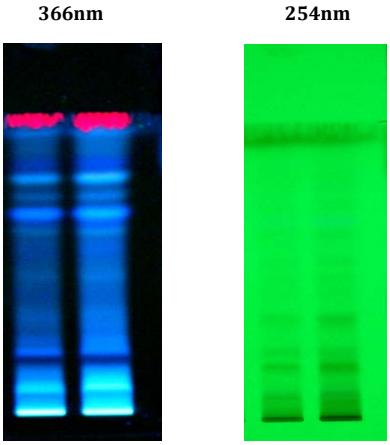


**Table 1: Rf values of the peak formed of *Rumex vesicarius* L. n-Hexane extract 5µl.**

| Peak | Rf | Height | Area |
|---|---|---|---|
| 1 | 0.1 | 0.2 | 813.1 |
| 2 | 0.15 | 7.9 | 203.9 |
| 3 | 0.21 | 0.3 | 215 |
| 4 | 0.31 | 3.4 | 1303 |
| 5 | 0.46 | 5.7 | 7583.6 |
| 6 | 0.62 | 6 | 11627.8 |
| 7 | 0.83 | 3 | 511.4 |

**Table 2: Rf values of the peak formed of *Rumex vesicarius* L. n-Hexane extract 10µl.**

| Peak | Rf | Height | Area |
|---|---|---|---|
| 1 | 0.04 | 0.1 | 90.6 |
| 2 | 0.11 | 0.5 | 1910.6 |
| 3 | 0.15 | 9.4 | 209.8 |
| 4 | 0.21 | 0.7 | 265.8 |
| 5 | 0.32 | 3.9 | 2498.6 |
| 6 | 0.46 | 6.7 | 13513.6 |
| 7 | 0.63 | 8.1 | 19848.9 |
| 8 | 0.72 | 5.3 | 233.5 |
| 9 | 0.83 | 1.7 | 778.2 |

**Table 3: Rf values of the peak formed of *Rumex vesicarius* L. aqueous extract 5µl**

| Peak | Rf | Height | Area |
|---|---|---|---|
| 1 | 0.13 | 35.8 | 4614.7 |
| 2 | 0.2 | 0.1 | 2050 |
| 3 | 0.32 | 0.1 | 2360.4 |
| 4 | 0.4 | 18.2 | 640.1 |
| 5 | 0.45 | 0.1 | 708 |
| 6 | 0.53 | 0 | 723.5 |
| 7 | 0.62 | 6.3 | 1037.6 |
| 8 | 0.67 | 0.1 | 237.2 |
| 9 | 0.73 | 0.1 | 173.7 |
| 10 | 0.78 | 9.8 | 258.8 |
| 11 | 0.84 | 0.2 | 365.7 |

**Table 4: Rf values of the peak formed of *Rumex vesicarius* L. aqueous extract 10µl.**

| Peak | Rf | Height | Area |
|---|---|---|---|
| 1 | 0.14 | 70 | 6748.5 |
| 2 | 0.19 | 0.1 | 1650 |
| 3 | 0.32 | 0.2 | 3912.2 |
| 4 | 0.4 | 21.3 | 1145.8 |
| 5 | 0.46 | 3.4 | 1440.5 |
| 6 | 0.53 | 0.9 | 1368.9 |
| 7 | 0.62 | 13.9 | 1706.3 |
| 8 | 0.67 | 0.1 | 413.7 |
| 9 | 0.72 | 0.9 | 270 |
| 10 | 0.78 | 14.6 | 497.6 |
| 11 | 0.84 | 0.4 | 499.7 |

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