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Original Article

A Complete Pharmacognostical Profile of *Rungia repens*

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ABSTRACT

Background: *Rungia repens* is one of the plants cited as 'Parpata' in Ayurvedic literature and indicated as febrifuge, antitussive and vermifuge. **Aim:** To generate and ensemble data of physical parameters for ascertaining the identification and develop validated HPTLC method for quantification of kaempferol in R. repens. **Materials and Methods:** *R. repens* was studied for establishing pharmacognostic standards including macro and microscopical characters, physico-chemical analysis and quantification of kaempferol by HPTLC method. **Results:** It is a small, much branched, prostrate or sub-erect herb with lanceolate leaf and violet flowers. Microscopically root can be characterized by collenchymatous phloem associated with wide lignified xylem; stem by epidermis with simple and glandular trichomes and collenchymatous hypodermis; and leaf by epidermis embedded with cystoliths and bearing covering and glandular trichomes. Powdered drug can be typified by cystoliths, trichomes of aforementioned type, pollen grains and fragments of cork. Further studies revealed that brunt of heavy metal and microbial load in plant material was within permissible limits. Flavonoids and phenolics were found be major components. HPTLC method was developed for quantification of kaempferol using precoated silica gel plates as a stationary phase, and toluene: ethyl acetate: dichloromethane: formic acid: methyl ethyl ketone (5: 1: 1.5: 0.5: 0.8) as a mobile phase and scanning the plate at 254 nm. **Conclusion:** The distinctive quality profile data and validated HPTLC method tailored for *Rungia repens* using kaempferol as a marker, would aid as expedient measures for its evaluation.

Key words: Ghati pitpapada, HPTLC, Kaempferol, Pharmacognostic study, *Rungia repens*.

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INTRODUCTION

Rungia repens Nees. (Acanthaceae), commonly known as *ghati pitpapada* or *khadsalio pitpapado* or parpata in Gujarat, is distributed on the sea coast of Saurashtra.[1] It is a spreading, decumbent weed, found throughout warmer parts of India in moist places and cultivated fields during winter.[2,3] The plant is categorically valued in traditional medicine in the treatment of fever, cough, and worms. The fresh leaves are bruished, mixed with castor oil, and applied to the scalp in cases of *tinea capitis*, a scaly fungoid infection.[3,4] In literature the plant is recorded to possess anti-inflammatory, diuretic and antimicrobial activities.[5] Also, it has been documented to contain flavonoids notably, chrysoeriol 4'-mono and 7,4'-diglucosides, luteolin 7-glucoside, apigenin 7-glucoside, kaempferol 3-O-α-L-rhamnopyranosyl (1 3) β-D-glucopyrano-side, delphinidin 3,5-diglucoside and lutein.[6,7] Kaempferol, a flavonol, is reported to be having manifold biological actions markedly antioxidant, anti-inflammatory, cardiotonic, antimicrobial, neuroprotective, antitumour etc.[8-16] In the present study, we here propose data for development of a comprehensive monograph, a requisite for quality assessment. Further, the HPTLC method for estimation of kaempferol has been validated for linearity, interday & intraday precision, repeatability, accuracy, specificity, limit of detection and limit of quantification.

MATERIALS AND METHODS

Plant Material

Fresh, fully-grown, flowering plants of *R. repens* were collected from Nadiad, Gujarat in the month of October 2010. The plants collected were authenticated by taxonomist of Gujarat University, Ahmedabad, Gujarat. Voucher specimen sample (LM 630) was deposited at the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat. The plant material was cleaned, dried, powdered to 60 # and used for the present study.

Chemicals and Reagents

Standards kaempferol was procured from Sigma Aldrich, India. All the solvents used were of chromatography grade and other chemicals used were of analytical (AR) grade.

Pharmacognostical Studies

The whole plant was studied for morphological characters. Microscopical study was performed for different plant parts (free hand transverse sections) and powdered material of entire plant. Quantitative microscopy was carried out for leaf.

Moisture content,[17] ash values and extractive values were determined.[18]

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Heavy Metal Analysis

Heavy metals analysis for lead (Pb), mercury (Hg) and arsenic (As) was carried out for powdered herb. Their concentrations were determined by Inductively Coupled Plasma Optical Emission Spectrometry (Perkin Elmer-Optima 3300 RL).

Determination of Microbiological Parameters

The microbiological parameters like total plate count, yeast and mould count, *E. coli, Staphylococcus aureus* and Salmonella spp. were investigated in powdered herb. Results obtained were compared with limits for each microorganism.

Phytochemical Studies

Phytochemical screening was performed,[19] and flavonoids and phenolics[20] were estimated.

Extraction and TLC Study

5 g drug powder was exhaustively extracted with 100 ml methanol, filtered and dried. The methanolic extract was hydrolysed by refluxing with 70 ml 1:1 mixture of toluene: 2N HCL for 2 h. After neutralizing with sodium carbonate, it was extracted with ethyl acetate (3X 25 ml) and vacuum dried to yield 12.9% w/w of extract (Ext A).[21]

Estimation of Kaempferol by HPTLC method

Chromatographic conditions

HPTLC was performed on 10×10 cm precoated silica gel 60 F_{254} plates (E. Merck, Germany). Before chromatography the plates were pre-washed by methanol and activated at 60°C for 5 min. Samples were applied to the plates as bands 6 mm wide and 12.2 mm apart using Camag Linomat V applicator (Muttenz, Switzerland) fitted with a 100 microlitre syringe (Camag, Switzerland). Linear ascending development was performed in Camag twin-trough glass chamber (10×10 cm) with mobile phase vapour [toluene: ethyl acetate: dichloromethane: formic acid: methyl ethyl ketone, 5: 1: 1.5: 0.5: 0.8] at room temperature (25 ± 2°C). The plate was dried and scanned in Camag TLC scanner using WinCATS software (version 1.4.3.6336) in absorption mode at 254 nm with slit dimensions 6.00×0.45 mm. The scanning speed was 20 mm/sec and source of radiation deuterium lamp.

The method was validated in terms of linearity, interday precision, intraday precision, repeatability, accuracy, specificity, limit of detection and limit of quantification. International Conference on Harmonization (ICH) guideline was employed for validation of analytical method.[22]

Calibration curve

A stock solution (400 µg ml^{-1}) of kaempferol was prepared by dissolving accurately weighed 4 mg in 10 ml methanol in a volumetric flask. Standard solutions for calibration were prepared by dilution of the stock solution with methanol; the concentrations were such that amounts of kaempferol between 400-2000 ng. The correlation coefficient, slope intercepts and regression equation were also calculated to provide mathematical estimate degree of linearity. A calibration curve was derived by plotting peak area (Y axis) versus concentration (X axis).

Quantification of kaempferol in extract

10 mg of Ext A was dissolved in 1 ml methanol in a volumetric flask. 5 µl of this solution was used for estimation of kaempferol. The peak area values of standards and sample were used to calculate the amount of kaempferol in the plant.

RESULTS AND DISCUSSION

Rungia repens is an annual, diffuse or sub-erect, branched, slender, 15-30 cm tall herb. Morphologically it can be characterized by its simple, opposite, sub-sessile, lanceolate leaf, with ciliate margin; slender, angular and glabrous stems, that often show rooting at the lower stem nodes; tortuous and branched roots and violet terminal spikes (Figure 1).

Microscopical Characters

Microscopical examination (TS) revealed a suberized cork (ck) made up of 2-4 layers of tangentially elongated cells; narrow thin walled parenchymatous cortex (ct) embedded with sclerenchymatous cells and lacuna; collenchymatous phloem (ph); lignified radially arranged xylem (xy) consisting of vessels and thick walled fibres (Figure 2) as diagnostic features of root. Further, a layer of tangentially elongated, thick walled epidermis (e) bearing multi-cellular simple and sessile glandular trichomes (t); collenchymatous hypodermis (hyp); parenchymatous cortex (ct) filled with chlorophyll; stele constituted of a layer of endodermis (en), narrow band of phloem (ph) and central xylem (xy) around thick walled, beaded parenchymatous pith (pi) (Figure 3) were typical structural components of stem. Moreover the presence of cystoliths (cys) along with short conical unicellular, long straight or bent multicellular simple covering trichomes (t) and sessile glandular trichomes in upper and lower epidermis (le); centrally located collateral meristele (mer); band of palisade cells (pal) on either side of the lamina being continuous below upper epidermis (ue); narrow spongy parenchymatous tissue traversed by obliquely cut vascular bundles (Figure 4) are archetypal of leaf. Presence of cystoliths of calcium carbonate (a and b); oval to somewhat elongated pollen grains with two pores and smooth exine; fragments of cork in surface view from root (d) and straight and bent thick walled trichomes (e) (Figure 5) are selected representative features of powdered plant material. Quantitative microscopy data for leaf are established and entered in Table 1.

Heavy Metal Analysis

It has been observed that all the heavy metals are found within permissible limits as per WHO guidelines.[23] The results have been summarized in Table 2.

Microbiological Parameters

The plant material is free of encumbrance of Salmonella spp. and microbial load is within the limits specified by WHO.[24] The results have been mentioned in Table 3.

Physicochemical Evaluations

Data of Physico-chemical parameters including moisture content, ash and extractive values are given in Table 4. Low acid insoluble ash value yielded confirmed presence of more amount of inorganic matter in the form of cystoliths. The plant showed higher water-soluble components than alcohol soluble components. Flavonoids and phenolics are among the major compounds present in the plant (Table 5).

Estimation of Kaempferol by HPTLC Analysis

In Co-TLC studies of extract with the reference standard, kaempferol was discernable at R_f 0.45 (Figure 6). Further, in HPTLC method developed the same spots in both the tracks (extract and reference standard) showed superimposable UV spectra approving the identity. The content of kaempferol was found to be 0.14-0.15% w/w. The LOD and LOQ, for signal-to-noise ratios were 3:1 and 10:1, respectively. The particulars of validation parameters are given in Table 6.

Figure 1: Herb of *Rungia repens* Nees.

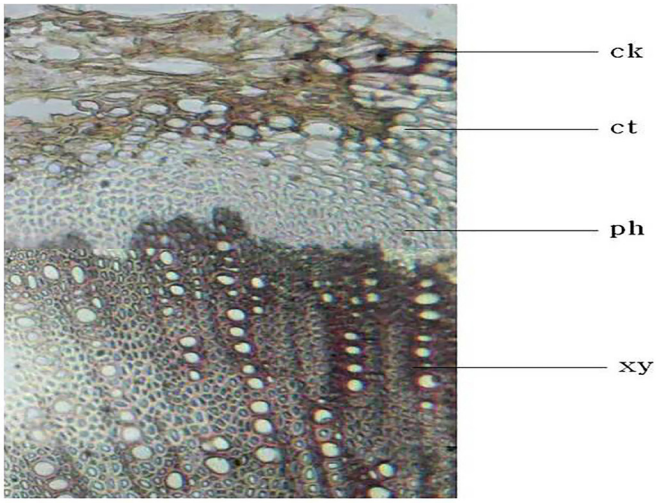

Figure 2: TS of *R. repens* root.

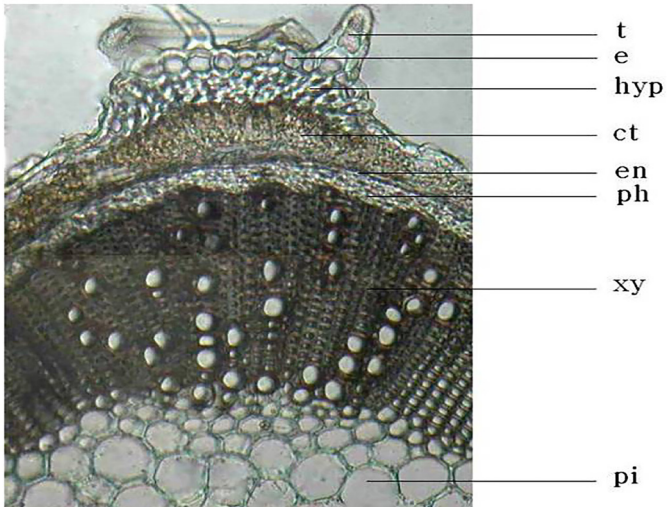

Figure 3: TS of *R. repens* stem.

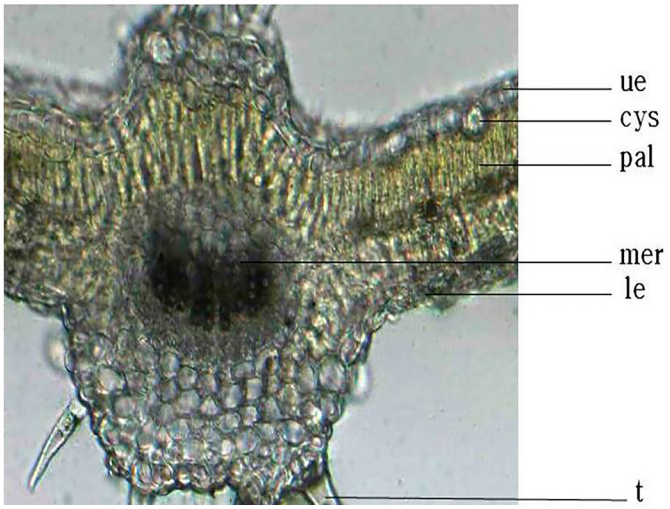

Figure 4: TS of *R. repens* leaf.

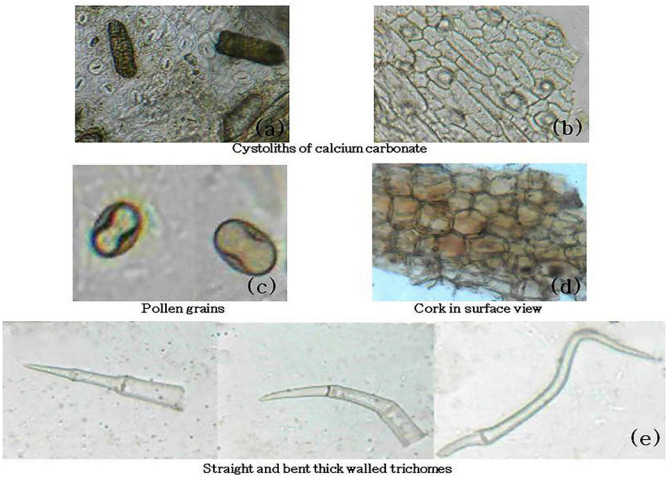

Figure 5: Powder characters of *R. repens* whole plant.

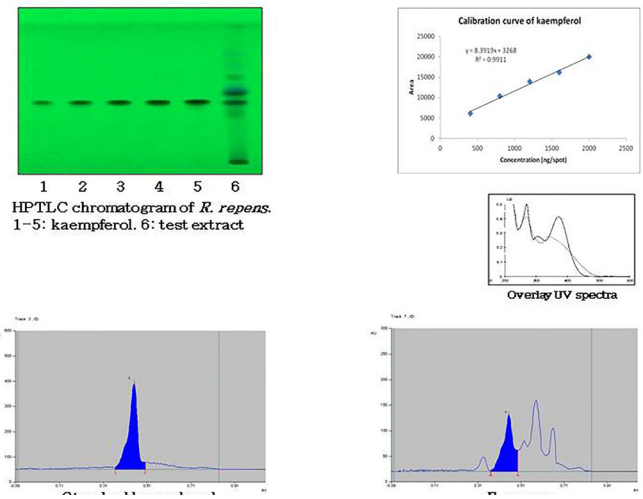

Figure 6: HPTLC study of kaempferol.

Table 1: Quantitative microscopy

Parameters	Values
Stomatal Index	
Upper surface	18.0 ± 0.73
Lower surface	23.48 ± 0.30
Palisade ratio	7.34 ± 0.91
Vein islets no.	11.20 ± 0.87
Vein termination no.	13.67 ± 0.93
Cystolith length in leaf	90.34 – 139.61 µ
Cystolith width in leaf	22.85 - 28 µ
straight covering trichome length	107.86 – 304.5 µ
bent covering trichome length	105.7 – 298.57 µ

Table 2: Heavy metal analysis

Elements	Results (ppm)
Lead (Pb)	BDL
Mercury (Hg)	BDL
Arsenic (As)	2.7710

Table 3: Microbiological parameters

Parameters	Results
Total Plate Count	1.73X104cfu/g
Yeast and Mould Count	<10 cfu/g
Escherichia coli	Absent
Salmonella spp.	Absent
Staphylococcus aureus	Absent

Table 4: Physico-chemical parameters

Sr. No.	Quality Parameters	% w/w ± S.D.
1.	Loss on drying	74.13 ± 0.38
2.	Ash value	
	a. Total ash value	14.5 ± 0.26
	b. Acid insoluble ash	1.75 ± 0.25
	c. Water soluble ash	4.5 ± 0.54
3.	Extractive value	
	a. Water soluble extractive	17.52 ± 0.32
	b. Alcohol Soluble extractive	5.5 ± 0.67

Table 5: Content of phytoconstituents

Sr. No.	Phytoconstituents	% w/w ± S.D.
1.	Flavonoids	2.96 ± 0.14
2.	Phenolics	7.87 ± 1.61

Table 6: Summary of validation parameters of kaempferol

Sr. No.	Parameters	Results
1	Linearity (R2)	0.9911
2	Precision (C.V.)	
	• Repeatability of Measurement	0.785
	• Repeatability of Application	1.875
	• Interday	1.14-2.78%
	• Intraday	1.09-1.63%
3	Range	400-2000 ng/spot
4	Limit of Detection	62.61 ng/spot
5	Limit of Quantification	189.73 ng/spot
6	Accuracy	98.21 - 99.92%
7	Specificity	Specific

CONCLUSION

This is the first report on the Pharmacognostic study expounded with HPTLC analysis for *Rungia repens*. The ensemble of data on standard parameters is useful for the endorsement of quality control and for documenting a monograph on this crude drug. The proposed HPTLC method for estimation of kaempferol was precise, accurate and selective. It does not suffer any positive or negative interference due to other common components present in the extract. Thus, it can be applied to obtain the necessary authentication of *R. repens* routinely, with good reliability and reproducibility. The present study proffers, an expedient tool to corroborate the drug through good constancy by addressing the pragmatic issue of qualitative analysis of the plant material in terms of botanical identification and quantification of kaempferol by HPTLC.

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CONFLICT OF INTEREST

No conflict of interest are declared.

ABBREVIATION USED

TLC: Thin layer Chromatography; **HPTLC:** High Performance Thin layer Chromatography; **WHO:** World Heatlh Organisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification.

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