

Marker-based standardization of *Ricinus communis* L. leaf and seed: pharmacognostic constants, reproducible chromatographic fingerprints and 90-day marker stability

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ARTICLE INFO	ABSTRACT
Article history: Received – 12 March 2026 Received in revised form – 22 March 2026 Accepted -25 March 2026 Available online – 08 April 2026	Pharmacological studies of <i>Ricinus communis</i> L. (Euphorbiaceae) report antioxidant, anti-inflammatory and hepatoprotective effects, yet few describe the material tested in terms that another laboratory could reproduce. A dose expressed in milligrams of extract per kilogram of body weight has no stable chemical meaning unless the extract itself has a stable composition. This study establishes a quality framework for the leaf and the seed of the species and reports what that framework detects. Authenticated material from three independent collections was characterized by macroscopic and microscopic examination, quantitative leaf constants, physicochemical constants and fluorescence behaviour. Sequential extraction with petroleum ether followed by ethanol or 70% hydroethanol gave four extracts whose yields varied between batches by 3.2 to 5.3%. Thin-layer chromatographic fingerprints were reproducible, no major band deviating by more than 0.02 retention-factor units from its batch mean and no coefficient of variation exceeding 2.6%. Column fractionation of the leaf petroleum ether extract was reported as a full mass balance: 62.5% of the load was recovered in pooled fractions, 5.4% as characterized pure compounds and 9.3% as an unresolved mixture. Lupeol and apigenin served as chromatographic markers, and marker content varied by less than 6% across batches. Over 90 days at 2-8 °C the alcoholic extracts retained 93 to 94% of marker content while the triterpenoid-rich fraction retained 98.6%, on which basis a 60-day shelf life was adopted for the alcoholic preparations. The framework is offered as a reproducible basis for pharmacological work on this species.
Keywords: <i>Ricinus communis</i> Pharmacognostic standardization Chromatographic fingerprint Marker compound Batch release Storage stability	

1. Introduction

Herbal drug research produces a large volume of pharmacological results that cannot be compared with one another. The reason is rarely the biology. It is that the material tested is described by its botanical name and its solvent, and by little else, so that two studies reporting the same extract of the same species may have worked with preparations of materially different composition (Mukherjee, 2002; World Health Organization, 2011). An extract is a mixture whose composition shifts with provenance, season, maturity, drying, particle size and extraction conditions. Where none of those variables is documented and no constituent is quantified, a reported dose-response relationship cannot be separated from a composition-response relationship.

Ricinus communis L. illustrates the problem well. The species has an extensive ethnomedicinal record and a modern literature attributing antioxidant, anti-inflammatory, antinociceptive and hepatoprotective activity to extracts of its leaves, roots and seeds (Ilavarasan et al., 2006; Taur et al., 2011; Nemudzhivadi & Masoko, 2014; Esfandiyari et al., 2018; Abomughaid et al., 2024). Its reported chemistry spans triterpenoids, sterols, flavonoids, phenolic acids and the alkaloid ricinine (Singh et al., 2009; Kumar, 2017; Farooq et al., 2018). Yet

published pharmacological studies of the plant seldom report a voucher number, a chromatographic fingerprint, a quantified marker, batch-to-batch variation or storage stability, and the seed carries the additional complication of ricin, so that the identity of the preparation matters for safety and not only for reproducibility (Abdul et al., 2018).

Regulatory and pharmacopoeial guidance has long been explicit on this point. Guidance on the quality control of herbal materials asks for botanical authentication with a deposited voucher, determination of ash and extractive values, a chromatographic profile, quantification of at least one constituent and evidence of stability (World Health Organization, 2011), and the same requirements underpin the batch release of any herbal preparation intended for pharmacological or clinical study (Mukherjee, 2002). The requirements are neither novel nor demanding. What is uncommon is their appearance in the primary literature on an individual species, where they are usually compressed into a sentence stating that the plant was identified by a taxonomist and the extract prepared by maceration.

This study reports the quality framework applied before any pharmacological experiment on the species, and the data it generated.

Four questions were addressed. Are the pharmacognostic constants of the leaf and the seed stable across independent collections? Are extractive yields reproducible? Are chromatographic fingerprints reproducible closely enough for batches to be pooled? And do the selected marker compounds hold their concentration over a realistic storage interval? The intention is not to report activity, which is described elsewhere, but to make the material on which such activity is claimed describable in numbers.

2. Materials and methods:

2.1 Collection, authentication and processing

Mature leaves and fully developed seeds were collected on three occasions from a documented, pesticide-free site, from several individuals across the population rather than from a single plant. Each collection recorded date, locality with coordinates, altitude, habitat, phenological stage and collector. Material was authenticated by a plant taxonomist, a voucher specimen was deposited in a recognized herbarium, and the voucher number was carried on every batch record, chromatogram and results table. Leaves and seeds were cleaned, shade-dried at ambient temperature to constant mass, powdered in a cleaned mill, passed through a defined sieve and stored in labelled, airtight, light-resistant containers. Seed processing was restricted to trained personnel working under institutional biosafety supervision.

2.2 Macroscopic, microscopic and physicochemical evaluation

Organoleptic and morphological characters were recorded for both plant parts. Transverse sections of the leaf were prepared by hand, cleared, stained and examined microscopically, and powder microscopy was carried out on chloral hydrate mounts. Quantitative leaf constants comprising stomatal number and index, vein-islet number, veinlet termination number, palisade ratio and epidermal cell number were determined on ten fields each. Loss on drying, total, acid-insoluble and water-soluble ash, alcohol-, water- and ether-soluble extractive values, swelling index, foaming index, pH of a 1% aqueous suspension and foreign organic matter were determined in triplicate on every batch (Khandelwal, 2008; Trease & Evans, 2009). Fluorescence behaviour of the powdered drugs was recorded in daylight and under ultraviolet light at 254 and 365 nm after treatment with a series of reagents.

2.3 Extraction and yield

A weighed quantity of each powdered plant part was defatted with petroleum ether (60-80 °C), the marc air-dried, and the defatted material extracted with ethanol for the leaf and 70% hydroethanol for the seed. Extractives were filtered, concentrated under reduced pressure at a controlled bath temperature and dried to constant mass. Yield was calculated as the mass of dried extract as a percentage of the powdered material extracted. Four extracts resulted: leaf petroleum ether (RCLP), leaf ethanolic (RCLE), seed petroleum ether (RCSP) and seed hydroethanolic (RCSE). Extraction was carried out on three independent batches so that reproducibility could be measured rather than assumed.

2.4 Qualitative screening and chromatographic fingerprinting

Extracts were screened for alkaloids, carbohydrates, glycosides, flavonoids, phenolics and tannins, saponins, proteins, steroids, triterpenoids, resins and fixed oils by standard procedures, each with a reagent blank, and responses were recorded on a four-point ordinal scale (Kokate et al., 2008). Analytical thin-layer chromatography used silica gel G with hexane:ethyl acetate (8:2 v/v) for the nonpolar extracts and ethyl acetate:formic acid:acetic acid:water (100:11:11:26 v/v) for the alcoholic extracts. Plates were examined in daylight and under ultraviolet light at 254 and 365 nm and after

class-specific derivatization with anisaldehyde-sulfuric acid, Dragendorff reagent, ferric chloride or natural product-polyethylene glycol reagent (Stahl, 1969; Wagner & Bladt, 1996). Retention factors, band colour and relative intensity were recorded for each of the three batches.

2.5 Column fractionation and mass balance

The leaf petroleum ether extract (4.2 g) was fractionated on silica gel (70-230 mesh) with a stepwise gradient from petroleum ether to methanol. Sixty-eight fractions were collected, monitored by thin-layer chromatography and pooled into seven combined fractions. Every mass entering and leaving the column was weighed, so that the proportion of the load recovered, characterized and left unresolved could be stated rather than inferred. Compounds were purified by repeated chromatography or recrystallization and identified by melting point, ultraviolet, infrared, proton and carbon-13 nuclear magnetic resonance spectroscopy and mass spectrometry, using convergent evidence rather than any single measurement (Harborne, 1998).

2.6 Quantitative phytochemistry and marker selection

Total phenolic content was determined by the Folin-Ciocalteu method against a gallic acid calibration curve (Singleton & Rossi, 1965) and total flavonoid content by the aluminium chloride colorimetric method against a quercetin curve (Chang et al., 2002), both in triplicate. Markers were selected on abundance, chromatographic resolution and stability: lupeol for the nonpolar preparations and apigenin for the alcoholic extracts. Marker content was quantified densitometrically against the isolated reference compound.

2.7 Batch release criteria and storage stability

A batch was released only when appearance, yield, chromatographic fingerprint, marker content, loss on drying and residual solvent all conformed to criteria fixed in writing before the work began. Failing batches were quarantined and investigated, never blended with conforming material. Storage stability was assessed on sealed amber containers held at 2-8 °C, with marker content, total phenolic content and the chromatographic fingerprint re-examined at 30, 60 and 90 days and expressed as a percentage of the day-0 value.

2.8 Data treatment

Determinations were made in triplicate and are reported as mean $\pm$ standard deviation with the coefficient of variation where between-batch consistency is the quantity of interest. Calibration curves are reported with their regression equation and coefficient of determination. Where a tolerance was applied, it was fixed before the measurement rather than after it.

3 Results:

3.1 Macroscopic and microscopic characters

The leaves were peltate and palmately seven- to nine-lobed, glabrous, dark green above and paler beneath, with a long hollow petiole; the seeds were ovoid, dorsally convex, 11.4 ± 1.6 mm long, with a mottled grey-brown testa and a prominent white caruncle. Transverse sections showed a single palisade layer, a loosely arranged spongy mesophyll traversed by collateral bundles, anomocytic stomata confined to the abaxial surface, non-articulated laticifers and prismatic calcium oxalate crystals. Powder microscopy of the sieved leaf material showed fragments of epidermis bearing anomocytic stomata, spiral and annular vessel elements, mesophyll fragments, crystal-bearing parenchyma with prismatic calcium oxalate and occasional laticifer fragments, and these elements were present in every batch examined. The quantitative leaf constants are given in Table 1.

Table 1

Quantitative microscopic constants of *Ricinus communis* leaf
(mean $\pm$ SD, n = 10 fields per batch, three batches)

Constant	Adaxial surface	Abaxial surface	CV (%)
Stomatal number (per mm ²)	Absent	165.4 $\pm$ 8.2	5.0
Stomatal index	Absent	21.4 $\pm$ 1.2	5.6
Vein-islet number (per mm ²)	18.6 $\pm$ 1.1	18.6 $\pm$ 1.1	5.9
Veinlet termination number (per mm ²)	22.4 $\pm$ 1.4	22.4 $\pm$ 1.4	6.3
Palisade ratio	6.8 $\pm$ 0.5	Not applicable	7.4
Epidermal cell number (per mm ²)	608.2 $\pm$ 21.4	607.5 $\pm$ 19.8	3.5

No constant varied by more than 7.4% between the three collections, which is the evidence that the batches could be treated as one population for the purposes of extraction.

3.2 Physicochemical constants

Constants for both plant parts are given in Table 2 and compared in Fig. 1. The leaf carried a total ash of 11.46% against 3.28% for the seed, consistent with the higher mineral content of photosynthetic tissue, while the seed gave an ether-soluble extractive value of 44.10% against 3.55% for the leaf, anticipating the extraction result reported in Section 3.4. Acid-insoluble ash remained below 2% in both parts, indicating that the material was substantially free of adherent siliceous contamination.

Table 2

Physicochemical constants of powdered leaf and seed (per cent w/w unless stated; mean $\pm$ SD, n = 3 batches)

Parameter	Leaf	Seed	Assessment against accepted ranges
Loss on drying at 105 °C	6.82 $\pm$ 0.21	5.14 $\pm$ 0.18	Within range (below 10%)
Total ash	11.46 $\pm$ 0.35	3.28 $\pm$ 0.14	Within range (8-15% for leaf drugs)
Acid-insoluble ash	1.92 $\pm$ 0.11	0.46 $\pm$ 0.05	Within range (below 2-5%)
Water-soluble ash	4.35 $\pm$ 0.19	1.12 $\pm$ 0.08	Not specified

Parameter	Leaf	Seed	Assessment against accepted ranges
Alcohol-soluble extractive	13.20 $\pm$ 0.44	9.85 $\pm$ 0.38	Within range (8-18% for leaf drugs)
Water-soluble extractive	17.65 $\pm$ 0.52	6.42 $\pm$ 0.27	Within range (10-25% for leaf drugs)
Ether-soluble extractive	3.55 $\pm$ 0.16	44.10 $\pm$ 1.05	Reflects seed oil content
Swelling index (mL/g)	4.2 $\pm$ 0.3	2.1 $\pm$ 0.2	Not specified
Foaming index	Below 100	Below 100	Below the reporting threshold
pH of 1% aqueous suspension	5.92 $\pm$ 0.08	6.34 $\pm$ 0.07	Not specified
Foreign organic matter	0.42 $\pm$ 0.09	0.18 $\pm$ 0.04	Within range (below 2%)

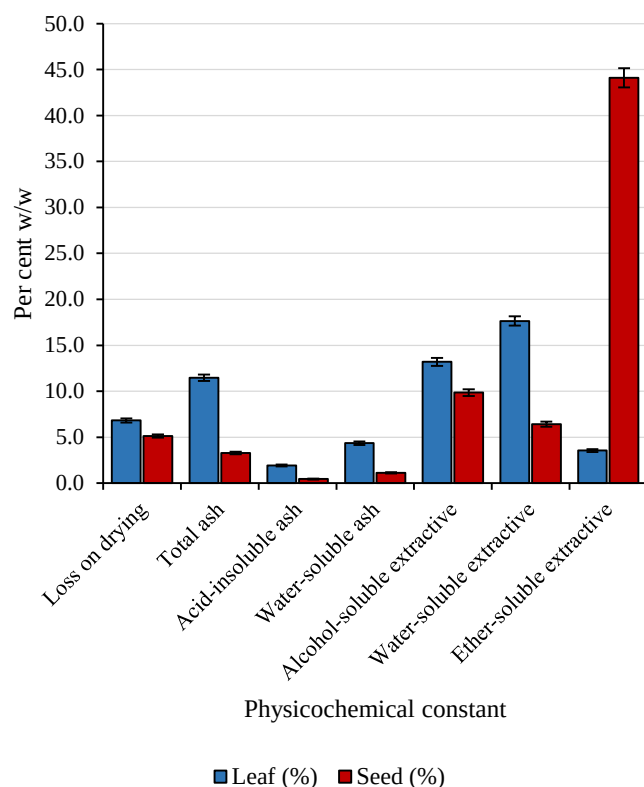


Fig. 1 Physicochemical constants of the leaf and seed of *Ricinus communis* (mean $\pm$ SD, n = 3 batches). The ether-soluble extractive value separates the two plant parts more sharply than any other constant.

3.3 Fluorescence behaviour

Fluorescence responses are given in Table 3. The bright fluorescent green produced by the leaf powder with methanolic sodium hydroxide under ultraviolet light at 365 nm was the most distinctive single response and was reproducible across all three batches, which makes it useful as a rapid comparative identity check before the more laborious chromatographic work is undertaken.

Table 3

Fluorescence analysis of leaf and seed powder

Treatment	Daylight (leaf)	UV 365 nm (leaf)	UV 365 nm (seed)
Powder as such	Dull green	Dark green	Pale brown
Powder + 1 N NaOH (aqueous)	Yellowish green	Fluorescent green	Greenish brown
Powder + 1 N NaOH (methanolic)	Deep yellow	Bright fluorescent green	Yellowish green
Powder + 1 N HCl	Light green	Dark brown	Brown
Powder + conc. H ₂ SO ₄	Brownish black	Black	Reddish black
Powder + conc. HNO ₃	Orange brown	Dark brown	Orange brown
Powder + 5% FeCl ₃	Dark green	Blackish green	Dull green
Powder + iodine solution	Brown	Dark brown	Dark brown

3.4 Extractive yields and between-batch reproducibility

Yields and their between-batch variation are given in Table 4 and shown in Fig. 2. Petroleum ether recovered 41.75% of the seed mass, a figure that corresponds closely to the ether-soluble extractive value determined independently on the powdered drug, and only 3.42% from the leaf. Ethanol recovered 12.86% from the defatted leaf marc against 8.94% for hydroethanol on the defatted seed marc. Every coefficient of variation fell below the 10% tolerance fixed in advance, and all twelve batches were accepted.

Table 4

Extractive yields and between-batch reproducibility (mean $\pm$ SD, n = 3 batches)

Extract	Solvent	Yield (%)	CV (%)	Physical description
RCLP	Petroleum ether 60-80 °C	3.42 $\pm$ 0.18	5.3	Dark green waxy semi-solid
RCLE	Ethanol (95%)	12.86 $\pm$ 0.54	4.2	Dark green sticky mass
RCSP	Petroleum ether 60-80 °C	41.75 $\pm$ 1.32	3.2	Pale yellow viscous oil
RCSE	Hydroethanol (70%)	8.94 $\pm$ 0.41	4.6	Brown amorphous solid

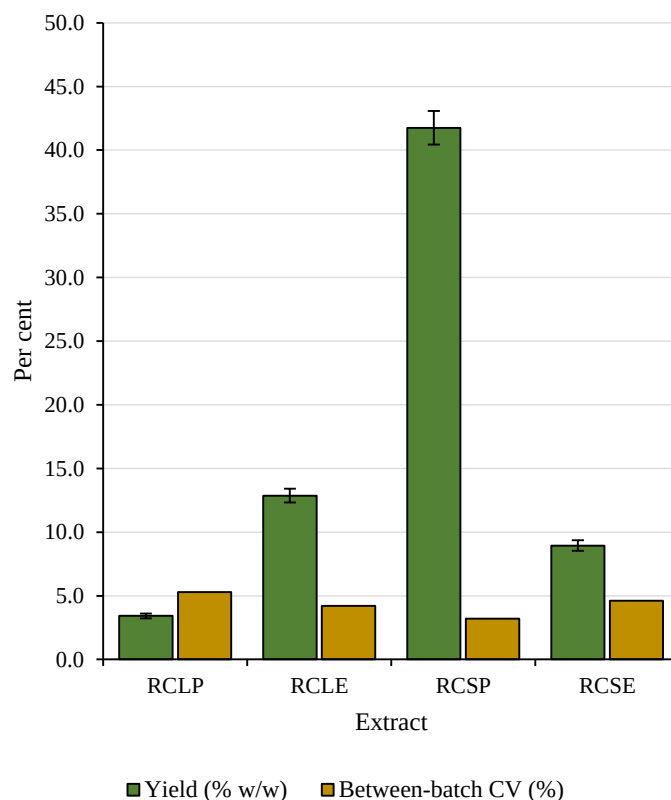


Fig. 2 Extractive yield of the four extracts with the between-batch coefficient of variation plotted on the same axis (n = 3 batches).

3.5 Qualitative class profile

The ordinal screening profile separated the extracts cleanly by solvent class (Fig. 3). The petroleum ether extracts occupied the terpenoid, steroid and fixed-oil axes almost exclusively, while the alcoholic extracts occupied the flavonoid, phenolic and alkaloid axes. The hydroethanolic seed extract was distinguished from all others by a prominent protein response, which is the earliest and cheapest signal that a seed preparation carries the protein fraction in which the toxic constituents of this species reside.

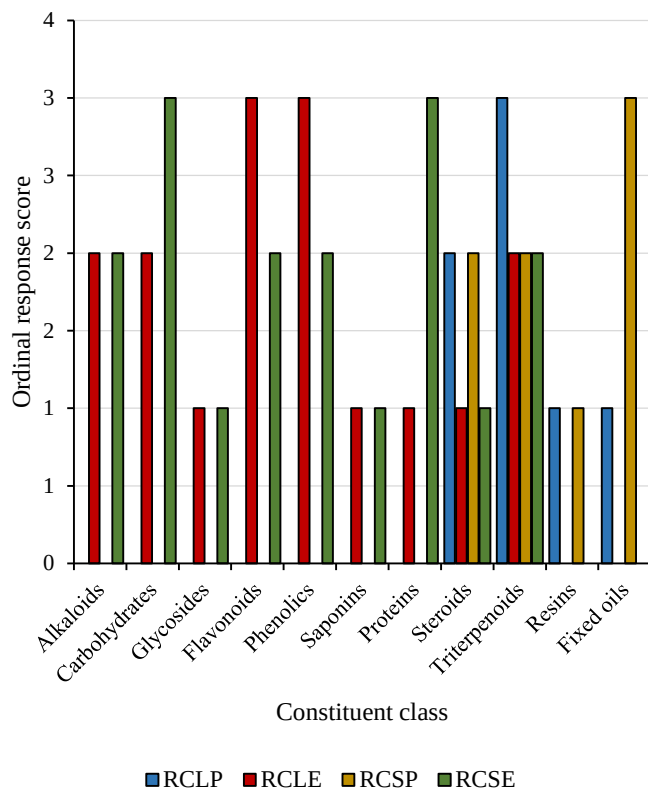


Fig. 3 Ordinal phytochemical class profile of the four extracts (0 = not detected, 1 = trace, 2 = moderate, 3 = prominent). The protein response separates RCSE from every other preparation.

3.6 Chromatographic fingerprints

Thin-layer chromatography of RCLP resolved six bands, three of them major, at retention factors of 0.62, 0.55 and 0.41, all responding violet to pink with anisaldehyde-sulfuric acid, a response characteristic of pentacyclic triterpenes. The alcoholic extracts resolved eight bands, four of which fluoresced after natural product-polyethylene glycol treatment. Between-batch reproducibility of the major bands is given in Table 5 and shown in Fig. 4.

Table 5

Between-batch reproducibility of the major chromatographic bands (n = 3 independent extraction batches)

Extract	Band	Mean R_f	Maximum deviation	CV (%)
RCLP	S2	0.62	0.02	1.9
RCLP	S3	0.55	0.01	1.2
RCLP	S4	0.41	0.02	2.6
RCLE	E3	0.71	0.02	1.7
RCLE	E4	0.63	0.01	1.1
RCLE	E5	0.52	0.02	2.3
RCSE	E3	0.71	0.02	1.8
RCSE	E5	0.52	0.02	2.4

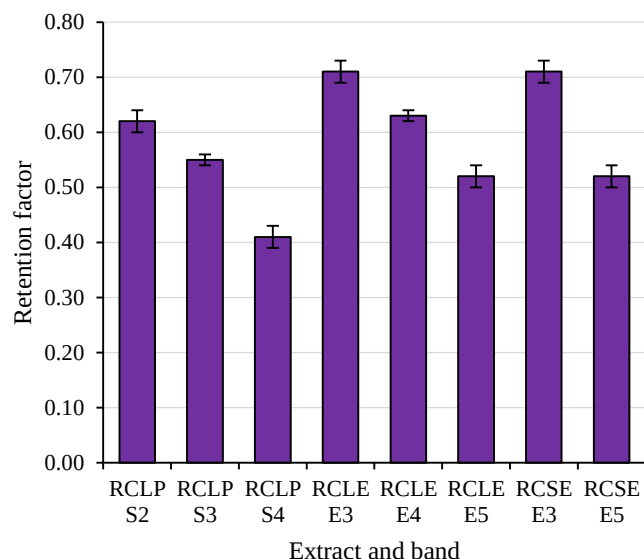


Fig. 4 Mean retention factor of the major chromatographic bands with the maximum between-batch deviation shown as error bars (n = 3 batches).

No band deviated by more than 0.02 retention-factor units from its batch mean and no coefficient of variation exceeded 2.6%. Densitometric estimation showed the relative intensity of the three major triterpenoid bands varying by less than 7% between batches. On that basis the batches were pooled.

3.7 Mass balance of the fractionation

Fractionation of the leaf petroleum ether extract is reported as a mass balance in Table 6. Of the 4.2 g loaded, 62.5% was recovered in the pooled fractions, 5.4% was obtained as characterized pure compounds and 9.3% remained as an unresolved mixture. Reporting the balance in this form makes plain what a list of isolated compounds conceals: the greater part of the extract remains chemically undefined, and any statement about the composition of the material must accommodate that residue.

Table 6

Mass balance of the column fractionation of the leaf petroleum ether extract

Item	Mass (mg)	Per cent of load	Status
Extract loaded on the column	4200	100.0	Reference
Recovered in pooled fractions F1 to F7	2626	62.5	Recovered
Compounds isolated in pure form (RC-1 to RC-5)	227	5.4	Characterized
Fraction F6, unresolved mixture	392	9.3	Uncharacterized
Fraction F1, waxy residue not examined	186	4.4	Not examined
Not recovered from the column	1574	37.5	Unaccounted

3.8 Quantitative phytochemistry and marker content

Gallic acid gave a linear response over 10 to 100 $\mu\text{g/mL}$ with $y = 0.0091x + 0.0214$ and a coefficient of determination of 0.9987;

quercetin gave $y = 0.0126x + 0.0158$ with 0.9974 (Fig. 5). The ethanolic leaf extract was the richest preparation, containing 86.4 ± 2.9 mg gallic acid equivalents and 42.7 ± 1.8 mg quercetin equivalents per gram, against 38.5 ± 1.6 and 15.3 ± 0.9 mg/g for the hydroethanolic seed extract. Marker content and the outcome of batch release testing are given in Table 7. Marker content varied by less than 6% across the batches of each preparation, and every batch satisfied all six release criteria.

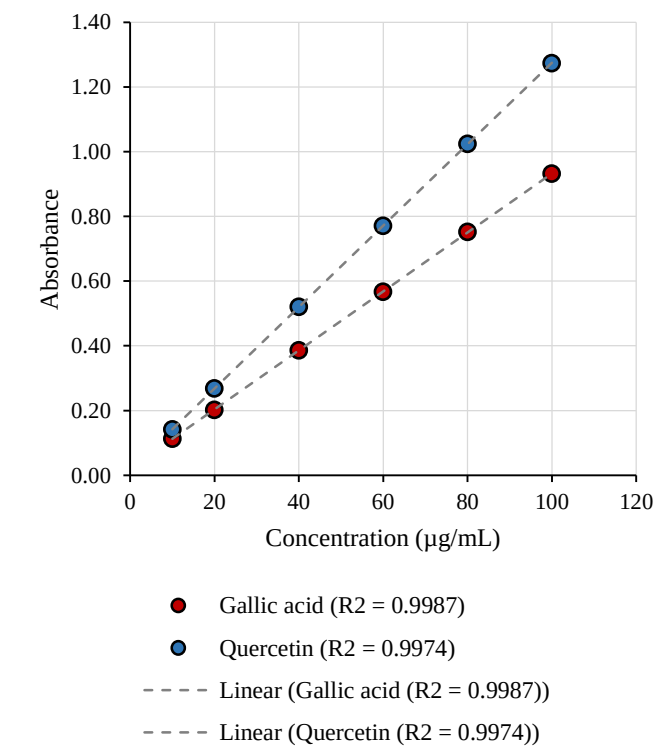


Fig. 5 Calibration curves for gallic acid and quercetin, each determined in triplicate over 10 to 100 µg/mL, with linear regression and coefficient of determination.

Table 7
Marker content and batch release outcome for the standardized preparations

Preparation	Batch	Marker	Marker content (mg/g)	Total phenolic (mg GAE/g)	Released
RCLE	L-01	Apigenin	2.86 ± 0.14	86.4 ± 2.9	Yes
RCLE	L-02	Apigenin	2.74 ± 0.12	84.9 ± 2.7	Yes
RCLE	L-03	Apigenin	2.91 ± 0.15	87.6 ± 3.1	Yes
RCSE	S-01	Apigenin	1.12 ± 0.09	38.5 ± 1.6	Yes
RCSE	S-02	Apigenin	1.08 ± 0.07	37.4 ± 1.5	Yes
RCSE	S-03	Apigenin	1.19 ± 0.10	39.6 ± 1.7	Yes
TRF	T-01	Lupeol	184.6 ± 6.2	Not applicable	Yes
TRF	T-02	Lupeol	179.2 ± 5.8	Not applicable	Yes

3.9 Batch release criteria

The six criteria applied, the basis of each and the action taken on failure are given in Table 8. Fixing them in writing before the work began is what distinguishes a release decision from a retrospective justification, and it is the reason the two batches that fell outside tolerance during method development could be quarantined and investigated rather than quietly blended with conforming material.

Table 8
Batch release criteria applied to every extract entering pharmacological study

Criterion	Basis of assessment	Action on failure
Appearance and organoleptic character	Visual comparison against the reference batch	Investigate; reject if unexplained
Extractive yield	Within the tolerance established across replicate batches	Investigate the extraction record
Chromatographic fingerprint	Retention factors and band pattern matching the reference chromatogram	Reject batch
Marker content	Densitometric quantification against the isolated reference compound	Reject batch
Loss on drying and residual solvent	Gravimetric determination	Re-dry and re-test
Storage stability	Fingerprint and marker re-examined at the defined interval	Restrict shelf life

3.10 Storage stability

Marker retention over 90 days at 2-8 °C is shown in Fig. 6. The alcoholic extracts lost 5.9% (RCLE) and 6.8% (RCSE) of marker content over the interval, with total phenolic content falling slightly faster at 7.4 and 8.5% respectively. The triterpenoid-rich fraction lost only 1.4%. Chromatographic fingerprints were unchanged at day 90 in every preparation. A 60-day shelf life was adopted for the alcoholic extracts, at which point marker loss remained below 5%, and 90 days for the triterpenoid-rich fraction.

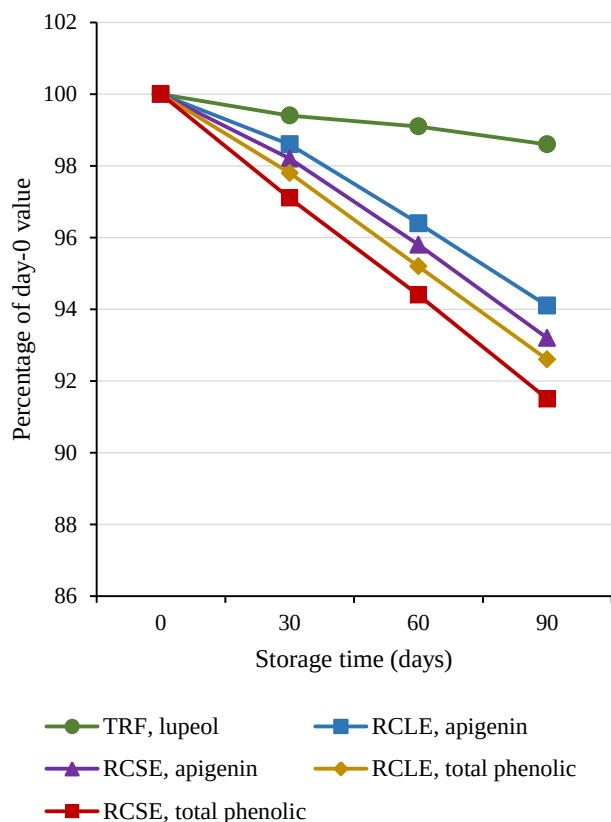


Fig. 6 Retention of marker and total phenolic content during storage at 2-8 °C, expressed as a percentage of the day-0 value.

4 Discussion:

4.1 What the constants establish and what they do not

The pharmacognostic constants reported here place the material within the ranges ordinarily accepted for crude drugs of these types and, more usefully, fix numerical criteria against which a future batch can be judged. Their value is comparative rather than absolute. A total ash of 11.46% for the leaf is unremarkable in itself; what matters is that the three collections agreed within 3.0%, so that a future batch falling outside that band signals a change in the material rather than noise in the method.

Two limits should be stated. Ash and extractive values are aggregate measures. They would not detect a shift in the proportion of one constituent class relative to another, which is precisely the change most likely to alter a pharmacological result. That question is answered only by the chromatographic fingerprint and the marker quantification, which is why both were made release criteria rather than optional refinements. The leaf constants, likewise, establish anatomical identity and not chemical equivalence.

4.2 Fingerprint reproducibility as the load-bearing measurement

The single most consequential number in this study is the 0.02 retention-factor unit maximum deviation across batches, with coefficients of variation between 1.1 and 2.6%. Reproducibility at that level is what allows independently prepared batches to be pooled, and pooling is what allows a dose to mean the same thing on the first day of an animal experiment and on the last. Where fingerprints drift between batches, an apparent dose-response relationship may in fact reflect a composition-response relationship, and no amount of statistical treatment applied afterwards can separate the two.

The densitometric finding that the relative intensity of the three major triterpenoid bands varied by less than 7% between batches adds a second dimension to the same argument. Retention-factor

agreement establishes that the same constituents were present; intensity agreement establishes that they were present in similar proportion. Both are needed before batches can be treated as interchangeable.

4.3 The case for reporting a mass balance

Fractionation results are conventionally reported as a list of isolated compounds with their yields. That convention flatters the work. In the present fractionation, 5.4% of the loaded extract was recovered as characterized pure compounds, 9.3% remained as an unresolved mixture and 37.5% was not recovered from the column at all. A reader given only the compound list would reasonably infer that the chemistry of the extract had been resolved, when what has been resolved is the identity of its most abundant crystallizable constituents.

The distinction matters for attribution. When a pooled fraction shows pharmacological activity, the activity belongs to whatever is in that fraction, not to the compounds that happened to crystallize from it. Publishing the balance makes the strength of any subsequent attribution visible to the reader instead of leaving it to be assumed, and the cost of doing so is a single table.

4.4 Marker selection and the value of a stability figure

Lupeol and apigenin were selected on abundance, chromatographic resolution and stability rather than on pharmacological interest. That order of criteria is deliberate. A marker is an index of batch identity, and a compound chosen because it is thought to be active will often prove to be present in quantities too small, or too labile, to serve that purpose reliably.

The stability data justify the choice and also qualify it. The triterpenoid-rich fraction retained 98.6% of its lupeol content over 90 days, which is consistent with the chemical stability of a pentacyclic triterpene in a protein-free, largely nonpolar matrix. The alcoholic extracts retained 93 to 94% of apigenin over the same interval, and total phenolic content fell slightly faster still, which is the expected behaviour of a phenol-rich mixture in which oxidation proceeds slowly even under refrigeration. A 6 to 7% drift is small, but across a long experimental programme run from a single batch it would introduce a systematic difference between animals dosed early and animals dosed late. Setting a 60-day shelf life and preparing fresh batches for each experimental block removes that confound at negligible cost.

4.5 The protein response as an early safety signal

The prominent protein response of the hydroethanolic seed extract on qualitative screening is the only result in this study that bears directly on safety, and it is worth reporting for that reason. Castor seed is the tissue in which ricin and the allergenic 2S albumins are concentrated (Abdul et al., 2018; Abomughaid et al., 2024), and a hydroethanolic extract is precisely the preparation in which residual toxic protein would be expected to appear. The Biuret, Millon and ninhydrin tests cost almost nothing and were the earliest indication that the seed preparation would need to be treated differently from the leaf preparation. Investigators working with seed extracts of this species would do well to report protein content alongside the phytochemical profile as a matter of routine.

4.6 Implications for reporting practice

Four items would materially improve the comparability of published work on this species, and none of them requires equipment or effort beyond what a study of this kind already expends.

- Report the voucher number, the collection site and the collection date, and carry the voucher number onto the batch record so that the chain from population to administered dose remains traceable.

- Report the between-batch variation of yield, fingerprint and marker content rather than a single mean, because it is the variation and not the mean that tells a reader whether a dose was reproducible.
- Report a mass balance for any preparative fractionation, so that the proportion of the material actually characterized is visible.
- Report the storage interval and temperature at which the tested material was held, and the marker retention over that interval, because an extract dosed on day 90 of a programme is not necessarily the extract that was released on day 0.

4.7 Limitations

The framework reported here is a quality framework and not a chemical resolution of the material. Thin-layer chromatography with densitometric estimation is adequate for fingerprint comparison but cannot quantify constituents present at low concentration, and a hyphenated technique such as liquid chromatography coupled to mass spectrometry would extend the description considerably. Only one marker was quantified per preparation; a multi-marker approach would be more informative and is the obvious next step. Stability was assessed at a single storage temperature over 90 days, so the data support a refrigerated shelf life and say nothing about ambient storage or about longer intervals. The constants and fingerprints apply in the first instance to material from the collection site studied, and their extension to other provenances requires independent measurement rather than assumption.

Conclusion:

Authenticated leaf and seed material of *Ricinus communis* from three independent collections proved consistent within 7.4% on every quantitative leaf constant, within 3.2% on physicochemical constants, and within 5.3% on extractive yield. Thin-layer chromatographic fingerprints were reproducible to within 0.02 retention-factor units, and the content of the selected markers, lupeol for the nonpolar preparations and apigenin for the alcoholic extracts, varied by less than 6% between batches. Over 90 days at 2-8 °C the triterpenoid-rich fraction retained 98.6% of its marker content and the alcoholic extracts 93 to 94%, supporting a 60-day shelf life for the latter. A full mass balance of the fractionation showed that 5.4% of the loaded extract was recovered as characterized compounds and 37.5% was not recovered at all.

None of these figures is a pharmacological result, and that is the point. They are the conditions under which a pharmacological result obtained from this material could be reproduced by another laboratory, and they cost a small fraction of the effort that the pharmacology itself demands.

The framework also produced one finding of direct practical consequence. The protein response of the hydroethanolic seed extract, obtained from three inexpensive colour tests, identified before any animal work that the seed preparation would need to be handled and dosed differently from the leaf preparation. Quality control is usually justified on grounds of reproducibility alone; in a species that carries a potent toxin in one of its organs, it also functions as an early safety screen. Reporting these measurements should be ordinary practice rather than a distinguishing feature of a study.

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