

## ORIGINAL ARTICLE

# Pharmacognostic and physicochemical standardization of homoeopathic drug: *Rumex crispus* L.

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### ABSTRACT

**Background:** *Rumex crispus* L., commonly called as “yellow dock” in English, “patience frisee” in French, and “Ampfer” in German, and ‘aceda de culebra’ in Spanish is a well-known herb belonging to *Polygonaceae*. Roots of the herb are used as medicine in homoeopathy.

**Objective:** The pharmacognostic and physicochemical studies on roots have been carried out to enable the use of correct species and standardize the raw material.

**Materials and Methods:** Pharmacognostic studies on roots of authentic raw drug have been carried out; physicochemical parameters, namely, extractive value, ash values, formulation besides weight per mL, total solids, alcohol content along with high-performance thin layer chromatography (HPTLC) and ultraviolet studies for mother tincture have been worked out.

**Results:** Roots are blackish-brown, wiry, rounded with irregular striations, tortuous; internally, it is softwood, light-yellow, and fracture fibrous. Phellem is 8–10 layered, discontinuous, and tanniferous. Phellogen is two-layered and contains inulin crystals in few. Outer phelloderm is 12–16 layered often containing spherocrystals and associated with stone cells. Secondary phloem is up to 25 layered. Xylem is in the form of strips. The physicochemical properties and HPTLC values of the drug are standardized and presented.

**Conclusion:** The powder microscopic features and organoleptic characters along with anatomical and physicochemical studies are diagnostic to establish standards for the drug.

**Keywords:** High-performance thin layer chromatography, Pharmacognosy, Roots, *Rumex crispus* L., Standardization

### INTRODUCTION

*Rumex crispus* L., popularly known as “yellow dock” in English, is a perennial herb belonging to the family *Polygonaceae*.<sup>[1]</sup> It is a native of the United States and introduced into Northern America, Mexico, Chile, and New Zealand. In India,<sup>[1]</sup> it is found in Nilgiris, Tamil Nadu.<sup>[2]</sup> The roots of this

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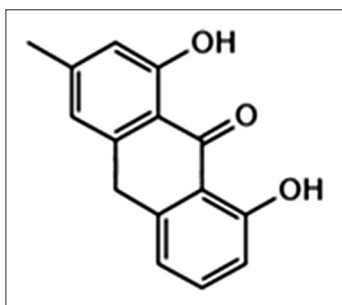
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plant are used as medicine in Homoeopathy for abortion, asthma, bronchitis, catarrh, corn, coryza, cough, diarrhea, pain in heart, indigestion, phthisis, rheumatism, and urticaria<sup>[3-5]</sup> The proving of this drug was done by Houghton, Joslin, H.M. Paire Bayard. *Rumex* species are rich in anthraquinones, naphthalenes, flavonoids, stilbenoids, triterpenes, carotenoids, and phenolic acids.<sup>[6]</sup> Chemically, the roots of *Rumex crispus* are reported to contain 1,8-dihydroxy-3-methyl-9-anthrone [Figure 1] as a major compound, chrysophanic acid and emodin as minor constituents, besides anthraquinones, nepodin, physcion, kaempferol, and quercetin. Some toxic substances, namely, hexadecanoic acid, gallic acid, and (+) catechin are also reported.<sup>[7-9]</sup>

Standardization is a tool in the process of quality control. By providing a set of standards of inherent characteristics, constant parameters, definite, qualitative and quantitative values. It is the cardinal responsibility of the authorities (pharmacopoeias) to ensure that the consumers get medication which guarantees purity, safety, potency, and efficacy. Chemical evaluation covers identification and characterization of crude drug with respect to phytochemical constituents. Chromatographic examination helps in identification of finished products based on the use of major chemical constituents as markers. Recent advantages in analytical instrumentation in the area of isolation, purification, and structure elucidation of naturally occurring metabolites made the quality control process of standardization more attractive. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of the chemicals or impurities present in the plant extract.

Pharmacognostic studies on roots of *Rumex* is confined to *Rumex hastatus*<sup>[10]</sup> and *Rumex vesicarius*.<sup>[11]</sup> However, studies on roots of *Rumex crispus* L. are fragmentary and confined.<sup>[1,12]</sup> Hence, considering



**Figure 1:** Chemical structure of 1,8-dihydroxy-3-methyl-9-anthrone

the medicinal importance of the drug and in absence of detailed pharmacognostic and physicochemical investigation, the present study is taken up.

## MATERIALS AND METHODS

### Pharmacognosy

The roots of plant drug of *Rumex crispus* L. was supplied by the Survey of Medicinal Plants and Collection Unit of CCRH, at Tamil Nadu. The roots were slightly boiled, cut into pieces, and fixed in formaldehyde-acetic acid-alcohol and processed for microtomy (paraffin method), sectioned, stained and permanent slides were prepared as per method of Johansen.<sup>[13]</sup> The sections were studied and photographs were taken on Olympus BX 53 Research Trinocular microscope. The powder microscopic characters were studied by boiling the powdered drug in distilled water, stained in safranin, and mounted in glycerin.

### Physicochemical

The air-dried roots were coarsely powdered to 10/44 (sieve size) and subjected to determination of loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physicochemical constants, ultraviolet (UV) aspects of mother tincture, following official methods.<sup>[14]</sup> Mother tincture was prepared as per Homoeopathic Pharmacopoeia of the United States Convention<sup>[15]</sup> by percolation method.<sup>[14]</sup> An amount of 100 g of coarse powder of the drug was suspended in 469 mL of 95% alcohol and 550 mL of purified water for 24 h at room temperature. It was filtered and made up to 1000 mL using the same solvent ratio.<sup>[16-18]</sup> UV-visible spectrophotometer, Perkin Elmer, Lambda 25 is used.

### High-performance Thin Layer Chromatography Analysis

A volume of 25 mL mother tincture was evaporated on water bath to remove alcohol. The residue was extracted thrice with 25 mL chloroform. Concentrated chloroform extract to 2 mL was used for the high-performance thin layer chromatography (HPTLC) study. The concentrated chloroform extract (10 µL and 15 µL) was spotted in the form of bands of length 7 mm with 100 µL syringe on pre-coated silica gel aluminum plate 60 F<sub>254</sub> (5 cm × 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat V sample applicator (Camag, Muttenz, Switzerland, supplied by Anchrom Technologists, Mumbai). A constant application rate of 6 µL/s was employed.

The space between the bands was fixed as 20 mm. The slit dimension was kept at 6 mm × 0.90 mm and scanning speed at 20 mm/s employed. The mobile phase consists of hexane:ethyl acetate (6:4 v/v) and 10 mL was used for chromatography. Linear ascending development was carried out in a 10 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase at room temperature for 20 min. The length of the chromatogram run was 8.5 cm, and subsequent to the development, the thin layer chromatography (TLC) plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III) at 254 nm (absorbance mode) and 366 nm (reflectance/emission mode) and operated by *win* CATS software (v 1.4.3 Camag) resident in the system.<sup>[12-14]</sup>

## OBSERVATIONS AND RESULTS

### Macroscopy

Roots are 0.4 mm to 1.3 cm thick, blackish-brown; rootlets are long, wiry, arising from root base, rounded with irregular vertical striations, tortuous, bark slightly thick, internally softwood, light-yellowish, porous, fracture fibrous.

### Microscopy

Transection (T.S.) shows that outermost phellem is 8–10 layered, shriveled, and discontinuous, in which outer 4–5 layers of densely tanniferous and lower 3–4 layers of barrel-shaped to tabular cells, contents scanty; phellogen is two-layered of barrel-shaped or tangentially elongated cells, thick-walled, contents slightly dense with inulin crystals in few; outer phelloderm is 12–16 layered, cells tangentially elongated, few polygonal and tabular in undulated layers containing resin and sphaerocrystals [Figure 2a], interspersed with isolated thick-walled stone cells; inner phelloderm is 10–12 layered cells of smaller, polygonal to elongated shaped, contents dense with tannins [Figures 3a, 2a and b]; secondary phloem is up to 25 cells thick with phloem parenchyma, sieve cells, phloem fibers, and interrupted by 1–3 seriate medullary rays becoming wider toward phelloderm [Figure 3a]; tracheary tissue in strips consists of vessels/tracheids arranged in radial rows, few isolated, and clustered besides xylem parenchyma and fibers [Figure 3a and b]. In longitudinal section, tracheary elements show wall thickenings mostly with bordered pits, few scalariform, helical and reticulate;

the vascular tissue is interrupted by medullary rays; pith is extensive [Figure 2c].

### Powder Microscopy

It shows isolated broken vessels with scalariform and bordered pits; pieces of cork with groups of tangentially long cells with few containing inulin crystals; pieces of phelloderm with sphaerocrystals in few; brownish resinous masses numerous, inulin crystals, oblong to oval in groups or isolated; pieces of phelloderm with attached ray cells.

### Organoleptic Characters

- Color – dark brown
- Taste – not characteristic
- Odor – slightly pungent
- Touch – smooth.

### Physicochemical Studies

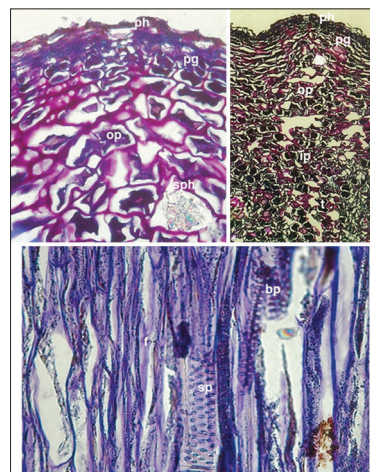
The determined data under the physicochemical study for the raw drug are summarized in Table 1 and that of mother tincture standardization in Table 2.

### Qualitative Phytochemical Tests

Loss on drying reveals the presence of water in the roots and also some volatile organic matter.

### High-performance Thin Layer Chromatography Finger Printing

The profile of chromatographic separation scanned at 254 nm (absorbance mode) reveals seven spots [Figures 4 and 5], out of which spot-3 (area 8461.5 AU and 11.24%), spot-6 (area 33713.0 AU and 44.76%), and spot-7 (area 28478.2 AU and



**Figure 2:** (a) Transsection of the root a portion enlarged with crystalliferous idioblasts ×343, (b) a portion of the root from phellem to cork ×139, (c) Longitudinal section of the root with vascular elements ×203. Ph: Phellem; pg: Phellogen; op: Outer phelloderm; ip: Inner phelloderm; sph: Sphaerocrystals; t: Tanniferous cells; bp: Bordered pits; sp: Scalariform pits; f: Fibers



**Table 1: Standardization of raw drug**

Parameters	Quantitative values% w/w
Loss on drying at 105°C	9.50-10.20*
Total ash	4.30-4.50**
Acid insoluble ash	0.45-0.47
Water soluble ash	1.13-1.70
Alcohol soluble extractive	28.5-30.0
Water soluble extractive	31.80-32.90
Extractive values in <sup>[19]</sup>	
Toluene	0.50-0.75
Chloroform	1.13-1.30
Methanol	32.30-33.15

\*Each test is repeated thrice, the mean value is reported (n=3), \*\*Each test is repeated six times, the mean value is reported (n=6)

**Table 2: Standardization of mother tincture**

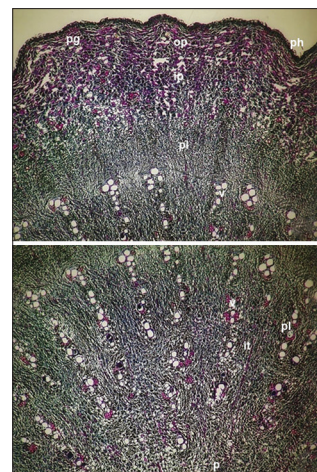
Parameters	Observations
Organoleptic profile	
Appearance	Viscous with blackish brown
Color	Blackish brown
Odor	Characteristic
Sediments	Absent
Weight (/mL)	0.91-0.95 g
Total solids	3.30-3.50% w/v
Alcohol content	42-45% v/v
pH	4.0-5.0
Ultraviolet absorbance (λ max)	206 and 326 nm

37.81%) possess maximum composition with  $R_f$  at 0.34, 0.67, and 0.87, respectively. Densitogram scanned at 366 nm (reflectance/emission mode) revealed 7 spots with spot-6 (area 27178.4 AU and 46.82%) and spot-7 (area 26542.9 AU and 45.73%) showing maximum composition at  $R_f$  0.67 and 0.87, respectively. It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the mother tincture. These are considered as valuable standards in pharmacopoeia. At 254 nm, seven spots appear at  $R_f$  0.08, 0.23, 0.34, 0.44, 0.54, 0.67, and 0.87 (all dark) [Figures 6 and 7] with various concentrations; however, at 366 nm, 7 spots appears at  $R_f$  0.23 (blue), 0.30 (blue), 0.35 (blue), 0.44 (blue), 0.54 (blue), 0.67 (yellow), and 0.87 (yellow). This is a vital fingerprint parameter to ensure the reliability and reproducibility of the drug.

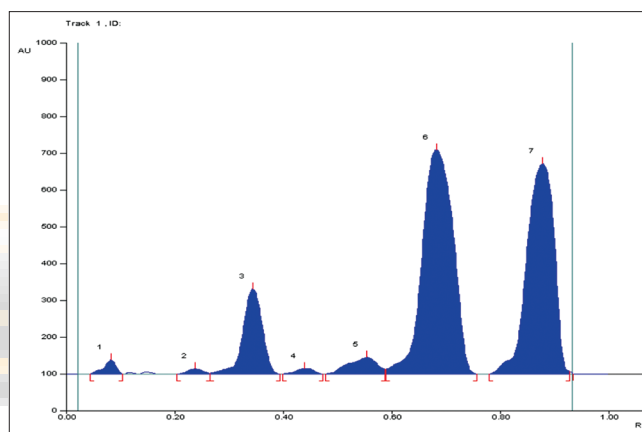
## DISCUSSION

### Pharmacognosy

*Rumex crispus* L. is a perennial herb belonging to the family *Polygonaceae*. The roots of the plant are



**Figure 3:** (a) Transsection of the root periphery to vascular region  $\times 38$ , (b) Transsection of the root from vascular region to center  $\times 53$ . Ph: Phellem; pg: Phellogen; op: Outer phelloderm; ip: Inner phelloderm; pl: Phloem; v: Vascular strand; it: Interfascicular tissue; p: Pith

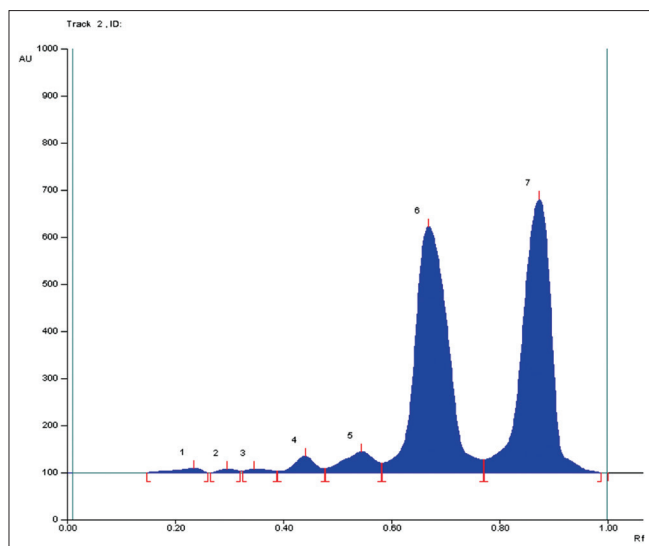


**Figure 4:** High-performance thin layer chromatography densitogram of *Rumex crispus*  $\Phi$  scanned at 254 nm in hexane: ethyl acetate (6:4 v/v)



**Figure 5:** High-performance thin layer chromatography image of *Rumex crispus* at 254 nm in hexane: Ethyl acetate (6:4 v/v)

useful in Homoeopathy for various ailments. The underground roots are reddish to blackish-brown



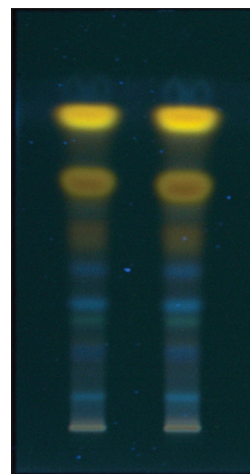
**Figure 6:** High-performance thin layer chromatography densitogram of *Rumex crispus* at 366 nm by using hexane:ethyl acetate (6:4 v/v)

as also reported<sup>[12]</sup> besides tortuous with irregular striations.

The outermost cork is reported light brown with a collapsed phellogen<sup>[1]</sup> while at present, it is 8–10 layered, shriveled, discontinuous with outer layers tanniferous followed by a two-layered phellogen containing inulin grains beneath it. A collenchymatous hypodermis of several rows has been reported<sup>[1]</sup> but is not presently found.

In T.S., the cortex is reported internally yellow and clearly distinct from wood and is now confirmed.<sup>[12]</sup> Further, it is reported as broad parenchymatous zone containing starch grains and rosette crystals of calcium oxalate<sup>[1]</sup> which is presently confirmed except without starch grains. The outer cortex is 12–16 layered with tangentially elongated cells in undulated form and often contains resin and spheraphidal (rosette) crystals and interspersed with stone cells. The inner cortex consists of smaller, polygonal to elongated cells with dense tanniferous contents. Secondary phloem is extensive and made of phloem parenchyma, sieve cells, and fibers. The vascular tissue is interrupted by 1–3 seriate medullary rays. Leaf trace bundles as reported earlier<sup>[1]</sup> are not found in the present study.

Earlier, fibrovascular bundles were reported as small in a circle with few tracheids and fibers.<sup>[1]</sup> However, at present, it is found as strips of tracheary tissue with vessels/tracheids arranged in radial rows and associated with xylem parenchyma and fibers.



**Figure 7:** High-performance thin layer chromatography image of *Rumex crispus* at 366 nm in hexane:ethyl acetate (6:4 v/v)

Pith is reported parenchymatous and extensive often with starch grains and rosette crystals.<sup>[1]</sup> However, at present, though it is extensive, it is devoid of rosette crystals and starch grains.

The salient pharmacognostic features presented along with powder microscopic and organoleptic characters are helpful in authentication of the drug.

### Physicochemical

The physicochemical properties [Tables 1 and 2] help to identify and estimate the active compounds present in the drugs. In HPTLC fingerprinting, the developed chromatogram and  $R_f$  values of bands will be specific with the selected solvent system. UV spectroscopic study exhibits two prominent peaks which serve as characteristic standards for *Rumex crispus* mother tincture.

### CONCLUSION

The macro and microscopical, organoleptic characters along with the anatomical and methodology used for the studies are diagnostic and establish the standards. HPTLC analysis of *Rumex crispus* L. leaves and twigs can provide standard finger prints and will be used as reference tool for identification, authentication, quality control and standardization of this important medicinal plant.

### Acknowledgment

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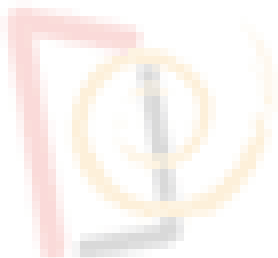
Nil.

## Conflicts of Interest

There are no conflicts of interest.

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### होम्योपैथिक औषधि रुमेक्स क्रिस्पस एल- का फार्माकोग्नोस्टिक एवं भौतिक रसायनिक मानकीकरण

**पृष्ठभूमि:** रुमेक्स क्रिस्पस एल, जिसे सामान्य तौर पर अंग्रेजी में येलो डॉक और फ्रेंच में पेशेंस फ्रीस व जर्मन में एम्पफेर कहते हैं, एक विख्यात जड़ी बूटी है जो पोलीगोनेसी से संबंधित है। इस की जड़ों को होम्योपैथी में दवाई के रूप में प्रयोग किया जाता है।

**उद्देश्य:** सही प्रजातियों को सक्षम करने के लिए और कच्चे माल को मानक करने के लिए जड़ों का फार्माकोग्नोस्टिक और भौतिक – रसायन अध्ययन।

**सामग्री और पद्धतियां:** विश्वसनीय औषधियों की जड़ों पर फार्माकोग्नोस्टिक अध्ययन किया गया है, मदर टिंचर के लिये भौतिक रासायनिक मानकों अर्थात्, खनन मूल्य, राख मूल्य, सूत्रीकरण के अतिरिक्त प्रति एम एल, कुल ठोस, एल्कोहल सामग्री सहित उच्च प्रदर्शन पतली परत क्रोमैटोग्राफी (एचपीटीएलसी) और पराबैंगनी अध्ययन भी किया गया है।

**परिणाम:** जड़ें काली भूरी, कड़ी, अनियमित रिसाव के साथ गोल, घुमावदार, अंदर से नरम लकड़ी वाली, हल्की पीली एवं टूटे रेशों वाली होती है। फेलेन 8–10 परत वाली, अनियंत्रित और टेनिफेरियस होती है। फेलोगेन दो परत वाला और कुछ मात्रा में इंडुलिन क्रिस्टल लिए होता है। फेलोडर्म द्विस्तरीय अक्सर स्फेरिक्रिस्टल युक्त और पत्थर कोशिकाओं के साथ जुड़ा होता है।

**निष्कर्ष:** औषधि के मानकों को स्थापित करने के लिए पाउडर माइक्रोस्कोपिक और आर्गनोलेप्टिक विशेषताओं के साथ शारीरिक एवं भौतिक-रसायनिक अध्ययन नैदानिक हैं।

### Farmacognosia y estandarización físico-química del medicamento homeopático: *Rumex crispus* L.

#### RESUMEN

**Fundamento:** *Rumex crispus* L., denominado comúnmente 'yellow dock' en inglés, 'Patience frisee' en francés, 'Ampfer' en alemán y 'aceda de culebra' en español. Pertenece a las Poligonáceas y sus raíces se utilizan como medicina en homeopatía.

**Objetivos:** Se han efectuado estudios farmacognósticos y físico-químicos de las raíces para poder utilizar la especie correcta y estandarizar la materia prima.

**Materiales y métodos:** Se han realizado estudios de farmacognosia sobre la raíces del medicamento crudo auténtico; se han establecido los parámetros físico-químicos, es decir, el valor de extracción, los valores de cenizas, formulación aparte de peso por ml, sólidos totales, contenido en alcohol junto con cromatografía de capa fina de alto rendimiento (HPTLC) y estudios con ultravioleta (UV) de la tintura madre.

**Resultados:** Las raíces negro-marrónáceas, pivotantes, redondas con estriaciones irregulares, sinuosas; madera interna blanda, ligeramente amarilla y fibrosa a la fractura. El felema posee 8 a 10 capas, es discontinuo y taninífero. El felógeno tiene dos capas y contiene algunos cristales de inulina. El feloderma posee hasta 25 capas, a menudo con esferocristales y está asociado con células piedra. El floema secundario tiene hasta 25 capas. El xilema tiene forma de tiras. Las propiedades físico-químicas y los valores de la HPTLC del medicamento están estandarizados y se han presentado.

**Conclusiones:** Las características microscópicas del polvo y las características organolépticas junto con los estudios anatómicos y físico-químicos son diagnósticos para establecer los estándares del medicamento.