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 tanniniferous. 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*.¹ It is a native of the United States and introduced into Northern America, Mexico, Chile, and New Zealand. In India,² it is found in Nilgiris, Tamil Nadu. The roots of this...
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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. 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.

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* and *Rumex vesicarius*. However, studies on roots of *Rumex crispus* L. are fragmentary and confined. Hence, considering 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. 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. Mother tincture was prepared as per Homoeopathic Pharmacopoeia of the United States Convention by percolation method. 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. 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 precoated silica gel aluminum plate 60 F 254 (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.

![Figure 1: Chemical structure of 1,8-dihydroxy-3-methyl-9-anthrone](http://www.ijrh.org)
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.¹²⁻¹⁴

**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 tanniniferous 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 spherocrystals [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 spherocrystals 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 121
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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 useful in Homoeopathy for various ailments. The underground roots are reddish to blackish-brown.
as also reported\cite{12} besides tortuous with irregular striations.

The outermost cork is reported light brown with a collapsed phellogen\cite{1} while at present, it is 8–10 layered, shriveled, discontinuous with outer layers tanniniferous followed by a two-layered phellogen containing inulin grains beneath it. A collenchymatous hypodermis of several rows has been reported\cite{1} but is not presently found.

In T.S., the cortex is reported internally yellow and clearly distinct from wood and is now confirmed.\cite{1,12} Further, it is reported as broad parenchymatous zone containing starch grains and rosette crystals of calcium oxalate\cite{1} 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 tanniniferous 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\cite{1} are not found in the present study.

Earlier, fibrovascular bundles were reported as small in a circle with few tracheids and fibers.\cite{1} 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.

Pith is reported parenchymatous and extensive often with starch grains and rosette crystals.\cite{1} 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.

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Conflicts of Interest
There are no conflicts of interest.

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

RESUMEN


Objetivos: Se han efectuado estudios farmacognósicos 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.


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.