

Leaf triterpenoids of *Ricinus communis* L. outperform the seed extract: Bioactivity-guided isolation and preclinical evaluation of hepatoprotective, anti-inflammatory and analgesic activity

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ARTICLE INFO	ABSTRACT
Article history: Received – 03 March 2026 Received in revised form – 25 March 2026 Accepted – 26 March 2026 Available online – 05 April 2026	<i>Ricinus communis</i> L. (Euphorbiaceae) carries both a substantial ethnomedicinal record and a serious toxicological liability, since its seed contains ricin. Published pharmacological studies differ in plant part, solvent, standardization and dose, and few report a quantified chemical marker for the preparation tested, which makes their results difficult to compare. We applied a sequential, gated design in which botanical authentication preceded chemical work, chemical characterization preceded toxicological screening, and toxicological screening preceded efficacy testing. Leaf and seed material were extracted separately along a polarity gradient. Nine constituents were isolated from the leaf and identified by convergent chromatographic and spectroscopic evidence: alpha-amyrin acetate, beta-amyrin acetate, lupeol, beta-sitosterol, stigmaterol, apigenin, and salicylic, vanillic and syringic acids. Extracts were standardized against a chromatographic marker and released only against written batch criteria. The ethanolic leaf extract and a triterpenoid-rich fraction were tolerated at 2000 mg/kg, whereas the hydroethanolic seed extract produced mortality at that dose. In a carbon tetrachloride model the seed extract afforded 75.0% protection against the rise in alanine aminotransferase at 200 mg/kg, against 85.9% for silymarin, with concordant improvement in oxidative-stress markers and blinded histopathology. The triterpenoid-rich fraction inhibited carrageenan-induced paw edema by 50.8% at 50 mg/kg, exceeding the crude leaf extract given at eight times that dose, and was active in both thermal and chemical nociception models. Phenolic content tracked the hepatoprotective endpoints and triterpenoid content tracked the anti-inflammatory and analgesic endpoints. On a risk-weighted view the leaf-derived triterpenoid fraction, and not the seed extract, is the preparation that merits further investigation.
Keywords: <i>Ricinus communis</i>, Triterpenoids, Hepatoprotection, Anti-inflammatory activity Antinociception, Extract standardization,	

1. Introduction

Ricinus communis L. (Euphorbiaceae), the castor plant, is cultivated throughout the tropics and subtropics and has a long record of use in Ayurveda, Unani and other traditional systems for constipation, inflammation, fever and skin disorders (Kumar, 2017; Abdul et al., 2018). Modern work attributes antioxidant, anti-inflammatory, antinociceptive, antimicrobial and hepatoprotective effects to extracts of its leaves, roots and seeds (Ilavarasan et al., 2006; Taur et al., 2011; Nemudzivhadi & Masoko, 2014; Esfandiyari et al., 2018; Abomughaid et al., 2024). Reported constituents span triterpenoids, sterols, flavonoids, phenolic acids and the pyridine alkaloid ricinine (Singh et al., 2009; Farooq et al., 2018).

Two problems limit what this body of work can support. The first is comparability. Studies differ in plant part, solvent system, extract standardization, dose, animal model and reference drug, and few report a chromatographic marker or a quantified marker content for the material actually administered. An extract is not a chemical

entity; its activity may arise from one dominant constituent, from several acting additively, from synergy, or from nonspecific toxicity. Where the preparation is not chemically defined, a pharmacological result obtained from it cannot be attributed to any constituent and cannot be reproduced from the published description (Mukherjee, 2002; World Health Organization, 2011).

The second is the coexistence of therapeutic promise and hazard within one plant. Castor seed contains ricin, a type 2 ribosome-inactivating protein, together with allergenic 2S albumins responsible for occupational sensitization (Abomughaid et al., 2024). A seed preparation may therefore be pharmacologically active and simultaneously unsuitable for development, and a study that reports efficacy without a matched toxicity assessment gives an incomplete account of it.

We addressed both problems with a design in which each stage gates the next. Leaf and seed were processed in parallel but strictly

separate streams so that part-specific conclusions remained available. Extraction followed a polarity gradient, so that the nonpolar terpenoid fraction and the polar phenolic fraction could be tested independently rather than as a single mixture. Extracts entering animal work were standardized against a marker and released only against written criteria. Acute oral toxicity was assessed before any efficacy experiment and fixed the doses used. Three therapeutic domains were then examined: hepatoprotection in a carbon tetrachloride model, acute inflammation by carrageenan-induced paw edema, and nociception by complementary thermal and chemical tests.

The aim was to determine which chemically defined fraction of *R. communis* carries which activity, and to place any efficacy finding against the tolerability of the preparation that produced it.

2. Materials and methods:

2.1 Plant material and pharmacognostic evaluation

Mature leaves and fully developed seeds were collected from a documented, pesticide-free site, from several individuals across the population. Material was authenticated by a plant taxonomist and a voucher specimen was deposited in a recognized herbarium; the voucher number was quoted on every batch record. Leaves and seeds were cleaned, shade-dried to constant mass, powdered, sieved and stored in labelled airtight containers. Macroscopic and microscopic characters, quantitative leaf constants, loss on drying, ash values, extractive values, swelling index and fluorescence behaviour were recorded for each of three collection batches (Khandelwal, 2008; Trease & Evans, 2009).

2.2 Extraction

Powdered material was first extracted with petroleum ether (60-80 °C) to recover fixed oils, waxes, sterols and free triterpenoids. The defatted marc was air-dried and then extracted with ethanol for the leaf and with 70% hydroethanol for the seed. Extractives were filtered, concentrated under reduced pressure at a bath temperature limited to protect thermolabile constituents, and dried to constant mass. Four extracts resulted: leaf petroleum ether (RCLP), leaf ethanolic (RCLE), seed petroleum ether (RCSP) and seed hydroethanolic (RCSE). Yield was calculated as the mass of dried extract as a percentage of the powdered material extracted, and extraction was performed in three independent batches.

2.3 Phytochemical screening and chromatography

Extracts were screened qualitatively for alkaloids, carbohydrates, glycosides, flavonoids, phenolics and tannins, saponins, proteins, steroids, triterpenoids, resins and fixed oils by standard procedures with reagent blanks (Kokate et al., 2008). Analytical thin-layer chromatography was performed on silica gel G, using 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,

with detection under ultraviolet light and after class-specific derivatization (Stahl, 1969; Wagner & Bladt, 1996). Preparative separation used silica gel (70-230 mesh) with a stepwise gradient from petroleum ether to methanol. Fractions were pooled on the basis of matching chromatographic profiles and purified by repeated chromatography or recrystallization. A triterpenoid-rich fraction (TRF) was prepared by pooling the three fractions containing the major triterpenoid bands.

2.4 Characterization of isolated constituents

Melting points were determined in triplicate and reported uncorrected. Ultraviolet spectra were recorded in methanol with diagnostic shift reagents for flavonoids; infrared spectra by the potassium bromide pellet technique; proton and carbon-13 nuclear magnetic resonance spectra in deuterated chloroform or dimethyl sulfoxide with tetramethyl silane as internal standard; and electron impact and fast atom bombardment mass spectra as appropriate. A constituent was reported as identified only where chromatographic behaviour in two systems, melting point and the spectroscopic data were individually consistent with and collectively sufficient to establish the structure; otherwise, it was described as a tentative assignment (Harborne, 1998).

2.5 Standardization and batch release

Lupeol was used as the chromatographic marker for the nonpolar preparations and apigenin for the alcoholic extracts. Total phenolic content was determined by the Folin-Ciocalteu method and expressed as gallic acid equivalents (Singleton & Rossi, 1965); total flavonoid content by the aluminium chloride method as quercetin equivalents (Chang et al., 2002). Batches were released only when appearance, yield, chromatographic fingerprint, marker content, loss on drying and storage stability all conformed to written criteria.

2.6 Animals and ethical approval

Adult Wistar rats and Swiss albino mice were obtained from a registered breeding facility and acclimatized for at least seven days at 22 ± 3 °C and 30-70% relative humidity on a 12 h light-dark cycle with food and water ad libitum except during protocol-defined fasting. All procedures were approved by the Institutional Animal Ethics Committee and conducted under the applicable national rules (Committee for Control and Supervision of Experiments on Animals, 2026). Reporting follows the ARRIVE guidelines 2.0 (Percie du Sert et al., 2020). Group sizes were supported by an a priori power calculation.

2.7 Acute oral toxicity

Acute oral toxicity was assessed by the acute toxic class method of OECD Test Guideline 423 (Organisation for Economic Co-operation and Development, 2002). Animals were fasted overnight, dosed by oral gavage with the extract suspended in aqueous carboxymethylcellulose, and observed continuously for 30 min,

periodically for 24 h and daily for 14 days, with body mass recorded and gross necropsy performed at termination.

2.8 Hepatoprotective activity

Rats were randomized to six groups (n = 6): normal control, carbon tetrachloride control, silymarin 100 mg/kg, RCSE 100 and 200 mg/kg, and RCLE 400 mg/kg. Treatments preceded the hepatotoxicant, so that the design tested protection rather than reversal. Serum alanine aminotransferase and aspartate aminotransferase were estimated by the method of Reitman and Frankel (1957), alkaline phosphatase by Kind and King (1954) and bilirubin by Malloy and Evelyn (1937). Hepatic malondialdehyde was measured as thiobarbituric acid reactive substances (Ohkawa et al., 1979), reduced glutathione with 5,5'-dithiobis-2-nitrobenzoic acid (Ellman, 1959), superoxide dismutase by pyrogallol autoxidation (Marklund & Marklund, 1974) and catalase by hydrogen peroxide decomposition (Aebi, 1984). Liver sections were stained with haematoxylin and eosin and scored blind on an ordinal scale (Bancroft & Gamble, 2008).

2.9 Anti-inflammatory activity

Carrageenan-induced hind-paw edema was used as the primary acute-inflammation model (Winter et al., 1962). Rats (n = 6) received vehicle, diclofenac sodium 10 mg/kg, RCLE 200 or 400 mg/kg, or TRF 50 mg/kg orally at a fixed interval before sub-plantar carrageenan. Paw volume was measured at baseline and at 1, 2, 3 and 4 h by a single operator using a calibrated plethysmometer.

2.10 Analgesic activity

Thermal nociception was assessed by the hot plate test at 55 ± 0.5 °C with a 20 s cut-off (Eddy & Leimbach, 1953) and chemical nociception by acetic acid-induced writhing counted over 20 min (Koster et al., 1959). Mice (n = 6) received vehicle, pentazocine 10 mg/kg or aspirin 100 mg/kg as reference, RCSE 100 or 200 mg/kg, or TRF 50 mg/kg.

2.11 Bias control and statistical analysis

Allocation followed a computer-generated random sequence produced by a person not involved in outcome assessment. Treatment preparation and outcome assessment were carried out by different investigators, and samples, cages and tissue cassettes carried coded identifiers only. Exclusion criteria were recorded in writing before each experiment. Data are expressed as mean $\pm$ SD. Assumptions were checked by the Shapiro-Wilk and Levene tests; group comparisons used one-way analysis of variance with Dunnett or Tukey post hoc tests, repeated measurements a repeated-measures model, and ordinal scores the Kruskal-Wallis test with Dunn correction. A two-sided $p < 0.05$ was taken as significant.

3. Result:

3.1 Extraction, screening and standardization

Extractive yields and the phenolic and flavonoid content of the four extracts are given in Table 1 and compared in Fig. 1. Petroleum ether

recovered 41.75% of the seed mass, consistent with the ether-soluble extractive value determined independently on the powdered drug, against 3.42% from the leaf. Ethanol recovered 12.86% from the defatted leaf marc, the richest polar fraction obtained. Between-batch coefficients of variation ranged from 3.2 to 5.3%.

Table -1

Extractive yield, total phenolic content and total flavonoid content of the four extracts (mean $\pm$ SD, n = 3 batches)

Extract	Yield (% w/w)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
RCLP (leaf, petroleum ether)	3.42 $\pm$ 0.18	9.2 $\pm$ 0.6	4.1 $\pm$ 0.4
RCLE (leaf, ethanolic)	12.86 $\pm$ 0.54	86.4 $\pm$ 2.9	42.7 $\pm$ 1.8
RCSP (seed, petroleum ether)	41.75 $\pm$ 1.32	4.8 $\pm$ 0.4	2.2 $\pm$ 0.3
RCSE (seed, hydroethanolic)	8.94 $\pm$ 0.41	38.5 $\pm$ 1.6	15.3 $\pm$ 0.9

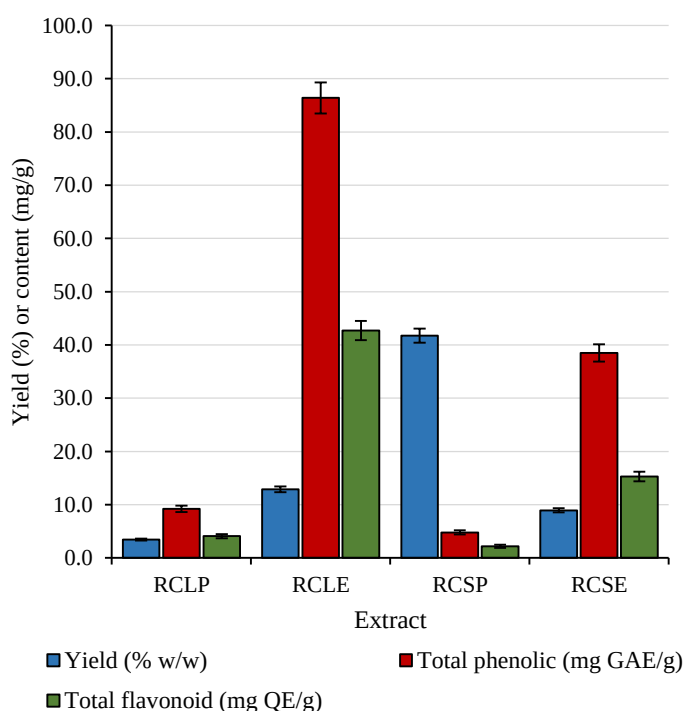


Fig. 1 Extractive yield and phenolic composition of the four extracts of *Ricinus communis* leaf and seed (mean $\pm$ SD, n = 3 batches). GAE gallic acid equivalents, QE quercetin equivalents.

Qualitative screening separated the extracts cleanly by solvent class. The petroleum ether extracts responded strongly in the Liebermann-Burchard and Noller tests and gave no response for polar constituents, while the alcoholic extracts responded for flavonoids, phenolics and alkaloids. The hydroethanolic seed extract gave a prominent protein response, which the acute toxicity result reported in Section 3.3 later placed in context.

Thin-layer chromatography of RCLP resolved six bands, three of them major, at retention factors of 0.62, 0.55 and 0.41; the alcoholic extract resolved eight, four of which fluoresced after treatment with natural product-polyethylene glycol reagent. Fingerprints were reproducible across the three extraction batches, no band deviating by more than 0.02 retention-factor units from its batch mean. Marker content varied by less than 6% between batches, and all batches satisfied the release criteria.

3.2 Isolated constituents

Nine constituents were obtained from the leaf and identified on convergent evidence (Table 2). All five nonpolar candidates named in the literature for this species were recovered in quantities sufficient for full spectroscopic characterization. No indole alkaloid was obtained, and no constituent was isolated from either seed extract in a state suitable for characterization. Of the 4.2 g of petroleum ether extract loaded, 5.4% was recovered as characterized pure compounds and 37.5% was not recovered.

Table -2

Constituents isolated from *Ricinus communis* leaf and the evidence supporting each identification

Code	Identified as	Class	Yield (mg)	m.p. (°C)	Basis of identification
RC-1	alpha-Amyrin acetate	Triterpenoid	42	224-226	m.p., IR, ¹ H, ¹³ C, MS
RC-2	beta-Amyrin acetate	Triterpenoid	38	240-242	m.p., IR, ¹ H, ¹³ C, MS
RC-3	Lupeol	Triterpenoid	56	214-216	m.p., IR, ¹ H, ¹³ C, MS
RC-4	beta-Sitosterol	Steroid	64	136-138	m.p., mixed m.p., IR, MS
RC-5	Stigmasterol	Steroid	27	168-170	m.p., IR, ¹ H, MS
RC-6	Apigenin	Flavone	22	345-347	UV with shift reagents, ¹ H, MS
RC-7	Salicylic acid	Phenolic acid	14	158-160	m.p., UV, IR, MS, co-TLC
RC-8	Vanillic acid	Phenolic acid	18	210-212	m.p., UV, IR, MS, co-TLC
RC-9	Syringic acid	Phenolic acid	12	204-206	m.p., UV, IR, MS, co-TLC

3.3 Acute oral toxicity and dose selection

RCLE and TRF produced no mortality and no clinical sign at 2000 mg/kg. RCSE killed two of three animals at that dose, with piloerection, hypoactivity and diarrhoea preceding death, and was tolerated at 300 mg/kg. Cut-off values and the doses selected for efficacy testing are given in Table 3.

Table- 3

Acute oral toxicity outcome and doses selected for efficacy testing

Preparation	Dose tested (mg/kg)	Mortality and clinical signs	Cut-off value (mg/kg)	Efficacy doses (mg/kg)
RCLE	2000	0/3; no signs	> 2000	200 and 400
RCSE	2000	2/3; piloerection, hypoactivity, diarrhoea	> 300 and ≤ 2000	100 and 200
RCSE	300	0/3; no signs	> 300 and ≤ 2000	100 and 200
TRF	2000	0/3; transient hypoactivity at 2 h	> 2000	50

3.4 Hepatoprotective activity

Carbon tetrachloride raised serum alanine aminotransferase 4.4-fold, aspartate aminotransferase 3.0-fold and alkaline phosphatase 2.7-fold, and raised total bilirubin from 0.42 to 1.86 mg/dL, with corresponding falls in total protein and albumin (Table 4, Fig. 2). All treated groups differed from the toxic control ($p < 0.01$ for silymarin, RCSE 200 and RCLE 400; $p < 0.05$ for RCSE 100). The effect of the seed extract was dose-related, protection of alanine aminotransferase rising from 47.1% at 100 mg/kg to 75.0% at 200 mg/kg against 85.9% for silymarin.

Table- 4

Effect of the extracts on serum markers of hepatic injury in carbon tetrachloride-treated rats (mean ± SD, n = 6)

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)	Albumin (g/dL)
Normal control	42.6 ± 3.4	96.3 ± 6.1	128.5 ± 8.2	0.42 ± 0.05	4.12 ± 0.18
CCl ₄ control	186.4 ± 11.2	292.5 ± 15.8	342.7 ± 18.6	1.86 ± 0.14	2.54 ± 0.15
Silymarin 100 mg/kg	62.8 ± 5.1	128.4 ± 9.2	171.2 ± 10.4	0.68 ± 0.07	3.78 ± 0.17
RCSE 100 mg/kg	118.7 ± 8.3	205.6 ± 12.4	253.8 ± 14.1	1.24 ± 0.11	3.02 ± 0.14
RCSE 200 mg/kg	78.5 ± 6.0	148.9 ± 10.1	196.4 ± 11.7	0.86 ± 0.08	3.51 ± 0.16
RCLE 400 mg/kg	92.4 ± 7.1	172.3 ± 11.0	219.6 ± 12.9	1.02 ± 0.09	3.28 ± 0.15

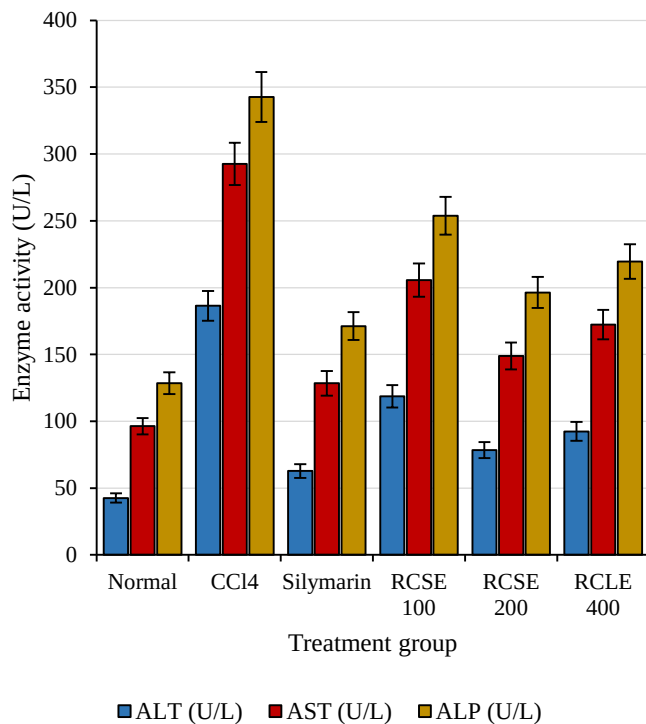


Fig. 2 Serum marker enzyme activity across treatment groups (mean $\pm$ SD, n = 6). ALT alanine aminotransferase, AST aspartate aminotransferase, ALP alkaline phosphatase.

Hepatic malondialdehyde rose 3.7-fold in the toxic control while reduced glutathione, superoxide dismutase and catalase fell to 38, 38 and 39% of normal respectively. Treatment restored all four towards normal in a dose-related manner (Table 5, Fig. 3). Blinded histopathological scoring agreed with the biochemical result: the toxic control scored 3.7 of 4, silymarin 0.8, RCSE 200 mg/kg 1.3 and RCLE 400 mg/kg 1.8.

Table- 5

Hepatic oxidative stress markers and blinded histopathological injury score (mean $\pm$ SD; score as median, n = 6)

Group	MDA (nmol/mg protein)	GSH (μ g/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	Injury score
Normal control	1.24 $\pm$ 0.11	32.6 $\pm$ 2.1	14.8 $\pm$ 1.1	62.4 $\pm$ 4.2	0.2
CCl ₄ control	4.62 $\pm$ 0.32	12.4 $\pm$ 1.2	5.6 $\pm$ 0.6	24.6 $\pm$ 2.3	3.7
Silymarin 100 mg/kg	1.78 $\pm$ 0.15	28.4 $\pm$ 2.0	12.9 $\pm$ 1.0	55.8 $\pm$ 3.9	0.8
RCSE 100 mg/kg	3.05 $\pm$ 0.24	19.6 $\pm$ 1.6	8.4 $\pm$ 0.8	36.2 $\pm$ 3.0	2.3
RCSE 200 mg/kg	2.14 $\pm$ 0.18	25.8 $\pm$ 1.9	11.2 $\pm$ 0.9	48.5 $\pm$ 3.5	1.3
RCLE 400 mg/kg	2.56 $\pm$ 0.21	22.4 $\pm$ 1.7	9.8 $\pm$ 0.8	41.7 $\pm$ 3.2	1.8

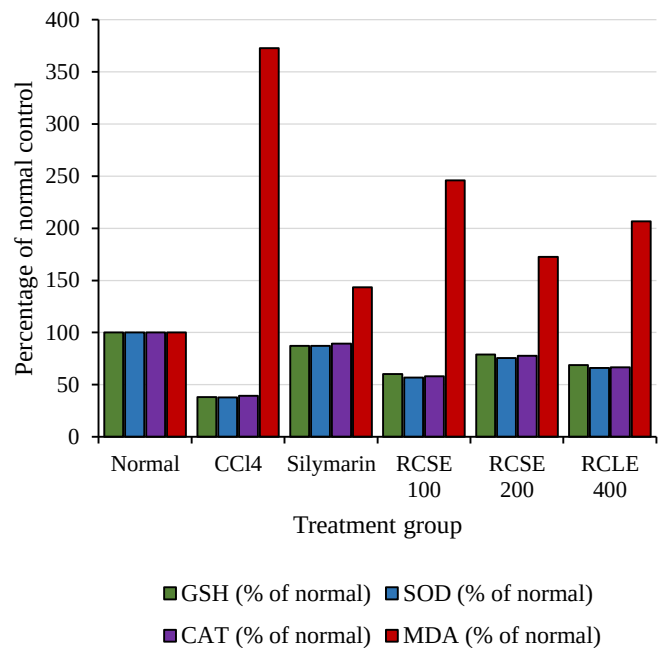


Fig. 3 Hepatic antioxidant status expressed as a percentage of the normal control, with the malondialdehyde concentration shown on the same scale (n = 6). GSH reduced glutathione, SOD superoxide dismutase, CAT catalase, MDA malondialdehyde.

3.5 . Anti-inflammatory activity

Paw edema in the vehicle group rose progressively to 0.68 mL at 4 h. All treated groups differed from vehicle from the second hour ($p < 0.01$), and the effect of the leaf extract was dose-related (Fig. 4). Inhibition at 3 h was 60.7% for diclofenac, 50.8% for TRF at 50 mg/kg, 44.3% for RCLE at 400 mg/kg and 27.9% for RCLE at 200 mg/kg. Expressed per unit mass administered, the fraction was approximately nine times as active as the crude extract.

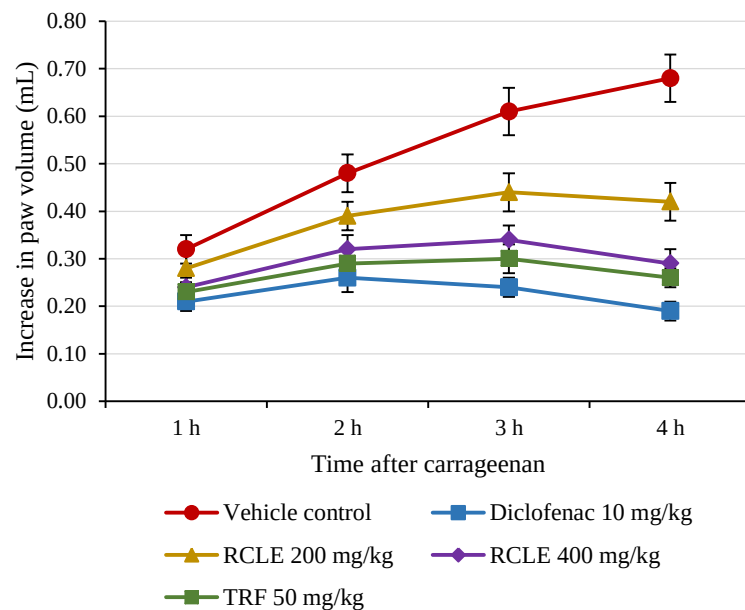


Fig. 4 Time course of carrageenan-induced hind-paw edema (mean $\pm$ SD, n = 6). TRF triterpenoid-rich fraction, RCLE leaf ethanolic extract.

3.6 Analgesic activity

Both nociception models showed activity (Table 6, Fig. 5). On the hot plate, maximum possible effect at 60 min was 51.6% for pentazocine, 33.3% for RCSE at 200 mg/kg, 25.3% for TRF at 50 mg/kg and 20.8% for RCSE at 100 mg/kg. In the writhing test, inhibition was 65.4% for aspirin, 51.3% for RCSE at 200 mg/kg, 44.6% for TRF and 31.4% for RCSE at 100 mg/kg. On a dose-normalized basis the fraction was three to six times as active as the crude seed extract.

Table- 6

Analgesic activity in the hot plate and acetic acid writhing tests (mean $\pm$ SD, n = 6)

Group	Latency at 60 min (s)	Maximum possible effect (%)	Writhes in 20 min	Writhing inhibition (%)
Vehicle control	4.3 $\pm$ 0.5	-	62.4 $\pm$ 4.2	-
Pentazocine 10 mg/kg	12.4 $\pm$ 0.9	51.6	-	-
Aspirin 100 mg/kg	-	-	21.6 $\pm$ 2.4	65.4
RCSE 100 mg/kg	7.4 $\pm$ 0.6	20.8	42.8 $\pm$ 3.6	31.4
RCSE 200 mg/kg	9.6 $\pm$ 0.7	33.3	30.4 $\pm$ 2.9	51.3
TRF 50 mg/kg	8.2 $\pm$ 0.6	25.3	34.6 $\pm$ 3.1	44.6

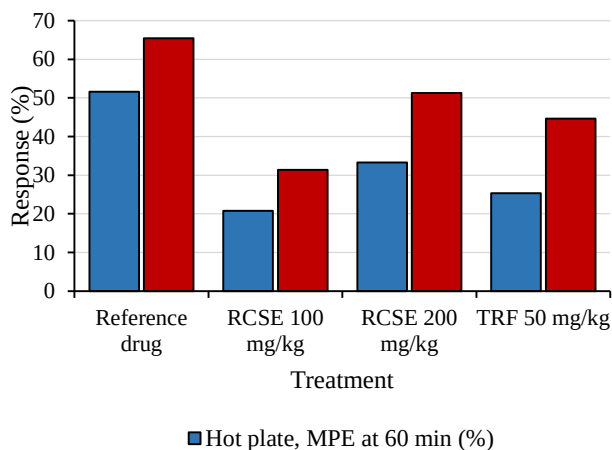


Fig. 5 Analgesic activity of the preparations expressed as maximum possible effect on the hot plate at 60 min and as inhibition of acetic acid-induced writhing (n = 6).

3.7 Relationship between composition and activity

Across the preparations tested, total phenolic content correlated with protection of alanine aminotransferase ($r = 0.91$) and total flavonoid content with restoration of hepatic glutathione ($r = 0.86$), while lupeol content correlated with inhibition of paw edema at 3 h ($r = 0.88$) and with maximum possible effect ($r = 0.79$). The cross-associations were weak, phenolic content against edema inhibition giving $r = 0.34$ (Fig. 6). With four preparations and two degrees of

freedom these coefficients describe the material tested and are not estimates of a population parameter.

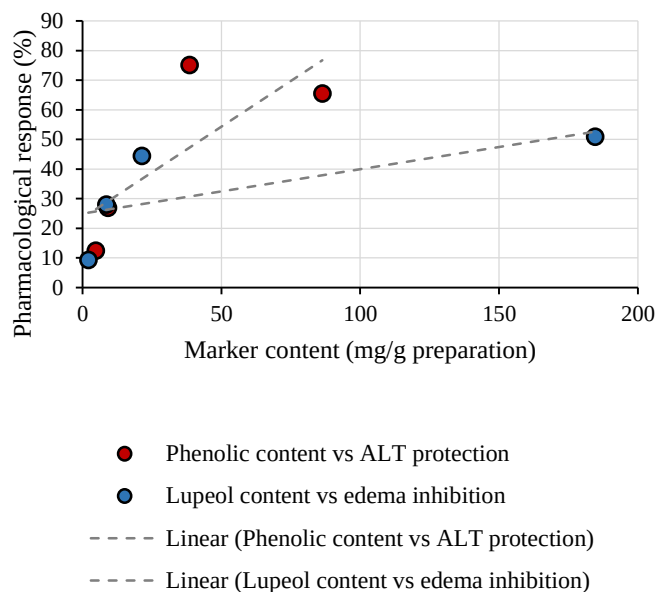


Fig. 6 Association between compositional markers and pharmacological response across the preparations tested.

Phenolic content tracks hepatoprotection while lupeol content tracks inhibition of edema.

4. Discussion:

4.1 Chemistry and pharmacology track together

The value of separating the extracts by polarity shows in the pattern of results rather than in any single measurement. The petroleum ether extract of the leaf was triterpenoid-rich on screening, yielded three pentacyclic triterpenes and two sterols on fractionation, and produced the strongest anti-inflammatory and dose-normalized analgesic responses. The ethanolic leaf extract was phenol-rich, gave the highest phenolic and flavonoid values, and was associated with restoration of hepatic glutathione and antioxidant enzyme activity. Neither chain proves causation, but each activity tracks the fraction whose chemistry would predict it, and the cross-associations are weak; a preparation that was simply more potent in every respect would have produced uniform associations along every axis.

The single most informative comparison is between the triterpenoid-rich fraction at 50 mg/kg and the crude ethanolic extract at 400 mg/kg. The fraction inhibited edema more effectively at every observation time at one-eighth of the dose. Comparisons of this kind, in which a defined fraction and the crude extract from which it derives are tested in the same experiment under one protocol, are uncommon in the literature on this species, where crude-extract screening predominates. They move the evidence from the observation that a plant contains something active to a statement about where in its chemistry that activity resides.

4.2 Mechanistic interpretation and its limits

The time course of edema inhibition, rising from 28.1% at 1 h to 61.8% at 4 h for the fraction, follows the shape produced by the cyclo-oxygenase inhibitor used as reference and points to an action on the later, prostaglandin-dependent phase of the response rather than on early mediator release (Winter et al., 1962; Vane, 1971). That reading is consistent with the published pharmacology of the isolated compounds: lupeol suppresses nuclear factor-kappa B activation and downstream cyclo-oxygenase-2 and cytokine expression (Fernández et al., 2001; Saleem, 2009), and the alpha,beta-amyrin pair has documented antinociceptive and anti-inflammatory activity in rodent models (Otuki et al., 2005; Vázquez et al., 2012).

The hepatoprotective result is more equivocal in mechanism. Carbon tetrachloride is bioactivated by cytochrome P450 2E1 to the trichloromethyl radical and thence to the trichloromethyl peroxy radical, which initiates lipid peroxidation and produces centrilobular necrosis (Recknagel et al., 1989; Weber et al., 2003). The 3.7-fold rise in malondialdehyde and the parallel depletion of glutathione and the antioxidant enzymes are the expected signature of that chain (Muriel, 2009). Restoration of those markers by the extracts is compatible with radical scavenging by the phenolic constituents, whose structure-activity relationships as chain-breaking antioxidants are well established (Rice-Evans et al., 1996; Middleton et al., 2000). It is equally compatible with inhibition of cytochrome P450 2E1 and hence reduced bioactivation of the toxicant, which would produce an identical profile. The present design cannot separate these, and attributing the protection to antioxidant activity would overstate what the data support. Measurement of cytochrome P450 2E1 activity, or use of a hepatotoxicant that does not require bioactivation, would resolve the question.

A second limitation attaches to the analgesic finding. Prolongation of hot plate latency is produced by sedation as reliably as by analgesia, and sedative activity has been reported for sesquiterpenes isolated from this species (Farooq et al., 2018). The transient hypoactivity recorded for the fraction in the toxicity study makes the alternative worth stating rather than dismissing. A rotarod or open-field assessment run alongside the thermal test would settle it, and its absence is a defect of the present design rather than a feature of the compound.

4.3 Efficacy read against tolerability

The toxicological result is not a peripheral observation. The seed extract delivered its 75.0% protection at a dose only 1.5-fold below the highest at which no mortality occurred, whereas the leaf extract delivered 65.4% at one-fifth of a tolerated dose and the triterpenoid fraction delivered its anti-inflammatory effect at one-fortieth of one. A preparation with the margin observed for the seed extract is not a therapeutic candidate in its present form, and reporting its efficacy without that context would misrepresent the finding.

The chemistry offers a testable explanation for the divergence. The hydroethanolic seed extract gave a prominent protein response on screening, and castor seed is the tissue in which ricin and the 2S albumins are concentrated (Abomughaid et al., 2024); the triterpenoid fraction, prepared from a petroleum ether extract, is protein-free and was tolerated at 2000 mg/kg. If the hazard resides in the protein fraction and the activity in the small-molecule fraction, then a protein-depleted seed extract should retain hepatoprotection while losing acute toxicity. That experiment is decisive either way and is the most direct extension of this work.

4.4 Comparison with published work

The findings agree in direction with the published record. Hepatoprotection in a carbon tetrachloride model has been reported for an aqueous extract in Wistar rats (Naveen et al., 2016), for leaf extracts (Visen et al., 1992) and for an isolated leaf constituent (Shukla et al., 1992). Anti-inflammatory and radical-scavenging activity has been reported for a methanolic root extract (Ilavarasan et al., 2006) and for leaf extracts in vitro (Nemudzivhadi & Masoko, 2014), and antinociceptive activity for leaf and seed extracts (Taur et al., 2011; Esfandyari et al., 2018). Direct numerical comparison would be inappropriate, since plant part, solvent, standardization, dose and hepatotoxicant schedule differ across those studies. What this work adds is a chain of evidence in which the preparation administered is chemically defined, the batches are demonstrably equivalent, the doses are anchored to a toxicity assessment, and the activity is located in a specific fraction.

4.5 Limitations

The study is preclinical and cannot establish clinical efficacy, therapeutic dose or human safety. The chemistry is characterized but not resolved: 37.5% of the loaded petroleum ether extract was not recovered, and the triterpenoid-rich fraction contains unresolved material alongside its five identified constituents, so its activity cannot be attributed to any named compound. The isolated quantities, 12 to 64 mg, allowed spectroscopic characterization but not animal dosing, and no pharmacokinetic data were generated. The compositional correlations rest on four preparations, and composition varies with provenance, season and processing, so the conclusions apply in the first instance to the batches studied.

5. Conclusion:

Sequential polarity-graded extraction of *Ricinus communis* leaf yields a nonpolar fraction dominated by pentacyclic triterpenes and sterols and a polar fraction dominated by flavonoids and phenolic acids, and the two are separable cleanly enough to be tested independently. Nine constituents were isolated and identified. Hepatoprotective and antioxidant effects tracked the phenol-rich material, while anti-inflammatory and antinociceptive effects tracked the triterpenoid-rich material, with the fraction outperforming the crude extract by roughly ninefold on a dose-normalized basis. Set against tolerability, the

leaf-derived triterpenoid fraction rather than the seed extract emerges as the preparation worth pursuing, notwithstanding that the seed extract produced the larger hepatoprotective effect. The immediate next steps are protein depletion of the seed extract, scale-up isolation for compound-level testing, and the mechanistic experiments that would distinguish radical scavenging from reduced .

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