

Protective Role of *Hygrophila auriculata* Seed Extract Against Carbon Tetrachloride Induced Hepatocellular Damage: An In Vitro Study

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ABSTRACT

Hepatic injury induced by xenobiotics is closely associated with oxidative stress-mediated cellular damage. The present study evaluated the phytochemical composition, antioxidant activity, and hepatoprotective efficacy of the aqueous seed extract of *Hygrophila auriculata* using a carbon tetrachloride (CCl₄) induced goat liver slice model. Preliminary screening confirmed the presence of flavonoids, saponins, tannins, and proteins. Quantitative estimation revealed abundant protein (231 ± 10.06 mg BSAE/g), substantial total phenolics (187 ± 20.29 mg GAE/g), and notable flavonoids (62.09 ± 7.23 mg QE/g). The extract exhibited a concentration-dependent DPPH radical scavenging effect with an IC₅₀ of approximately 72–73 µg/mL, indicating moderate yet significant antioxidant potential. Whereas, CCl₄ exposure (15 mM) markedly elevated SGOT, SGPT, and LDH activities (p < 0.001), reflecting hepatocellular membrane disruption. Post-treatment with the extract (0.02–0.10 mg QE/mL) significantly attenuated enzyme leakage in a dose-responsive manner (p < 0.05 vs. CCl₄). At 0.10 mg QE/mL, enzyme levels declined by 59.9% (SGOT), 47.3% (SGPT), and 31.7% (LDH), with corresponding hepatoprotection values of 71.7%, 62.0%, and 51.0%. The protective effect approached that of quercetin. These findings suggest that the polyphenol-rich seed extract confers hepatoprotection primarily through antioxidant-mediated mechanisms

Keywords: *Hepatic Injury, Cytotoxicity, Hepatoprotection, CCL4, Anti-Oxidant Property.*

INTRODUCTION

Medicinal plants have served as primary therapeutic resources for centuries, particularly in low- and middle-income countries where access to modern healthcare may be limited. The World Health Organization estimates that nearly 80% of the global population relies partly on traditional herbal medicine for primary health care (WHO, 2019). Plant derived bioactive compounds, especially flavonoids, phenolics, and terpenoids, are widely recognized for their antioxidant and cytoprotective properties, which contribute to their long-standing use in managing chronic diseases, including liver disorders.

Hygrophila auriculata (syn. *Asteracantha longifolia*), commonly known as Neermulli or Kulekhara, is a marshy annual herb belonging to the Acanthaceae family. It is distributed across South and Southeast Asia and parts of tropical Africa. Traditionally, its roots, seeds, and aerial parts have been used to treat jaundice, hepatic obstruction, anemia, inflammation, urinary tract infections, and rheumatism. Phytochemical investigations have identified flavonoids (apigenin derivatives), triterpenes (lupeol, betulin), alkaloids, sterols, and essential minerals, which are associated with antioxidant, anti-inflammatory, androgenic, and hepatoprotective activities (Hussain et al., 2009; Sethiya et al., 2018). Experimental studies report that methanolic and hydroalcoholic extracts of *H. auriculata* exhibit significant free radical scavenging activity in DPPH and lipid peroxidation models (Rastogi et al., 2014).

The liver plays a central role in metabolism, detoxification, and redox balance. Excessive generation of reactive oxygen species (ROS) can overwhelm endogenous antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), resulting in oxidative stress and hepatocellular damage (Finkel &

Holbrook, 2000). Drug-induced liver injury accounts for a substantial proportion of acute liver failure cases worldwide (Holt & Ju, 2006).

Carbon tetrachloride is widely used to experimentally induce hepatotoxicity because it is metabolized by cytochrome P450 enzymes into trichloromethyl radicals, initiating lipid peroxidation and membrane disruption (Manibusan et al., 2007).

Although several parts of *H. auriculata* have demonstrated hepatoprotective effects, limited evidence is available regarding the antioxidant and hepatoprotective potential of its aqueous seed extract using an in vitro liver slice model. Therefore, the present study aims to evaluate the protective effect of aqueous seed extract of *H. auriculata* against CCl₄-induced oxidative liver damage in goat liver slice culture.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade. The chemicals used included tannic acid, Folin–Denis reagent, sodium carbonate, bovine serum albumin (BSA), alkaline copper reagent (Copper A and Copper B), Folin–Ciocalteu reagent, quercetin, aluminium chloride, methanol, potassium acetate, ascorbic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), HEPES, sodium chloride (NaCl), potassium chloride (KCl), calcium chloride (CaCl₂), magnesium sulphate (MgSO₄), glucose, potassium dihydrogen phosphate (KH₂PO₄), sodium pyruvate, phosphate buffer, 2,4-dinitrophenylhydrazine (DNPH), sodium hydroxide (NaOH), α -aspartic acid, α -ketoglutarate, alanine, glycine buffer, lithium lactate, NAD solution, and carbon tetrachloride (CCl₄).

Preparation of Aqueous Seed Extract

A 20% (w/v) aqueous extract of *Hygrophila auriculata* seeds was prepared using freshly cleaned and dried seeds. The seeds were weighed accurately, crushed to obtain a coarse powder, and soaked in distilled water (1:5 w/v) overnight at room temperature to facilitate extraction of water-soluble constituents. The soaked material was then homogenized thoroughly to ensure maximum release of bioactive compounds. The homogenate was centrifuged at appropriate speed to remove insoluble debris, and the supernatant was collected. The extract was further filtered through muslin cloth followed by Whatman filter paper to obtain a clear filtrate. The final extract was stored in airtight containers at 4 °C and freshly prepared for each experimental assay to maintain stability and reproducibility.

Preliminary Qualitative Phytochemical Characterization

- **Alkaloids (Dragendorff's test):** 1 mL extract was acidified with concentrated HCl and treated with Dragendorff's reagent. A reddish-brown colour/precipitate indicated alkaloids.
- **Flavonoids (alkaline reagent test):** 1 mL extract was treated with dilute NaOH, producing a yellow colour that disappeared after adding dilute acid, indicating flavonoids.
- **Saponins (foam test):** 2 mL extract was diluted with 20 mL water and shaken for 15 min; persistent foam indicated saponins.
- **Phenols (Liebermann-type test):** sodium nitrite followed by dilute acid and dilute alkali produced a deep red/green/blue colour, indicating phenols.
- **Tannins (ferric chloride test):** diluted FeCl₃ produced red/blue/green/purple colour, indicating tannins/phenolics.
- **Anthraquinones (ammonia test):** extract treated with 10% ammonia; pink colour/precipitate indicated anthraquinones.
- **Cardiac glycosides (Keller–Killiani test):** glacial acetic acid containing FeCl₃ followed by conc. H₂SO₄; a brown ring at the interface indicated cardiac glycosides.
- **Phlobatannins:** extract treated with dilute HCl; red precipitate indicated phlobatannins.
- **Cardenolides (Kedde's test):** dried residue in methanol treated with alcoholic KOH and 3,5-dinitrobenzene and heated; characteristic colour reaction supported cardenolides.
- **Terpenoids (Salkowski-type):** chloroform layer followed by careful addition of conc. H₂SO₄; reddish-brown coloration indicated terpenoids.
- **Proteins (Millon's test):** white precipitate turning red on heating indicated proteins.

Quantitative Estimations of Phytochemicals

Total Tannins (Folin–Denis Method)

Extract was made up to 1 mL with distilled water, then 5 mL Folin–Denis reagent and 1 mL sodium carbonate were added. Absorbance was recorded at 700 nm against a reagent blank. Tannic acid standards (10–50 µg/mL) were used to generate a calibration curve, and results were expressed as tannic acid equivalents.

Total Protein (Lowry Method)

Extract was adjusted to 1 mL with distilled water and incubated for 10 min at room temperature. Alkaline copper reagent (5 mL) and Folin–Ciocalteu reagent (0.6 mL; 1:1) were added and incubated for 30 min. Absorbance was measured at 660 nm. BSA standards (40–200 µg/mL) were used, and results were expressed as BSA equivalents.

Total Flavonoids (Aluminium Chloride Method)

Total flavonoids were quantified following the aluminium chloride colorimetric protocol. The extract was adjusted to 2 mL with methanol, followed by addition of 0.1 mL 10% AlCl₃, 0.1 mL 1 M potassium acetate, and 2.8 mL distilled water. After

30 min incubation at room temperature, absorbance was measured at 415 nm. Quercetin standards (20–100 µg/mL) were used to construct the standard curve, and results were expressed as quercetin equivalents.

In Vitro Antioxidant Activity: DPPH Free Radical Scavenging Assay

DPPH scavenging activity was determined using the method described for plant antioxidants. Ascorbic acid stock (1000 µg/mL) was diluted to 100–500 µg/mL for the reference curve. For the test, different volumes of aqueous extract (0.1–3.0 mL) were taken and brought to a final volume of 3 mL using DPPH solution (2000 µg/100 mL). Where needed for assay compatibility, extract aliquots were diluted with methanol prior to reaction. Samples were incubated for 10 min at room temperature and absorbance was read at 517 nm.

Scavenging activity was calculated as:

$$\text{Scavenging (\%)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

In Vitro Hepatoprotective Evaluation Using Goat Liver Slice:

a. Liver Tissue Collection and Washing

Fresh goat liver was obtained from a licensed slaughterhouse (Shivajinagar, Bengaluru, Karnataka, India). Tissue was immediately rinsed in buffer to remove blood and clots and processed for slice culture.

b. Incubation Medium (KRHB–HEPES Buffer)

Krebs–Ringer HEPES buffer (KRHB–HEPES) contained HEPES (2.5 mM), NaCl (118 mM), KCl (2.85 mM), KH₂PO₄ (1.5 mM), MgSO₄ (1.18 mM), and glucose (4 mM). pH was adjusted to 7.4 using 1 N NaOH. Medium was sterilized by autoclaving.

c. Experimental Design: The hepatoprotective activity of the aqueous seed extract of *Hygrophila auriculata* (HSE) was evaluated using a modified goat liver slice model. Fresh liver slices (100 mg per group) were randomly assigned to eight experimental groups:

- Group A (Normal control): Liver slices incubated in Krebs–Ringer–HEPES buffer at 37 °C.
- Group B (Toxic control): Liver slices exposed to CCl₄ (15 mM) for 1 h at 4 °C.
- Group C: CCl₄ (15 mM) + HSE (0.02 mg QE/mL), incubated for 2 h at 37 °C.
- Group D: CCl₄ (15 mM) + HSE (0.04 mg QE/mL), 2 h at 37 °C.
- Group E: CCl₄ (15 mM) + HSE (0.06 mg QE/mL), 2 h at 37 °C.
- Group F: CCl₄ (15 mM) + HSE (0.08 mg QE/mL), 2 h at 37 °C.
- Group G: CCl₄ (15 mM) + HSE (0.10 mg QE/mL), 2 h at 37 °C.
- Group H (Reference control): CCl₄ (15 mM) + quercetin (0.01 mg/mL), 2 h at 37 °C.

Following incubation, the media were collected and analyzed for SGOT, SGPT, and LDH activities as biomarkers of hepatocellular damage.

$$\% \text{ Cytotoxicity} = (\text{enzyme activity in media} / \text{total enzyme activity}) \times 100,$$

where total enzyme activity = activity in media + activity in tissue fraction.

Biochemical Marker Assays

a. SGOT and SGPT Activity

Transaminase activities were determined using the DNPH-based colorimetric approach of Reitman and Frankel. Pyruvate standards (0.05–0.25 mL working standard) were prepared for calibration. Reaction mixtures were incubated at 37 °C, treated with DNPH, alkalized with 0.4 N NaOH, and absorbance was recorded at 540 nm.

b. LDH Activity

LDH release into the medium was estimated using King's routine colorimetric method. Buffered substrate, NAD, and sample were incubated at 37 °C; the reaction was terminated with DNPH, developed with 0.4 N NaOH, and absorbance was measured at 540 nm. Sodium pyruvate (80 µg/mL) was used to prepare the standard curve.

RESULTS

Qualitative Phytochemical Screening of the Aqueous Seed Extract of *Hygrophila auriculata*

Qualitative screening of the aqueous seed extract indicated the presence of flavonoids, saponins, tannins, and proteins, while alkaloids, phenols, anthraquinones, cardiac glycosides, phlobatannins, cardenolides, and terpenoids were absent. This profile suggests enrichment of polar bioactive constituents potentially contributing to antioxidant activity.

Table 1. Qualitative Screening of the Phytoconstituents of Aqueous Extract of the Seeds of *H. auriculata*

Phytochemical class	Aqueous extract
Alkaloids	–
Flavonoids	+
Saponins	+
Phenols	–

Tannins	+
Anthraquinones	-
Cardiac glycosides	-
Phlobatannins	-
Cardenolides	-
Terpenoids	-
Proteins	+

(‘+’ indicates presence of the respective constituent and ‘-’ indicates absence of the same.)

Quantitative Determination of Protein, Total Phenolic, Total Tannin, and Total Flavonoid Contents in the Aqueous Seed Extract of *Hygrophila auriculata*

Quantitative analysis of the aqueous seed extract of *Hygrophila auriculata* revealed high protein content (231 ± 10.06 mg BSAE/g), considerable total phenolics (187 ± 20.29 mg GAE/g), and appreciable flavonoid levels (62.09 ± 7.23 mg QE/g), while tannins were comparatively lower (18.37 ± 2.76 mg TAE/g). Significant differences were observed among the phytochemical classes ($p < 0.05$).

Table 2. Quantitative Phytochemical Composition of *Hygrophila auriculata* Seed Aqueous Extract

Quantitative Estimations	Value (Mean \pm SD)	Unit	Equivalent Standard
Total Phenols	187 ± 20.29	mg GAE/g	Gallic acid
Total Proteins	231 ± 10.06	mg BSAE/g	Bovine Serum Albumin
Total Tannins	18.37 ± 2.76	mg TAE/g	Tannic acid
Total Flavonoids	62.09 ± 7.23	mg QE/g	Quercetin

Values represent Mean \pm SD (n = 3). GAE: Gallic Acid Equivalent; BSAE: Bovine Serum Albumin Equivalent; TAE: Tannic Acid Equivalent; QE: Quercetin Equivalent. Statistical analysis was performed using one-way ANOVA ($p < 0.05$ considered significant).

In Vitro Antioxidant Activity Assessed by DPPH Radical Scavenging Assay

The aqueous seed extract of *Hygrophila auriculata* showed a progressive, concentration-dependent increase in DPPH radical scavenging activity, with inhibition values rising from $18.64 \pm 1.4\%$ at $20 \mu\text{g/mL}$ to $69.07 \pm 5.6\%$ at $100 \mu\text{g/mL}$. In comparison, ascorbic acid exhibited stronger scavenging activity across the same concentrations ($36.83 \pm 5.5\%$ to $91.94 \pm 0.07\%$). The calculated IC_{50} value of the extract was approximately $72\text{--}73 \mu\text{g/mL}$, whereas the standard demonstrated a lower IC_{50} ($\sim 36 \mu\text{g/mL}$), indicating comparatively higher antioxidant potency.

Table 3. DPPH Radical Scavenging Activity and IC_{50} Values of Ascorbic Acid and Aqueous Seed Extract of *Hygrophila auriculata*

Concentration ($\mu\text{g/MI}$)	Ascorbic Acid (% Scavenging \pm SD)	Aqueous <i>H. auriculata</i> Extract (% Scavenging \pm SD)
20	36.83 ± 5.50	18.64 ± 1.40
40	53.32 ± 3.80	27.15 ± 2.50
60	69.92 ± 2.50	39.40 ± 1.01
80	86.70 ± 1.07	56.12 ± 4.20
100	91.94 ± 0.07	69.07 ± 5.60
IC_{50} ($\mu\text{g/mL}$)	35.8 ± 1.2	72.6 ± 2.8

Values are expressed as Mean \pm SD (n = 3). Statistical analysis was performed using one-way ANOVA ($p < 0.05$ Considered significant). IC_{50} values were calculated using nonlinear regression analysis.

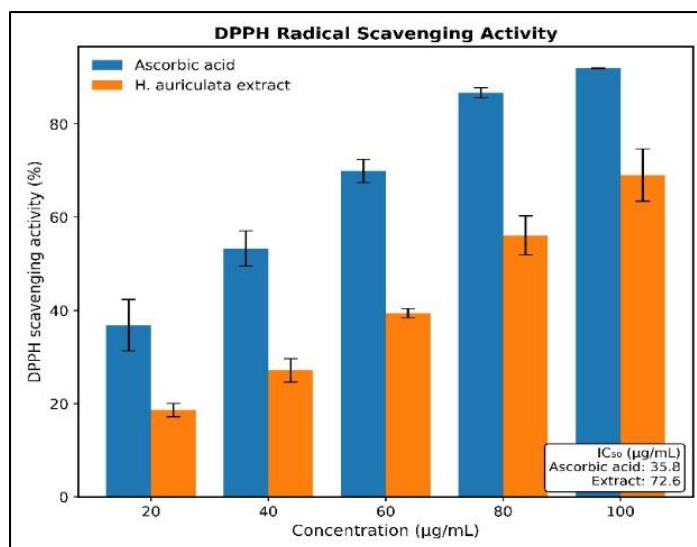


Figure 1. Graphical Representation of DPPH Radical Scavenging Activity of Aqueous *Hygrophila auriculata* Seed Extract Compared with Ascorbic Acid

Grouped bar chart shows DPPH scavenging (%) at 20–100 µg/mL (Mean ± SD, n = 3). The aqueous extract exhibited concentration-dependent scavenging, while ascorbic acid showed higher inhibition at all concentrations. IC₅₀ values (µg/mL) were: ascorbic acid 35.8 ± 1.2 and aqueous extract 72.6 ± 2.8.

The Hepatoprotective Evaluation of *Hygrophila auriculata* Seed Extract against CCl₄-Induced Cytotoxicity

a. Effect on SGOT, SGPT, and LDH Release

Table 4 summarizes the activities of hepatic marker enzymes in goat liver slices exposed to carbon tetrachloride (CCl₄) with and without treatment. In the normal control group (buffer only), baseline release of SGOT, SGPT, and LDH was low (16.2 ± 4.1, 18.8 ± 5.3 and 56.4 ± 8.2 U/L, respectively). Exposure to 15 mM CCl₄ caused a significant increase in enzymatic leakage (98.6 ± 9.5, 79.3 ± 8.7 and 148.7 ± 11.4 U/L), indicative of hepatocellular membrane damage consistent with oxidative stress-induced injury (Li et al., 2015).

Administration of the aqueous seed extract of *H. auriculata* (HSE) resulted in a dose-dependent decrease in enzyme release compared to CCl₄ alone. At 0.10 mg QE/mL, SGOT, SGPT, and LDH were significantly reduced (39.5 ± 2.6, 41.8 ± 2.9 and 101.6 ± 5.4 U/L), approaching the effect seen with quercetin (0.01 mg/mL), a standard antioxidant (31.7 ± 5.3, 32.4 ± 3.9 and 79.7 ± 7.4 U/L). This trend demonstrates the extract’s ability to stabilize hepatocellular membranes and reduce cytotoxic leakage.

Table 4. Effect of *Hygrophila auriculata* Aqueous Seed Extract on Serum Hepatic Marker Enzymes in CCl₄-Induced Goat Liver Slices

Group	Treatment	Concentration	SGOT (U/L)	SGPT (U/L)	LDH (U/L)
A	Control (Buffer)	—	16.2 ± 4.1	18.8 ± 5.3	56.4 ± 8.2
B	CCl ₄	15 mM	98.6 ± 9.5	79.3 ± 8.7	148.7 ± 11.4
C	CCl ₄ + HSE	0.02 mg QE/ml	83.3 ± 7.4	70.6 ± 6.8	141.3 ± 10.1
D	CCl ₄ + HSE	0.04 mg QE/ml	72.4 ± 6.2	66.1 ± 6.3	133.6 ± 9.5
E	CCl ₄ + HSE	0.06 mg QE/ml	59.5 ± 5.8	57.3 ± 5.1	121.4 ± 8.6
F	CCl ₄ + HSE	0.08 mg QE/ml	46.6 ± 4.3	49.4 ± 3.9	113.2 ± 7.8
G	CCl ₄ + HSE	0.10 mg QE/ml	39.5 ± 2.6	41.8 ± 2.9	101.6 ± 5.4
H	CCl ₄ + Quercetin	0.01 mg/mL	31.7 ± 5.3	32.4 ± 3.9	79.7 ± 7.4

Values represent Mean ± SD (n = 3) of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and lactate dehydrogenase (LDH) activities expressed in U/L. Liver slices exposed to carbon tetrachloride (15 mM) exhibited significant elevation in enzyme release compared to the control group, indicating

hepatocellular injury. Co-treatment with increasing concentrations of HSE (0.02–0.10 mg QE/mL) resulted in a dose-dependent reduction in enzyme levels. Quercetin (0.01 mg/mL) served as the positive control. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with significance considered at $p < 0.05$.

Table 5. Percentage Cytotoxicity in CCl₄-Induced Goat Liver Slices

Group	Treatment	SGOT (%)	SGPT (%)	LDH (%)
A	Control	16.4	23.7	37.9
B	CCl ₄	100.0	100.0	100.0
C	0.02 mg QE/mL	84.5	89.0	95.0
D	0.04 mg QE/mL	73.4	83.4	89.8
E	0.06 mg QE/mL	60.4	72.2	81.6
F	0.08 mg QE/mL	47.3	62.3	76.1
G	0.10 mg QE/mL	40.1	52.7	68.3
H	Quercetin (0.01 mg/mL)	32.2	40.9	53.6

Percentage enzyme release of SGOT, SGPT, and LDH was calculated relative to the CCl₄ toxic control (set as 100%). Values represent the proportion of enzyme activity in each treatment group compared to the CCl₄ group, using the formula: %Enzyme Release = $\left(\frac{E_{\text{treatment}}}{E_{\text{CCl}_4}}\right) \times 100$. CCl₄ (15 mM) served as the toxic control, while quercetin (0.01 mg/mL) was used as the reference standard.

b. Hepatoprotective Effect of *Hygrophila auriculata* Seed Extract Against CCl₄-Induced Cytotoxicity

CCl₄ exposure (15 mM) significantly elevated SGOT, SGPT, and LDH levels compared to the normal control ($p < 0.001$), indicating severe hepatocellular damage. Treatment with the aqueous seed extract (0.02–0.10 mg QE/mL) resulted in a statistically significant, dose-dependent reduction in enzyme leakage ($p < 0.05$ vs. CCl₄). At 0.10 mg QE/mL, SGOT, SGPT, and LDH levels declined by approximately 59.9%, 47.3%, and 31.7%, respectively, relative to the toxic control. Correspondingly, hepatoprotection increased progressively, reaching 71.7%, 62.0%, and 51.0% for SGOT, SGPT, and LDH. Quercetin (0.01 mg/mL) demonstrated superior protection across all parameters. These findings confirm that the extract mitigates CCl₄-induced oxidative hepatotoxicity in a concentration-dependent manner.

Table 6. Percentage Hepatoprotection of *Hygrophila auriculata* Seed Extract in CCl₄-Induced Goat Liver Slices

Group	Treatment	Concentration	SGOT Protection (%)	SGPT Protection (%)	LDH Protection (%)
C	CCl ₄ + HSE	0.02 mg QE/mL	18.6	14.4	8.0
D	CCl ₄ + HSE	0.04 mg QE/mL	31.8	21.8	16.4
E	CCl ₄ + HSE	0.06 mg QE/mL	47.4	36.4	29.6
F	CCl ₄ + HSE	0.08 mg QE/mL	63.1	49.4	38.5
G	CCl ₄ + HSE	0.10 mg QE/mL	71.7	62.0	51.0
H	CCl ₄ + Quercetin	0.01 mg/mL	81.2	77.5	74.8

Hepatoprotection (%) was calculated using: $\left(\frac{E_{\text{CCl}_4} - E_{\text{treatment}}}{E_{\text{CCl}_4} - E_{\text{control}}}\right) \times 100$. Values are expressed relative to toxic control (CCl₄ = 0% protection).

Grouped bar graph showing SGOT, SGPT, and LDH activities (U/L) expressed as Mean \pm SD ($n = 3$). Exposure to CCl₄ (15 mM) significantly elevated enzyme levels compared to the control group, indicating hepatocellular damage. Co-treatment

with increasing concentrations of aqueous seed extract (0.02–0.10 mg QE/mL) resulted in a dose-dependent reduction in enzyme release. Quercetin (0.01 mg/mL) served as the positive control. Statistical analysis was performed using one-way ANOVA followed by Tukey’s post hoc test, with differences considered significant at $p < 0.05$.

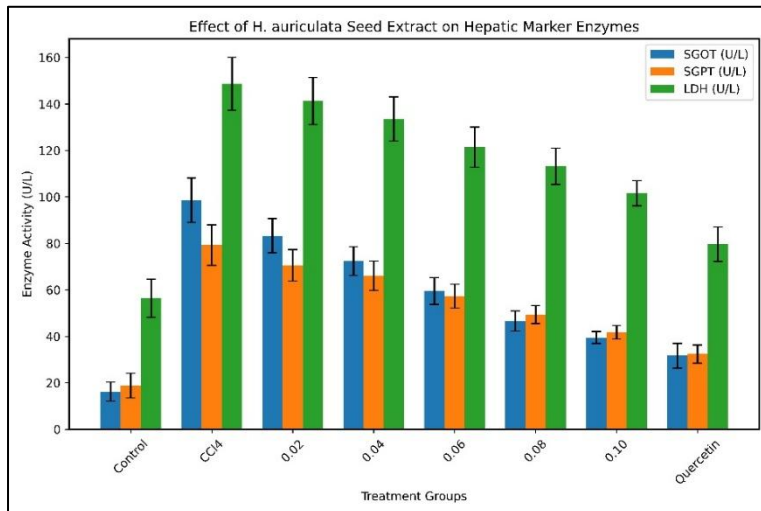


Figure 2. Graphical Representation of Effect of Aqueous Seed Extract of *Hygrophila auriculata* on Hepatic Marker Enzymes in CCl₄-Induced Goat Liver Slice Model

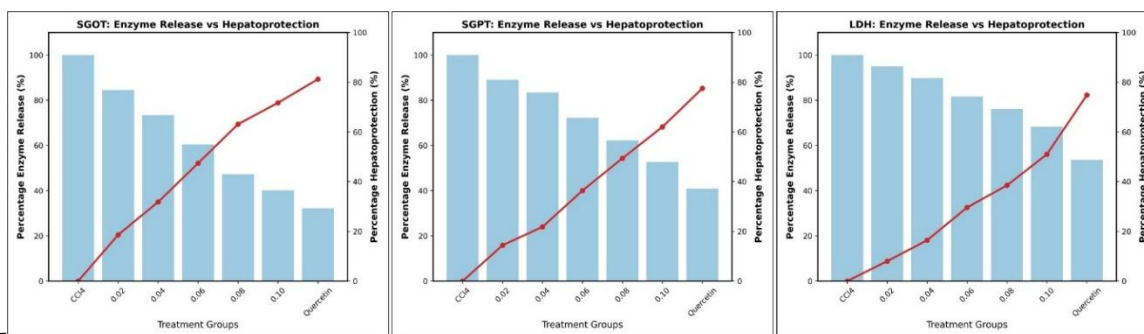


Figure 3. Graphical Representation of Enzyme Release and Hepatoprotection in CCl₄-Induced Goat Liver Slice Model Treated with Aqueous Seed Extract of *Hygrophila auriculata*

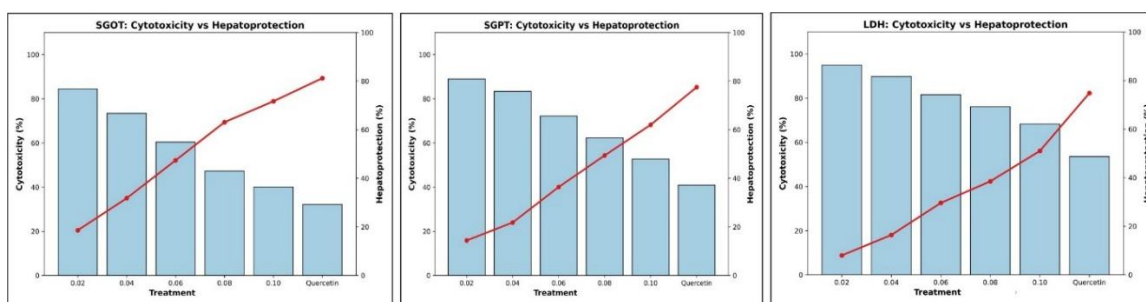


Figure 4. Graphical Representation of Cytotoxicity % and Hepatoprotection % in CCl₄-Induced Goat Liver Slice Model Treated with Aqueous Seed Extract of *Hygrophila auriculata*

SGOT, SGPT and LDH. Bars represent percentage enzyme release relative to the CCl₄ group (set as 100%), and the red line indicates percentage hepatoprotection. Enzyme release (%) was calculated as: $\left(\frac{E_{treatment}}{E_{CCl_4}}\right) \times 100$ and Hepatoprotection (%)

was calculated as: $\left(\frac{E_{CCl_4} - E_{treatment}}{E_{CCl_4} - E_{control}}\right) \times 100$. Data are mean \pm SD (n = 3). The extract produced a dose-dependent reduction in enzyme leakage with a corresponding increase in hepatoprotection, comparable to quercetin (0.01 mg/mL).

SGOT, SGPT, and LDH. Light blue bars (left Y-axis) represent percentage cytotoxicity relative to the CCl₄ toxic control (set

as 100%), while the red line (right Y-axis) indicates percentage hepatoprotection. Cytotoxicity (%) was calculated as: $\left(\frac{E_{treatment}}{E_{CCl_4}}\right) \times 100$ and Hepatoprotection (%) was calculated using: $\left(\frac{E_{CCl_4} - E_{treatment}}{E_{CCl_4} - E_{control}}\right) \times 100$. The extract (0.02–0.10 mg QE/mL) produced a dose-dependent reduction in enzyme leakage with a corresponding increase in hepatoprotection. Quercetin (0.01 mg/mL) served as the reference standard

Table 7. Summary of Effect of *Hygrophila auriculata* Seed Extract on Enzyme Release, Cytotoxicity and Hepatoprotection in CCl₄-Induced Goat Liver Slices

Gro up	Treatm ent	Conc .	SGO T (U/L)	SGOT Cytotoxi city (%)	SGOT Protecti on (%)	SGP T (U/L)	SGPT Cytotoxi city (%)	SGPT Protecti on (%)	LD H (U/ L)	LDH Cytotoxi city (%)	LDH Protecti on (%)
A	Control	—	16.2 ± 4.1	16.4	—	18.8 ± 5.3	23.7	—	56.4 ± 8.2	37.9	—
B	CCl ₄	15 mM	98.6 ± 9.5	100	0	79.3 ± 8.7	100	0	148.7 ± 11.4	100	0
C	CCl ₄ + HSE	0.02 mg QE/mL	83.3 ± 7.4	84.5	18.6	70.6 ± 6.8	89.0	14.4	141.3 ± 10.1	95.0	8.0
D	CCl ₄ + HSE	0.04 mg QE/mL	72.4 ± 6.2	73.4	31.8	66.1 ± 6.3	83.4	21.8	133.6 ± 9.5	89.8	16.4
E	CCl ₄ + HSE	0.06 mg QE/mL	59.5 ± 5.8	60.4	47.4	57.3 ± 5.1	72.2	36.4	121.4 ± 8.6	81.6	29.6
F	CCl ₄ + HSE	0.08 mg QE/mL	46.6 ± 4.3	47.3	63.1	49.4 ± 3.9	62.3	49.4	113.2 ± 7.8	76.1	38.5
G	CCl ₄ + HSE	0.10 mg QE/mL	39.5 ± 2.6	40.1	71.7	41.8 ± 2.9	52.7	62.0	101.6 ± 5.4	68.3	51.0
H	CCl ₄ + Querceti n	0.01 mg/mL	31.7 ± 5.3	32.2	81.2	32.4 ± 3.9	40.9	77.5	79.7 ± 7.4	53.6	74.8

$$\text{Cytotoxicity (\%)} = (E_{treatment} / E_{CCl_4}) \times 100$$

$$\text{Hepatoprotection (\%)} = [(E_{CCl_4} - E_{treatment}) / (E_{CCl_4} - E_{control})] \times 100$$

Values are expressed as Mean ± SD (n = 3). CCl₄ group was considered 100% cytotoxicity and 0% protection

DISCUSSION

The present findings demonstrate that the aqueous seed extract of *Hygrophila auriculata* is enriched with polar phytoconstituents, particularly flavonoids, tannins, saponins, and proteins, which are widely recognized for their biological relevance in oxidative stress modulation. The substantial levels of total phenolics (187 mg GAE/g) and flavonoids (62.09 mg QE/g) observed in the extract are consistent with reports indicating that polyphenol-rich plant extracts exhibit strong redox-modulating capacity and hepatoprotective potential (Zhang et al., 2022; Cory et al., 2018). Phenolic compounds are

known to donate hydrogen atoms or electrons, neutralize reactive oxygen species (ROS), and interrupt lipid peroxidation cascades, which are central to CCl₄-induced hepatic injury.

The DPPH assay further confirmed the antioxidant competence of the extract, with a concentration-dependent increase in radical scavenging activity and an IC₅₀ of ~72–73 µg/mL. Although less potent than ascorbic acid, the extract demonstrated meaningful free-radical quenching ability. Current evidence suggests that antioxidant capacity correlates strongly with total phenolic and flavonoid content, reinforcing the functional contribution of these constituents (Shahidi & Ambigaipalan, 2015; Xu et al., 2023). Such antioxidant action is particularly relevant in hepatotoxicity models where oxidative stress is a primary mechanism of injury.

CCl₄ toxicity is mediated through cytochrome P450-dependent bioactivation to the trichloromethyl radical (CCl₃•), which initiates lipid peroxidation, disrupts membrane integrity, and leads to leakage of hepatic marker enzymes (SGOT, SGPT, LDH) into the extracellular medium (Li et al., 2015; Weber et al., 2020). In the present study, CCl₄ exposure markedly elevated enzyme release, confirming severe hepatocellular damage. Treatment with the extract significantly attenuated this effect in a dose-dependent manner, with maximal reductions observed at 0.10 mg QE/mL. The corresponding increase in hepatoprotection percentage further supports membrane stabilization and mitigation of oxidative injury.

Notably, the extract's protective trend approached that of quercetin, a well-established flavonoid antioxidant known to enhance endogenous defense systems and suppress lipid peroxidation (Boots et al., 2008; Salehi et al., 2020). The moderate tannin content may also contribute synergistically, as condensed tannins have been reported to exert hepatoprotective and anti-inflammatory effects at controlled concentrations.

Collectively, these findings suggest that the hepatoprotective activity of *H. auriculata* seeds is closely associated with its polyphenolic composition and antioxidant capacity. The dose-dependent reduction in cytotoxicity and restoration of enzyme levels highlight its potential as a natural therapeutic candidate for oxidative stress-mediated liver disorders.

CONCLUSION

The present study demonstrates that the aqueous seed extract of *Hygrophila auriculata* possesses significant antioxidant and hepatoprotective properties. The extract, rich in phenolics and flavonoids, exhibited concentration-dependent radical scavenging activity and effectively attenuated CCl₄-induced elevation of hepatic marker enzymes in goat liver slices. The marked reduction in SGOT, SGPT, and LDH levels, along with increased hepatoprotection percentages, indicates stabilization of hepatocellular membranes and mitigation of oxidative damage. These findings support the therapeutic potential of *H. auriculata* seeds as a natural agent for managing oxidative stress-associated liver disorders.

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