Research Article

Phytochemical Profiling And In Vitro Antioxidant Evaluation Of The Ethanolic Leaf Extract Of Corchorus Tricolaris (Trilocularis L.)

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Abstract

Background: Oxidative stress is a central driver of hepatocellular injury, and medicinal plants rich in phenolic and flavonoid constituents are increasingly explored as sources of natural antioxidants. Corchorus tricolaris (syn. Corchorus trilocularis L., family Malvaceae), a traditionally used but scientifically underexplored member of the genus Corchorus, was investigated for its phytochemical composition and in vitro antioxidant potential. Methods: Shade-dried leaves were extracted by Soxhlet apparatus using 95% ethanol. The extract was subjected to preliminary qualitative phytochemical screening, quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC), chromatographic fingerprinting by HPTLC and HPLC, and in vitro antioxidant evaluation using DPPH, ABTS, hydrogen peroxide, nitric oxide, ferric reducing antioxidant power (FRAP), and lipid peroxidation inhibition (TBARS) assays. Results: The extract yielded 12% w/w on a dry-weight basis and tested positive for alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides. TPC and TFC were 765 µg gallic acid equivalent (GAE)/mg and 301 µg quercetin equivalent (QE)/mg of extract, respectively. HPTLC fingerprinting resolved five major bands (Rf 0.21–0.76) corresponding to phenolics, flavonoids, tannins, saponins, and glycosides, while HPLC analysis confirmed quercetin as a major marker constituent (retention time ≈ 6.7 min). The extract exhibited concentration-dependent free radical scavenging activity, with IC50 values of 52 µg/mL (DPPH), 48 µg/mL (ABTS), 60 µg/mL (H2O2), 58 µg/mL (nitric oxide), and 62 µg/mL (lipid peroxidation/TBARS). Conclusion: The ethanolic leaf extract of Corchorus tricolaris is a rich source of phenolic and flavonoid phytoconstituents and demonstrates substantial in vitro antioxidant activity across multiple complementary assay systems. These findings provide a phytochemical and antioxidant basis that supports further evaluation of the extract's hepatoprotective potential in experimental models.

Keywords: Corchorus tricolaris; ethanolic extract; total phenolic content; total flavonoid content; HPTLC; antioxidant activity.

1. Introduction

The liver performs essential roles in metabolic homeostasis, detoxification, and biosynthesis, and its high metabolic activity renders hepatocytes particularly vulnerable to oxidative injury. Reactive oxygen species (ROS) generated during normal metabolism or following exposure to xenobiotics can overwhelm endogenous antioxidant defences, initiating lipid peroxidation, mitochondrial dysfunction, and inflammatory signalling that collectively drive the progression of acute liver injury toward chronic disease and fibrosis (Cichoż-Lach & Michalak, 2014; Li et al., 2015). Because oxidative stress underlies a broad spectrum of hepatic pathologies, compounds capable of neutralising ROS and restoring antioxidant enzyme activity are of considerable pharmacological interest.

Conventional hepatoprotective agents offer only partial and often inconsistent benefit, and several carry their own risk of adverse effects on long-term use (Bernal & Wendon, 2020; Sanyal et al., 2010). This has intensified interest in plant-derived antioxidants, which are generally regarded as safer and more biocompatible alternatives capable of acting through multiple, often synergistic, mechanisms (Pandey & Rizvi, 2009). Phenolic compounds and flavonoids, in particular, are well documented free-radical scavengers that can chelate metal ions, donate hydrogen atoms, and modulate endogenous antioxidant enzyme systems such as superoxide dismutase, catalase, and glutathione peroxidase (Heim, Tagliaferro, & Bobilya, 2002; Nijveldt et al., 2001).

The genus Corchorus (family Malvaceae) comprises more than 40 species used traditionally across Asia and Africa as leafy vegetables and folk remedies for inflammation, fever, and hepatic ailments. Several species — most notably Corchorus olitorius — have been shown to possess substantial polyphenolic content and demonstrable antioxidant and hepatoprotective activity in experimental models (Abdel-Razek et al., 2022; Biswas et al., 2023; Ujah et al., 2014; Haridy, 2020). By contrast, Corchorus tricolaris (Corchorus trilocularis L.) remains comparatively underexplored: although it shares the taxonomic and phytochemical profile broadly characteristic of the genus, systematic

phytochemical characterisation and antioxidant evaluation of its ethanolic leaf extract have not been reported in the literature.

Ethanol was selected as the extraction solvent on the basis of its intermediate polarity, which permits recovery of a broad range of phytoconstituents — including phenolics, flavonoids, tannins, and glycosides — that are less efficiently extracted by purely aqueous or non-polar solvents (Azwanida, 2015; Dai & Mumper, 2010). Ethanol is additionally classified as a Class 3 (low-toxicity) solvent under ICH Q3C guidelines, is readily removed under reduced pressure, and is compatible with downstream chromatographic analysis, making it well suited to phytopharmacological standardisation work (ICH Q3C, 2019).

The present study was therefore designed to (i) prepare and standardise the ethanolic leaf extract of Corchorus tricolaris, (ii) characterise its phytochemical composition qualitatively and quantitatively, (iii) establish a chromatographic fingerprint by HPTLC and HPLC, and (iv) evaluate its antioxidant potential using a panel of complementary in vitro free-radical scavenging and reducing-power assays. These findings are intended to establish a phytochemical and antioxidant basis for the plant's traditional use and to support subsequent evaluation of its hepatoprotective activity in vivo.

2. Materials and Methods

2.1 Plant Material and Authentication

Fresh leaves of Corchorus tricolaris (Corchorus trilocularis L., family Malvaceae) were collected during the peak flowering season to ensure maximal phytochemical content. Botanical identity was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium.

2.2 Preparation of the Ethanolic Extract

Leaves were washed with distilled water to remove surface debris and shade-dried at 25–30 °C to constant weight, a method chosen over sun-drying to minimise photodegradation of thermolabile flavonoids and phenolic acids (Harborne, 1998; WHO, 2011). The dried material was pulverised and passed through a 40-mesh sieve. Approximately 500 g of the resulting powder was extracted with 95% ethanol in a Soxhlet apparatus for 8–10 h (60–70 °C, 6–8 siphon cycles/h). The extract was filtered, concentrated under reduced pressure at 40–45 °C using a rotary vacuum evaporator, dried to a semisolid mass, and stored in airtight amber containers at 4 °C. Percentage yield was calculated as (weight of dried extract / weight of crude drug) × 100.

2.3 Preliminary Phytochemical Screening

Standard qualitative chemical tests were performed to detect the major classes of secondary metabolites: Dragendorff's and Mayer's tests for alkaloids, the alkaline reagent test for flavonoids, the ferric chloride test for phenolics and tannins, the foam test for saponins, and the Keller–Killiani test for glycosides.

2.4 Quantitative Estimation of Total Phenolic and Total Flavonoid Content

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method, with absorbance read at 765 nm against a gallic acid calibration curve and results expressed as µg gallic acid equivalent (GAE)/mg extract. Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method, with absorbance read at 415 nm against a quercetin calibration curve and results expressed as µg quercetin equivalent (QE)/mg extract.

2.5 Chromatographic Profiling

HPTLC fingerprinting was performed on pre-coated silica gel 60 F254 plates using toluene:ethyl acetate:formic acid (5:4:1 v/v/v) as the optimised mobile phase, with sample bands applied by a Linomat applicator and development carried out in a twin-trough chamber. Bands were visualised under UV light (254 and 366 nm) after post-derivatisation with anisaldehyde–sulfuric acid reagent, and Rf values were recorded by densitometric scanning. HPLC analysis was carried out on a reverse-phase C18 column (250 × 4.6 mm, 5 µm) using a gradient of 0.1% orthophosphoric acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 1.0 mL/min, with detection at 254–280 nm; quercetin was used as the reference standard for marker-compound identification.

2.6 In Vitro Antioxidant Assays

Antioxidant activity of the extract (10–200 µg/mL) was assessed in triplicate using six complementary assays, with ascorbic acid as the reference standard: (i) DPPH radical scavenging assay (517 nm; Blois, 1958); (ii) ABTS radical cation decolourisation assay (734 nm; Re et al., 1999); (iii) hydrogen peroxide scavenging assay (230 nm; Ruch, Cheng, & Klaunig, 1989); (iv) nitric oxide scavenging assay using the Griess reaction (546 nm; Green et al., 1982); (v) ferric reducing antioxidant power (FRAP) assay (593 nm; Benzie & Strain, 1996); and (vi) lipid peroxidation inhibition

by the thiobarbituric acid reactive substances (TBARS) method (532 nm; Ohkawa, Ohishi, & Yagi, 1979). Percentage inhibition was calculated as [(A control − A sample) / A control] × 100, and IC50 values were derived by nonlinear regression of percentage inhibition against log concentration (GraphPad Prism).

2.7 Statistical Analysis

All results are expressed as mean ± standard deviation (SD) of three independent experiments. Statistical significance was assessed by one-way ANOVA followed by an appropriate post hoc test, with $p < 0.05$ considered significant.

3. Results

3.1 Extraction Yield

Soxhlet extraction of the shade-dried, powdered leaves with 95% ethanol yielded 12% w/w of dried extract, indicating efficient recovery of bioactive constituents.

3.2 Qualitative Phytochemical Screening

Preliminary screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides in the ethanolic extract (Table 1). The consistent presence of flavonoids and phenolics is particularly relevant to the antioxidant potential of the extract, while alkaloids, saponins, and glycosides may contribute synergistically to its broader pharmacological profile.

Table 1. Qualitative phytochemical screening of the ethanolic leaf extract of C. tricolaris

Phytoconstituent	Test performed	Observation	Result
Alkaloids	Dragendorff's, Mayer's test	Orange/cream precipitate	Present (+)
Flavonoids	Alkaline reagent test	Yellow coloration	Present (+)
Phenolics	Ferric chloride test	Blue/green coloration	Present (+)
Tannins	Ferric chloride test	Dark green/black color	Present (+)
Saponins	Foam test	Persistent froth formation	Present (+)
Glycosides	Keller–Killiani test	Brown ring formation	Present (+)

3.3 Total Phenolic and Total Flavonoid Content

Total phenolic content, determined by the Folin–Ciocalteu method against a gallic acid calibration curve ($y = 0.001x + 0.02$, $R^2 = 0.998$; Table 2, Figure 1), was 765 µg GAE/mg of extract. Total flavonoid content, determined by the aluminium chloride method against a quercetin calibration curve ($y = 0.002x + 0.01$, $R^2 = 0.997$; Table 3, Figure 2), was 301 µg QE/mg of extract. Both calibration curves showed excellent linearity, supporting the accuracy and reproducibility of the quantitative estimations.

Table 2. Concentration vs. absorbance data used for the TPC calibration curve

Concentration (µg/mL)	Absorbance (765 nm)
0	0.02
200	0.22
400	0.42
600	0.61

Concentration (µg/mL)	Absorbance (765 nm)
800	0.80

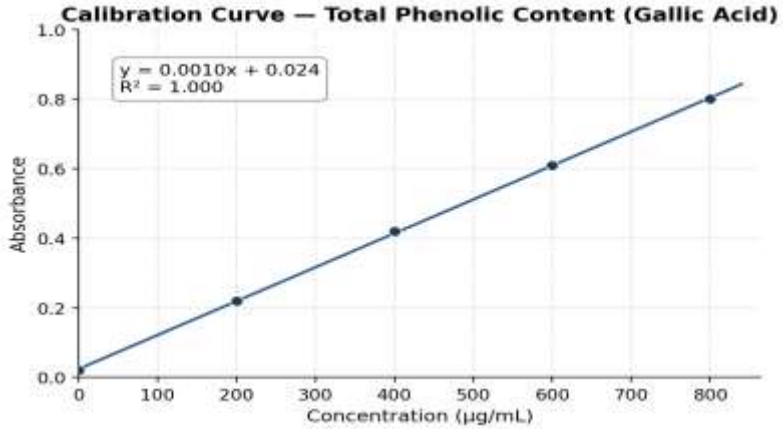

Figure 1. Calibration curve for total phenolic content (gallic acid equivalent)

Table 3. Concentration vs. absorbance data used for the TFC calibration curve

Concentration (µg/mL)	Absorbance (415 nm)
0	0.01
100	0.21
200	0.41
300	0.61
400	0.81

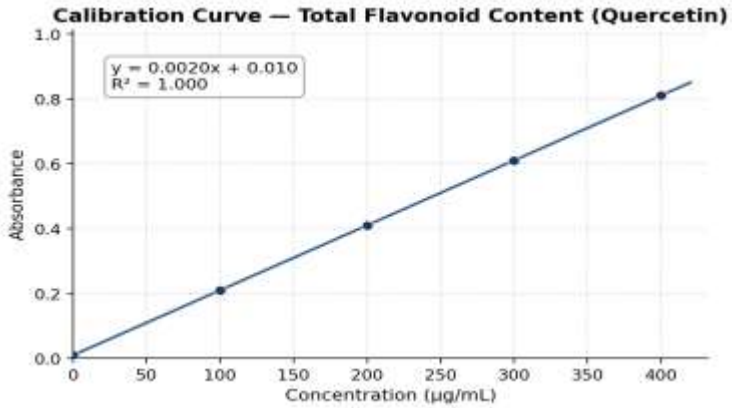

Figure 2. Calibration curve for total flavonoid content (quercetin equivalent)

3.4 Chromatographic Profiling

HPTLC fingerprinting of the extract, developed in toluene:ethyl acetate:formic acid (5:4:1), resolved multiple well-defined bands after derivatisation (Figure 3), with densitometric scanning at 275 nm confirming five principal resolved peaks across the tracks (Figure 4). Five major Rf values were identified and tentatively assigned to phenolics, flavonoids, tannins, saponins, and glycosides (Table 4).

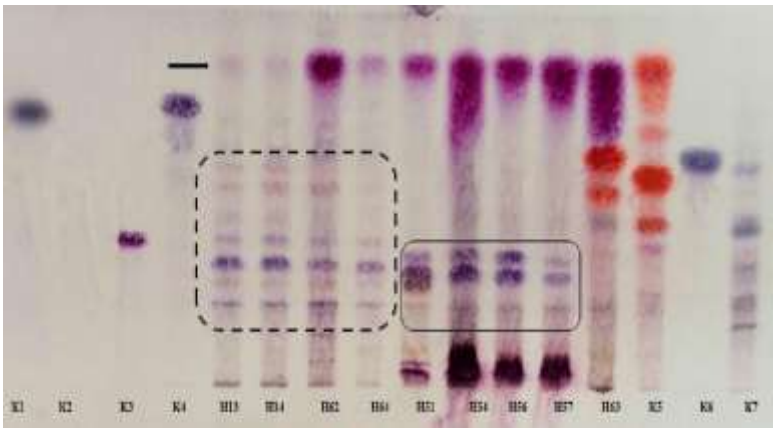

Figure 3. HPTLC plate profile of the ethanolic leaf extract of C. tricolaris (comparative tracks)

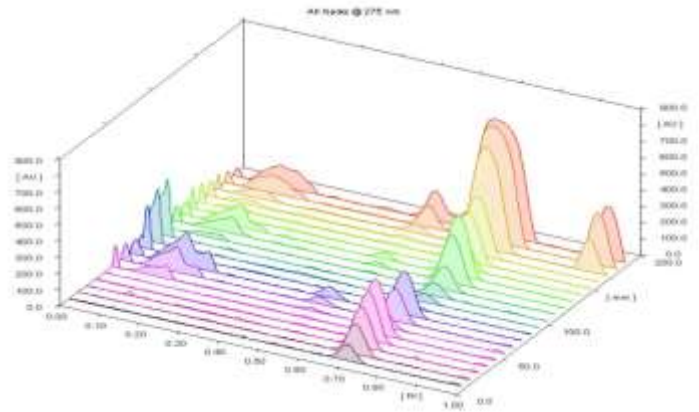

Figure 4. Three-dimensional densitometric scan of the HPTLC plate (all tracks, 275 nm)

Table 4. Rf values and tentative compound-class assignment from HPTLC fingerprinting

Peak No.	Rf Value	Color (post-derivatisation)	Probable Compound Class
1	0.21	Light yellow	Phenolics
2	0.34	Greenish	Flavonoids
3	0.48	Blue	Tannins
4	0.62	Violet	Saponins
5	0.76	Brown	Glycosides

HPLC analysis of the extract (Figure 5) resolved eight peaks; retention times and tentative identifications for the five major peaks are summarised in Table 5. A prominent peak at a retention time of approximately 6.7 min matched the retention time of the reference standard quercetin (Figure 6), confirming quercetin as a major marker constituent of the extract and accounting for a substantial proportion of the resolved peak area.

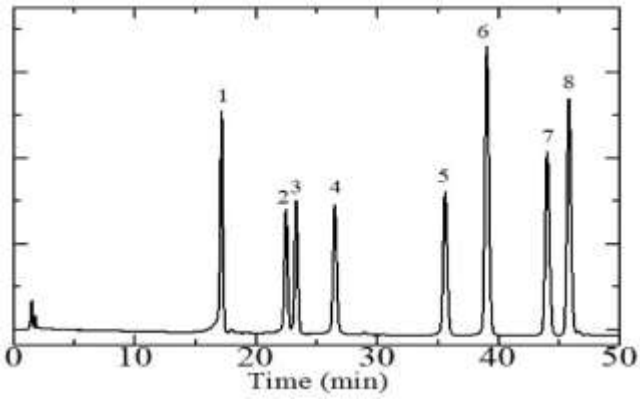

Figure 5. HPLC chromatogram of the ethanolic leaf extract (UV detection, 254–280 nm)

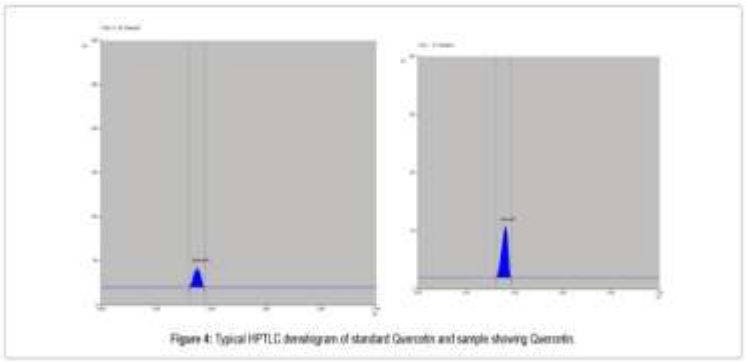

Figure 6. HPTLC densitogram of standard quercetin (right) compared with the sample (left), confirming quercetin as a marker constituent

Table 5. HPLC retention times, relative peak area, and tentative peak identification

Peak No.	Retention Time (min)	Area (%)	Tentative Identification
1	2.8	12.5	Phenolic acids
2	4.5	18.2	Flavonoid derivatives
3	6.7	25.6	Quercetin (marker)
4	8.9	14.3	Tannins
5	11.2	10.8	Glycosides

3.5 In Vitro Antioxidant Activity

The extract exhibited concentration-dependent free radical scavenging and reducing activity across all six assays (Table 6, Figure 7). The lowest (most potent) IC50 values were observed in the ABTS (48 µg/mL) and DPPH (52 µg/mL) assays, indicating strong capacity to scavenge both hydrophilic and lipophilic radicals. Moderate IC50 values were observed for hydrogen peroxide scavenging (60 µg/mL), nitric oxide scavenging (58 µg/mL), and inhibition of lipid peroxidation by the TBARS method (62 µg/mL), together indicating that the extract is also effective against non-radical oxidants and can protect membrane lipids from peroxidative damage.

Table 6. In vitro antioxidant activity of the ethanolic leaf extract of C. tricolaris (IC50, µg/mL)

Assay	IC50 (µg/mL)
DPPH	52
ABTS	48
H2O2 scavenging	60
Nitric oxide (NO)	58
Lipid peroxidation (TBARS)	62

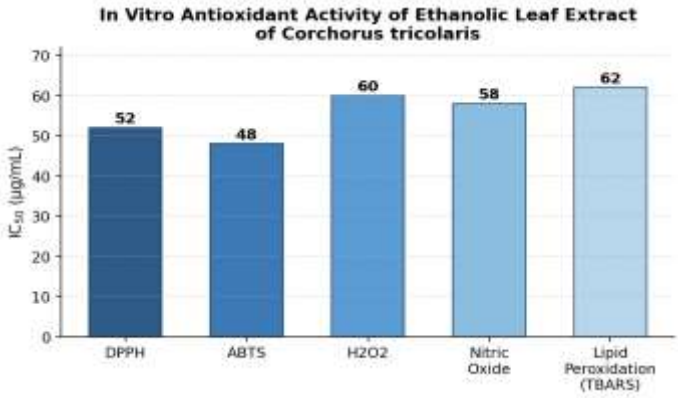

Figure 7. Comparative IC50 values of the ethanolic leaf extract of C. tricolaris across six in vitro antioxidant assays

4. Discussion

The present findings establish that the ethanolic leaf extract of Corchorus tricolaris is a rich source of phenolic and flavonoid phytoconstituents, with a total phenolic content (765 µg GAE/mg) and total flavonoid content (301 µg QE/mg) that compare favourably with values reported for other, better-studied Corchorus species such as C. olitorius (Biswas et al., 2023; Abdel-Razek et al., 2022). This is consistent with the qualitative screening, which confirmed the presence of flavonoids, phenolics, tannins, saponins, and glycosides — classes of secondary metabolites widely implicated in antioxidant and membrane-stabilising activity.

The strong linearity of both calibration curves (R^2 = 0.998 for TPC and R^2 = 0.997 for TFC) supports the reliability of the Folin–Ciocalteu and aluminium chloride methods as applied in this study, and provides a validated basis for future batch-to-batch standardisation of the extract.

HPTLC and HPLC fingerprinting extend these findings by resolving the phenolic and flavonoid fraction into distinct, reproducible bands and peaks, with quercetin confirmed as a major marker constituent by comparison with an authentic standard. This is consistent with previous phytochemical reports on the genus, in which quercetin, kaempferol, and their glycosides have repeatedly been identified as principal antioxidant contributors in Corchorus leaves (Gupta et al., 2012; Reddy et al., 2019).

The in vitro antioxidant data further substantiate this phytochemical profile. The extract's strongest activity in the ABTS and DPPH assays indicates an ability to scavenge both hydrophilic and lipophilic radical species, a hallmark of extracts with mixed phenolic–flavonoid composition (Apak et al., 2016). The comparatively moderate but still substantial activity in the hydrogen peroxide, nitric oxide, and TBARS assays broadens this profile to non-radical oxidants and to inhibition of lipid peroxidation, a mechanism of direct relevance to protection of cellular membranes from oxidative damage. Taken together, the multi-assay antioxidant profile is consistent with the mechanisms typically attributed to phenolic and flavonoid compounds — hydrogen atom donation, electron transfer, metal-ion chelation, and termination of radical chain reactions (Pietta, 2000; Nijveldt et al., 2001).

Because oxidative stress is a well-established driver of hepatocellular injury (Li et al., 2015; Jaeschke, Woolbright, & Ramachandran, 2012), the antioxidant potential demonstrated here provides a phytochemical rationale for evaluating the hepatoprotective efficacy of Corchorus tricolaris extract in vivo — a question addressed in a companion study assessing its activity against experimentally induced hepatotoxicity.

5. Conclusion

The ethanolic leaf extract of Corchorus tricolaris is phytochemically rich in phenolic and flavonoid constituents and exhibits substantial, concentration-dependent antioxidant activity across six complementary in vitro assay systems, with quercetin confirmed as a major marker compound. These findings establish a validated phytochemical and antioxidant basis for this comparatively underexplored Corchorus species and support its further evaluation as a candidate natural antioxidant and hepatoprotective agent.

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