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# Standardization parameters and HPTLC fingerprinting of the roots of *Ricinus communis* Linn

***Abhilasha Mittal[1]* and Mohammed Ali[2]**

[1]Faculty of Pharmaceutical Sciences, Jyoti Vidyapeeth Women's University, Vedant Gyan Valley, Ajmer Express Way, NH-8, Jaipur – 302019 (Rajasthan), India

[2]Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi - 110062, India

**Abstract**

*Ricinus communis* L. (Euphorbiaceae), known as castor plant, is widespread throughout tropical regions of India and grows as an annual or perennial soft wooded small tree. A decoction of the root is administered to relieve lumbago, and a root paste is applied to alleviate toothache. The roots contained glycosides, phenolic compounds, steroids and acidic components. The root bark of R. *communis* furnished 5.74% of the total ash, 2.03% of the acid insoluble ash and 2.98% of the water soluble ash. There was 9.46 % of water content. Successive extraction of the roots (50 g) yielded 0.362 g, 0.482 g, 1.243 g and 2.643 g of the extracts in petroleum ether, chloroform, methanol and water, respectively. Individual extractions of the roots (15 g) with these solvents produced 0.235 g, 0.727 g, 7.425 g and 12.017 g of the extracts, respectively. Fluorescence behavior of powdered root bark of *R. communis* indicated that the light yellow powder of the bark powder changed to black at $\lambda_{max}$ 366 nm. Among the nine elements, calcium (350.45 ppm) was present in the maximum amount followed by followed by potassium (330.50 ppm), magnesium (230.68 ppm) and cadmium (198.57 ppm). Sodium (56.26 ppm) and lead (8.18 ppm) were detected in trace amounts. The HPTLC scanning of the petroleum ether, chloroform and methanol extracts of the roots exhibited 5, 4 and 4 major bands, respectively.

*Corresponding author, Mailing address:
**Abhilasha Mittal**
E-Mail: abhilashamittal24@gmail.com

***Key words:***

*Ricinus communis*, roots, phytochemical screening, HPTLC fingerprint.

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## INTRODUCTION

*Ricinus communis* L. (Euphorbiaceae)is commonly known as 'palm of Christ', Jada (Oriya), Verenda (Bengali), Endi (Hindi), Errandi (Marathi) and Diveli (Guajarati)[1]. It is an annual or perennial soft

wooded small tree up to 6 m in height. The plant is widespread throughout tropics and warm temperate regions of the world and cultivated in India and other countries up to 2,000 m altitude [2]. The seeds contained 45% of fixed oil which consist glycosides of ricinoleic, isoricinoleic, stearic and dihydroxy stearic acids [3,4]. The GC-MS analyses of *R. communis* essential oil using capillary columns has shown compounds like α-thujone (31.71%), α-pinene (16.88%), camphor (12.92%) and camphene (7.48%)[5]. The oil is useful in the treatment of eye inflammation and in the manufacture of sebacic acid, surface coating, disinfectants, cosmetic and pharmaceutical preparations[6]. Castor bean has also been proposed as a potential source of biodiesel, the high oil content of its seeds [7]. It is believed that castor oil was first used as an ointment 4,000 years ago in Egypt, from where it spread to other parts of the world, including Greece and Rome, where it was prescribed as a laxative 2,500 years ago[8]. The plant possessed beneficial effects such as anti-oxidant,[9,10] antiulcer[11], anti-inflammatory, [12,13]Antidiabetic, [14] hepatoprotective, [15,16,17] antifertility[18] and many other medicinal properties. The roots furnished phenolic methyl esters and fatty acid glycosides[19, 20]. The present manuscript describes quality control parameters of the roots.

## MATERIALS AND METHODS

### Plant material

The fresh roots of *R. communis* were collected from the arid waste land of Jaipur (Rajasthan). The plant material was identified on the basis of exomorphic characters and reviews of literature by Prof. M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen No. PRL/ JH / 09 / 12 is deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

### Physicochemical evaluation

The dried roots of *R. communis* were coarsely powdered in a grinder and stored in an air tight container. Physicochemical parameters were calculated according to the prescribed methods. Preliminary phytochemical analysis of the alcoholic extract of the powdered roots was performed.

### HPTLC fingerprinting

The root powder (5 g) was extracted with 25 ml each of petroleum ether, chloroform and methanol separately for 1 hr. The extracts were dried individually to get the dark brown residues. Each residue was dissolved in petroleum ether, chloroform and methanol (5 ml), respectively, and applied on pre-coated silica gel 60F254 TLC plates.

### Optimization of HPTLC solvent system

A number of solvent systems were tried for different extracts. The most satisfactory resolution was obtained in the solvent as summarized in Table 6.

### Sample application

The spots of each sample extract was applied in concentration of 10µl in duplicate on pre-coated silica gel 60F254 TLC plates with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

### Development of chromatogram

The chromatogram was developed in a Twin trough glass chamber 20x 10 cm saturated with different solvents till a suitable height of the solvent front was obtained.

### Detection of spots

The air-dried plates were viewed in ultraviolet radiation to mid day light. The chromatograms were scanned by densitometer at 400nm after treatment with anisaldehyde- sulfuric acid reagent. The $R_f$ values and finger print data were recorded by WIN CATS software. The 3D display of all tracks and fingerprints of all different samples at 400nm for petroleum ether, chloroform and methanol extracts.

## RESULTS AND DISSCUSSION

The preliminary phytochemical screening of the methanolic and aqueous extract was carried out by chemical testing with different specific chemical

reagents. It responded positively to the chemical tests of glycosides, phenolic compounds, steroids and acidic components (Table 1).

Significant amount of acid insoluble ash has been detected which indicates presence of various silicacious substances. Cellulosic substances also contribute markedly in the total ash as indicated by water soluble ash. The root bark of *R. communis* furnished 5.74% of the total ash, 2.03% of the acid insoluble ash and 2.98% of the water soluble ash. There was 9.46 % of water content as indicated by loss of drying (Table 2). Small amount of non-polar substances in comparison to polar solvents was observed in the roots. However, alcoholic and aqueous extractives showed significant yields of the extracts (Table 3). The root bark of *R. communis* (50 g) furnished 0.362 g petroleum ether extract, 0.482 g of chloroform extract, 1.243 g of methanol and 2.643 g of water extractive on successive extraction. Individual extractions of the roots (25 g) with these solvents produced 0.235 g, 0.727 g, 7.425 g and 12.017 g, respectively.

Fluorescence behavior of powdered root bark of *R. communis* indicated that the light yellow powder of the bark powder changed to black at $\lambda_{max}$ 366 nm. It remained identical when the powder was reacted with Conc. $HNO_3$, Conc. $H_2SO_4$, iodine and ferric chloride solution (10%).

The colour of the bark powder appeared green on treatment with Conc. HCl, chloroform and picric acid and reddish brown with sodium hydroxide (10%) and methanol at $\lambda_{max}$ 366 nm (Table 4).

Elemental analysis of the ashes of the root bark of *R. communis* showed the maximum amount of calcium (350.45 ppm) followed by potassium (330.50 ppm), magnesium (230.68 ppm) and cadmium (198.57 ppm). Sodium (56.26 ppm), iron (45.86 ppm), copper (33.20 ppm), Zinc (23.25 ppm) and lead (8.18 ppm) were detected in trace amounts. Mercury and arsenic could not be detected in the ashes of R. *communis* (Table 5).

HPTLC is an invaluable 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 this technique is more versatile than ordinary TLC methods as the spots were well resolved. HPTLC fingerprinting of the root extracts of *R. communis* was determined for the petroleum ether, chloroform and methanol extracts after spraying with anisaldehyde-sulphuric acid reagent using CAMAG HPTLC system. The phytochemical variations were observed in chromatograms as shown in Figures 1, 2 and 3. The chloroform extract of the root sample at 366 nm showed the presence of highest number of TLC spots (Table 6) and purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the bands. The HPTLC scanning of the petroleum ether extract of the roots of *R. communis* showed five major bands at $R_f$ 0.46, 0.50, 0.75, 0.81 and 0.86 corresponding to the peak areas 13.36%, 10.30%, 25.97% and 32.16%. The chloroform extract of roots exhibited prominent bands at $R_f$ 0.49, 0.54, 0.74, 0.85 and 0.91 with peak areas of 21.97%, 11.84%, 13.25%, 17.32% and 9.61%, respectively. The methanol extract of the roots showed the presence of four important bands at $R_f$ 0.49, 0.54, 0.83 and 0.87 having peak areas of 25.34%, 14.07%, 14.70% and 28.25%, respectively.

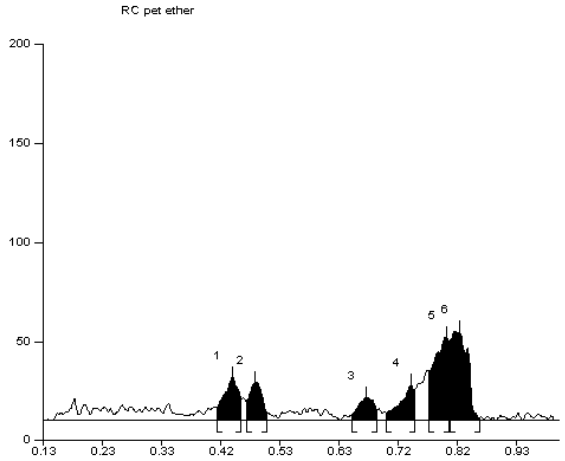

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**Fig 1:** HPTLC scan of petroleum ether extract of *R. communis* roots

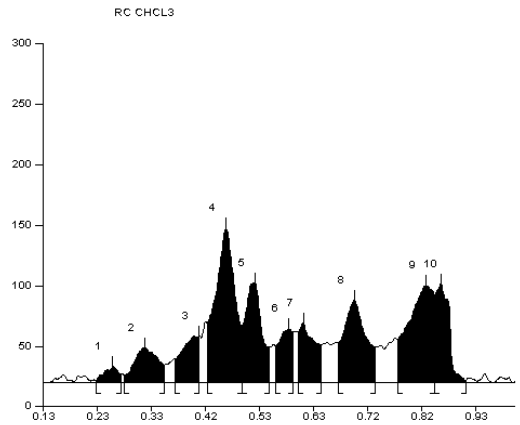


**Fig 2:** HPTLC scan of chloroform extract of *R. communis* roots

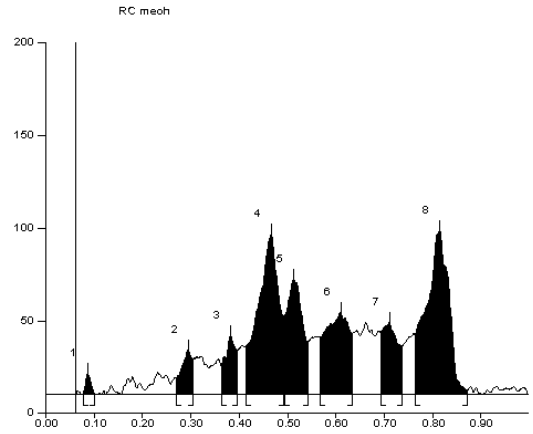


**Fig 3:** HPTLC scan of methanol extract of *R. communis* roots

**Table 1:** Detection of phytoconstituents in roots of *R. communis*

| S. No. | Constituents | Methanol | Water |
|---|---|---|---|
| 1 | Alkaloids | - | - |
| 2 | Carbohydrates | - | - |
| 3 | Glycosides | + | + |
| 4 | Phenolic compounds and tannins | + | + |
| 5 | Flavonoids | - | - |
| 6 | Proteins and free amino acids | - | - |
| 7 | Saponins | - | - |
| 8 | Steroids | + | + |
| 9 | Acidic compounds | + | + |
| 10 | Mucilage | - | - |
| 11 | Lipids and fats | + | - |

**Table 2:** Physiochemical parameter of root bark powder of *Ricinus communis*

| Physiochemical parameter | Value (% w/w) |
|---|---|
| Ash value | |
| Total ash | 5.74 |
| Acid insoluble ash | 2.03 |
| Water soluble ash | 2.98 |
| Loss on drying | 9.46 |

**Table 3:** Extractive values in different solvents of the *Ricinus communis* roots (15 g)

| S. No. | Individual | Successive (50g) |
|---|---|---|
| Petroleum ether | 0.235 | 0.362 |
| Chloroform | 0.727 | 0.482 |
| Methanol | 7.425 | 1.243 |
| Water | 12.017 | 2.643 |

**Table 4:** Fluorescence behavior of powdered roots of *Ricinus communis*

| S. No. | Chemical Treatment | Observation | | |
|---|---|---|---|---|
| | | Day light | U.V. 254 | U.V. 366 |
| 1 | Drug powder | Light Yellow | Black | Black |
| 2 | Conc. HCl | Light green | Green | Green colour |
| 3 | Conc. HCl+D.W. | Greenish yellow | Light yellow | Light green yellow |
| 4 | Conc. $HNO_3$ | Red brown colour | Green colour | Black colour |
| 5 | Conc. $HNO_3$+D.W | Light Red brown | Green colour | Black |
| 6 | Conc. $H_2SO_4$ | Greenish black | Green black | Dark black |
| 7 | Conc. $H_2SO_4$+D.W | Brown Black | Green | Black |
| 8 | Iodine | Reddish brown | Dark green | Black |
| 9 | 10% NaOH | Light yellow | Greenish yellow | Reddish brown |
| 10 | $CHCl_3$ | Reddish brown | Green | Dark green |
| 11 | Methanol | Light reddish yellow | Light yellow | Light reddish yellow |
| 12 | 10% $FeCl_3$ | Green | Dark green | Black |
| 13 | Picric acid | Yellow | Yellow | Green |

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**Table 5:** Elemental analysis of ashes of *Ricinus communis* roots

| S. No. | Elements | Amount (ppm) |
|---|---|---|
| 1 | Sodium | 56.26 ppm |
| 2 | Potassium | 330.50 ppm |
| 3 | Calcium | 350.45 ppm |
| 4 | Magnesium | 230.68 ppm |
| 5 | Iron | 45.86 ppm |
| 6 | Copper | 33.20 ppm |
| 7 | Zinc | 23.25 ppm |
| 8 | Mercury | ___ |
| 9 | Lead | 8.18 ppm |
| 10 | Arsenic | ___ |
| 11 | Cadmium | 198.57 ppm |

**Table 6:** TLC profile of *Ricinus communis* root extracts

| Extract | Solvent system | No of peaks | $R_f$ value | Visualizing agents |
|---|---|---|---|---|
| Petroleum ether | Toluene: ethylacetate : acetic acid (8.5:2.5: 0.5) | 6 | 0.46, 0.50, 0.69, 0.75, 0.81, 0.86 | UV 366 nm |
| Chloroform | Toluene: ethyl acetate :acetic acid(7.5:2.5:7) | 10 | 0.27, 0.35, 0.41, 0.49, 0.54, 0.59, 0.64, 0.74, 0.85, 0.91 | Anisaldehydein sulphuric acid |
| Methanol | Toluene : ethyl acetate : acetic acid (7.5:2.5: 0.6) | 8 | 0.10, 0.30, 0.40, 0.49, 0.54, 0.63, 0.74, 0.87 | Anisaldehyde in sulphuric acid |

## CONCLUSION

The physicochemical study, preliminary phytochemical screening and HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. Adulterants, if any, in this plant material can be easily identified by using these parameters.

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