RESEARCH PAPER

Integrated Characterization and Mechanistic In Vitro Assessment of Hepatoprotective and Wound-Repair Potential of *Picrorhiza Kurroa* in Experimental Models

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ABSTRACT

Background: Picrorhiza kurroa has long been employed in traditional medicine for liver ailments and skin-related disorders; however, integrated in vitro evidence combining pharmacognostic standardisation with mechanistic bioactivity evaluation remains limited.

Methods: The rhizomes of P. kurroa were subjected to comprehensive pharmacognostic, physicochemical, and phytochemical characterisation. A hydroethanolic extract was evaluated for hepatoprotective activity using tert-butyl hydroperoxide–induced oxidative stress in HepG2 cells, while wound-healing potential was assessed through fibroblast migration (scratch assay) and collagen synthesis in L929 cells. Antioxidant status, enzyme leakage, cell viability, and extracellular matrix–related parameters were quantified using standard in vitro assays.

Results: The extract exhibited high phenolic and flavonoid content and demonstrated significant cytoprotection against oxidative hepatic injury by restoring cell viability, reducing intracellular reactive oxygen species, and normalising alanine and aspartate aminotransferase leakage. In fibroblast cultures, the extract markedly enhanced cell migration and collagen synthesis in a concentration-dependent manner.

Conclusion: The integrated in vitro findings substantiated the hepatoprotective and wound-healing potential of P. kurroa, supporting its traditional use and highlighting its promise as a standardised phytopharmaceutical candidate for further translational development.

Keywords: Picrorhiza Kurroa; Hepatoprotective Activity; Wound Healing; In Vitro Models; Pharmacognostic Standardisation; Phytochemicals

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Introduction

Liver disorders and impaired wound healing continue to represent major global health challenges, contributing substantially to morbidity, healthcare expenditure, and reduced quality of life. Oxidative stress–mediated hepatocellular injury is a central pathogenic mechanism underlying a wide spectrum of liver diseases, including drug-induced hepatotoxicity and metabolic liver dysfunction. Similarly, delayed wound healing is often associated with excessive reactive oxygen species generation, impaired fibroblast migration, and insufficient collagen deposition, leading to chronic non-healing wounds (Dewidar et al., 2020; Zambrano-Vásquez et al., 2025; Zhou et al., 2025). Conventional pharmacological interventions for hepatic protection and wound management are often limited by adverse effects, restricted long-term safety, and inadequate efficacy in complex oxidative and inflammatory conditions. These limitations have stimulated growing interest in plant-derived therapeutics capable of targeting multiple molecular pathways simultaneously. Medicinal plants rich in polyphenols and glycosides are particularly attractive due to their antioxidant, cytoprotective, and regenerative properties (Ebrahimi et al., 2025; El-Deeb et al., 2025; Hoseini et al., 2025; Jangra et al., 2025; Jie et al., 2025).

Picrorhiza kurroa Royle ex Benth., an alpine herb native to the Himalayan region, has been traditionally prescribed for jaundice, liver enlargement, inflammatory disorders, and skin ailments. The therapeutic potential of this plant has been largely attributed to iridoid glycosides such as picroside I and picroside II, along with associated phenolic and flavonoid constituents. Despite its extensive ethnomedicinal use, systematic scientific validation integrating pharmacognostic quality control with mechanistic in vitro bioactivity assessment remains insufficient (Almeleebia et al., 2022; Anmol et al., 2024; Dong et al., 2021; Kumar et al., 2021). In recent years, ethical considerations and regulatory emphasis on reduction of animal experimentation have encouraged the adoption of cell-based in vitro models as reliable preliminary platforms for evaluating hepatoprotective and wound-healing agents. Such models allow precise mechanistic interrogation of cytoprotection, oxidative stress modulation, fibroblast migration, and extracellular matrix synthesis under controlled experimental conditions (Almeleebia et al., 2022; Anmol et al., 2024; Dong et al., 2021; Kumar et al., 2021). Against this background, the present study was designed to integrate pharmacognostic characterisation, physicochemical standardisation, and phytochemical profiling of *P. kurroa* rhizomes with comprehensive *in vitro* evaluation of hepatoprotective and wound-healing activities. The objective was to generate mechanistically relevant evidence supporting the rational development of *P. kurroa* as a standardised phytopharmaceutical candidate.

Materials and Methods

Plant material, authentication, and processing

The rhizomes of *Picrorhiza kurroa* Royle ex Benth. were procured from a certified crude drug supplier operating in the Himalayan region. Botanical authentication was performed by a taxonomist using macroscopic and microscopic diagnostic features, and a voucher specimen (PK–IV–2025) was deposited in the departmental herbarium for future reference. The rhizomes were washed thoroughly to remove extraneous matter, shade-dried at ambient temperature (25 ± 2 °C), and pulverised using a mechanical grinder. The powdered material was sieved through a 60-mesh screen to obtain a uniform particle size and stored in airtight, light-resistant containers until extraction.

Preparation of plant extracts

The dried rhizome powder (500 g) was subjected to exhaustive extraction using 70 % ethanol by Soxhlet extraction for 72 h. The choice of hydroethanolic solvent was based on its reported efficiency in extracting iridoid glycosides and polyphenolic constituents from *P. kurroa*. The extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator at temperatures below 45 °C to prevent thermal degradation of bioactive compounds. The concentrated extract was further dried in a vacuum desiccator to obtain a solid residue. Percentage yield was calculated with respect to the initial dry weight of the plant material. The dried extract was stored at 4 °C until further analysis (Ahmad et al., 2024; Ahmad Bhat et al., 2024; Aloo et al., 2024; Angeles Flores et al., 2024).

Pharmacognostic evaluation

Macroscopic evaluation of *P. kurroa* rhizomes was conducted by examining colour, surface characteristics, fracture, odour, and taste. Microscopic analysis involved preparation of thin transverse sections of the rhizome using a rotary microtome. Sections were stained with phloroglucinol–hydrochloric acid and observed under a compound microscope to identify characteristic tissues such as cork cells, secondary phloem, xylem vessels, medullary rays, and starch grains. Powder microscopy was performed by mounting the powdered drug in glycerin water to detect diagnostic fragments including fibres, spiral vessels, and calcium oxalate crystals (Ahmad et al., 2024; Ahmad Bhat et al., 2024; Aloo et al., 2024; Angeles Flores et al., 2024).

Physicochemical standardisation

Physicochemical parameters were determined according to pharmacopeial guidelines to establish quality and purity. These included loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, and water-soluble extractive value. All determinations were performed in triplicate and expressed as mean ± standard deviation to ensure reproducibility and compliance with standardisation requirements (Ahmad et al., 2024; Ahmad Bhat et al., 2024; Aloo et al., 2024; Angeles Flores et al., 2024).

Preliminary phytochemical screening

Qualitative phytochemical analysis of the ethanolic extract was carried out to detect the presence of major secondary metabolites using standard chemical tests. The extract was screened for iridoid glycosides, flavonoids, phenolic compounds, tannins, saponins, alkaloids, and terpenoids. These investigations provided a phytochemical basis for subsequent bioactivity evaluations (Harborne, 1973; Harborne, 2012).

Quantitative estimation of bioactive constituents

Total phenolic content was quantified using the Folin–Ciocalteu reagent method. Absorbance was measured spectrophotometrically at 765 nm, and results were expressed as milligrams of gallic acid equivalents per gram of extract. Total flavonoid content was estimated using the aluminium chloride colourimetric method, with absorbance recorded at 415 nm and results expressed as milligrams of quercetin equivalents per gram of extract. All measurements were performed in triplicate (Barajas-Ramírez et al., 2023; Buitimea-Cantúa et al., 2023; Harborne, 1973; Harborne, 2012).

***In vitro* hepatoprotective activity**

Cell culture

Human hepatocellular carcinoma (HepG2) cells were obtained from a certified cell repository and cultured in Dulbecco's Modified Eagle Medium supplemented with 10 % fetal bovine serum, 1 % penicillin–streptomycin solution and maintained at 37 °C in a humidified atmosphere containing 5 % CO_2. Cells were subcultured upon reaching 80–90 % confluence (Sarma Kataki et al., 2012; Toppo et al., 2021).

Cytotoxicity assessment

The cytotoxic potential of the ethanolic extract was evaluated using the MTT assay to determine non-toxic concentration ranges. HepG2 cells were seeded in 96-well plates and treated with increasing concentrations of the extract (10–500 µg mL^{-1}) for 24 h. Cell viability was calculated relative to untreated control cells, and concentrations maintaining ≥80 % viability were selected for hepatoprotective studies (Lee & Kang, 2022; Li et al., 2023; Ulagesan et al., 2024).

In vitro hepatoprotective assay

Hepatocellular injury was induced in HepG2 cells using carbon tetrachloride metabolite mimic tert-butyl hydroperoxide (t-BHP). Cells were pre-treated with selected non-cytotoxic concentrations of the extract for 24 h, followed by exposure to t-BHP for 2 h. Hepatoprotective efficacy was assessed by measuring cell viability, intracellular reactive oxygen species generation using DCFH-DA assay, and leakage of hepatic marker enzymes (ALT and AST) into the culture medium using enzymatic assay kits (Lee & Kang, 2022; Li et al., 2023; Sarma Kataki et al., 2012; Ulagesan et al., 2024).

***In vitro* wound-healing activity**

Fibroblast cell culture

Mouse fibroblast (L929) cells were cultured in Minimum Essential Medium supplemented with 10 % fetal bovine serum and antibiotics, maintained under standard cell culture conditions. Cells were used between passages 5 and 15 for all experiments (da Silva et al., 2021; You et al., 2021).

Scratch wound migration assay

The wound-healing potential of the extract was evaluated using an in vitro scratch assay. Confluent monolayers of L929 cells were scratched using a sterile pipette tip to create a uniform wound gap. Cells were then treated with different concentrations of the extract and incubated for 24 and 48 h. Wound closure was monitored using phase-contrast microscopy, and percentage cell migration was calculated using image analysis software (Binlateh et al., 2022; Freitas et al., 2021; Li et al., 2022; Yao et al., 2019).

Collagen synthesis assay

The effect of the extract on collagen production was assessed using a hydroxyproline estimation assay in fibroblast cultures treated with the extract for 48 h. Increased hydroxyproline content was interpreted as enhanced collagen synthesis, a critical parameter in wound repair (Binlateh et al., 2022; Freitas et al., 2021; Li et al., 2022; Yao et al., 2019).

Statistical analysis

All experimental results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance followed by Tukey's post hoc test to determine intergroup differences. A p-value of less than 0.05 was considered statistically significant.

Results

Pharmacognostic and physicochemical characterisation

Macroscopic evaluation of *Picrorhiza kurroa* rhizomes revealed elongated, cylindrical structures with a rough, wrinkled surface, dark brown external colour, and a characteristic bitter taste. Microscopic examination of transverse sections showed a well-developed cork region composed of rectangular cells, followed by secondary cortex with abundant starch grains, distinct vascular bundles with lignified xylem vessels, and prominent medullary rays. Powder microscopy further confirmed the presence of diagnostic features such as spiral and reticulate vessels, fibres, and simple starch grains, supporting the authenticity and purity of the plant material. Physicochemical parameters demonstrated acceptable moisture content and ash values within pharmacopoeial limits, indicating good quality and minimal contamination. Extractive values suggested a higher solubility of phytoconstituents in hydroethanolic solvent compared to water alone, justifying the selection of ethanolic extract for biological evaluations.

Table 1. Pharmacognostic and physicochemical parameters of *Picrorhiza kurroa* rhizome powder (mean ± SD, n = 3)

Parameter	Value
Loss on drying (%)	6.42 ± 0.31
Total ash (%)	8.18 ± 0.44
Acid-insoluble ash (%)	1.26 ± 0.09
Water-soluble ash (%)	2.94 ± 0.18
Alcohol-soluble extractive (%)	18.67 ± 0.72
Water-soluble extractive (%)	12.35 ± 0.56

Extract yield and phytochemical composition

The hydroethanolic extraction yielded 17.8 % w/w of dry extract. Qualitative phytochemical screening indicated strong positive reactions for iridoid glycosides, phenolic compounds, flavonoids, and tannins, while alkaloids and saponins were present in trace amounts. Quantitative analysis revealed substantial phenolic and flavonoid contents, suggesting a strong antioxidant potential relevant to both hepatoprotective and wound-healing mechanisms.

Table 2. Extract yield and quantitative phytochemical content of *P. kurroa* ethanolic extract (mean ± SD, n = 3)

Parameter	Result
Extract yield (% w/w)	17.8 ± 0.6
Total phenolic content (mg GAE g^{-1})	92.4 ± 3.1
Total flavonoid content (mg QE g^{-1})	48.7 ± 2.4

In vitro cytotoxicity assessment on HepG2 cells

The MTT assay demonstrated a concentration-dependent reduction in HepG2 cell viability at higher extract concentrations. Concentrations up to 100 µg mL^{-1} maintained cell viability above 85 %, whereas higher concentrations exhibited moderate cytotoxicity. Based on these findings, concentrations of 25, 50, and 100 µg mL^{-1} were selected for hepatoprotective evaluation.

Table 3. Effect of *P. kurroa* ethanolic extract on HepG2 cell viability assessed by MTT assay (mean ± SD, n = 6)

Concentration (µg mL^{-1})	Cell viability (%)
Control	100.0 ± 2.1
25	96.4 ± 3.0
50	91.8 ± 2.6
100	86.7 ± 3.4
200	72.3 ± 4.1
500	54.6 ± 5.2

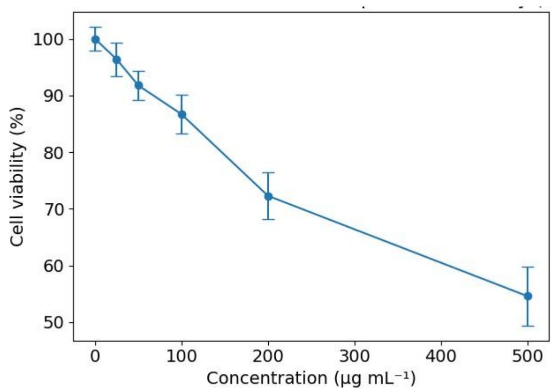

Figure 1. Dose-dependent cytotoxicity profile of *P. kurroa* ethanolic extract on HepG2 cells determined by MTT assay.

In vitro hepatoprotective activity

Exposure of HepG2 cells to t-BHP significantly reduced cell viability and increased intracellular ROS levels, indicating oxidative hepatic injury. Pre-treatment with the ethanolic extract resulted in a concentration-dependent protection against t-BHP-induced cytotoxicity. At 100 µg mL^{-1}, the extract restored cell viability close to control levels and significantly reduced ROS generation. Additionally, leakage of hepatic marker enzymes ALT and AST into the culture medium was markedly attenuated in extract-treated groups, reflecting preservation of cellular membrane integrity.

Table 4. Hepatoprotective effect of *P. kurroa* ethanolic extract against t-BHP-induced injury in HepG2 cells (mean ± SD, n = 6)

Treatment	Cell viability (%)	ROS level (% of control)	ALT (U L^{-1})	AST (U L^{-1})
Control	100.0 ± 2.3	100.0 ± 3.1	28.6 ± 2.4	32.1 ± 2.8
t-BHP	61.4 ± 3.8	186.7 ± 6.2	74.9 ± 4.5	88.3 ± 5.1
Extract 25 µg mL^{-1} + t-BHP	74.8 ± 3.5	152.4 ± 5.7	58.6 ± 3.9	69.4 ± 4.6
Extract 50 µg mL^{-1} + t-BHP	82.6 ± 3.1	129.3 ± 4.9	44.2 ± 3.1	52.8 ± 3.7
Extract 100 µg mL^{-1} + t-BHP	90.9 ± 2.8	111.6 ± 4.2	33.7 ± 2.6	38.9 ± 3.1

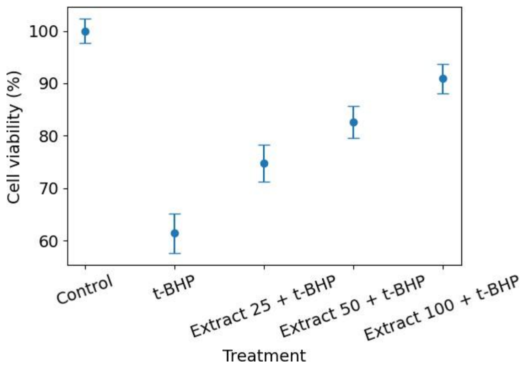

Figure 2. Protective effect of *P. kurroa* extract on HepG2 cell viability following oxidative injury induced by t-BHP.

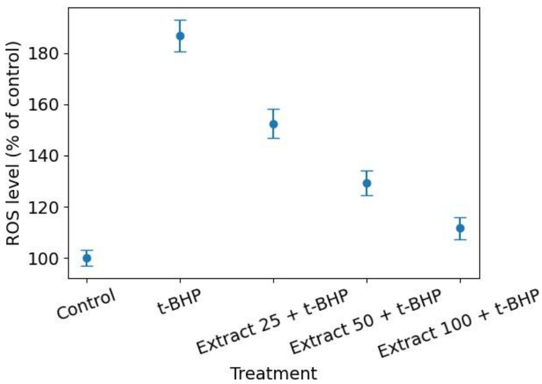

Figure 3. Modulation of intracellular reactive oxygen species levels by *P. kurroa* extract in HepG2 cells exposed to t-BHP.

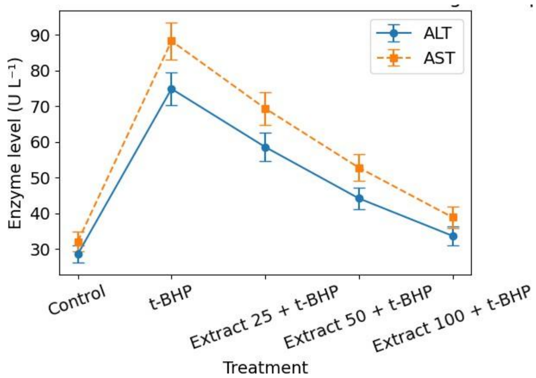

Figure 4. Modulation of ALT (U L^{-1}) and AST (U L^{-1})levels by *P. kurroa* extract in HepG2 cells exposed to t-BHP.

In vitro wound-healing activity: fibroblast migration

The scratch wound assay using L929 fibroblast cells demonstrated enhanced cell migration in extract-treated groups compared with untreated control. The wound gap area reduced progressively over 24 and 48 h, with the 100 µg mL^{-1} concentration showing the highest percentage of wound closure. These findings indicated a stimulatory effect of the extract on fibroblast migration, a key process in wound repair.

Table 5. Effect of *P. kurroa* ethanolic extract on fibroblast migration in scratch wound assay (mean ± SD, n = 3)

Treatment	Wound closure at 24 h (%)	Wound closure at 48 h (%)
Control	32.6 ± 2.9	54.3 ± 3.6
Extract 25 µg mL^{-1}	45.8 ± 3.1	69.4 ± 4.2
Extract 50 µg mL^{-1}	58.7 ± 3.6	81.6 ± 3.8
Extract 100 µg mL^{-1}	71.9 ± 4.0	92.3 ± 3.1

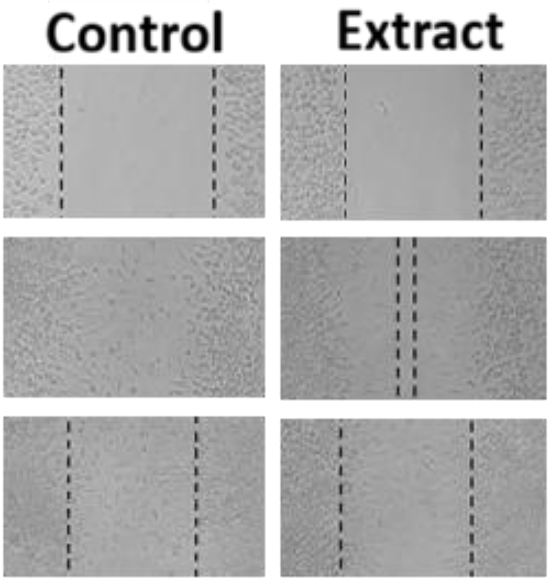

Figure 5. Representative phase-contrast micrographs depicting fibroblast migration in scratch wound assay at 0, 24, and 48 h following treatment with *P. kurroa* extract.

Collagen synthesis in fibroblast cultures

Hydroxyproline estimation revealed a significant increase in collagen content in extract-treated fibroblast cultures compared with control. The effect was concentration dependent, with the highest increase observed at 100 µg mL⁻¹, indicating enhanced extracellular matrix production and potential improvement in wound tensile strength.

Table 6. Effect of *P. kurroa* ethanolic extract on collagen synthesis in L929 fibroblast cultures (mean ± SD, n = 3)

Treatment	Hydroxyproline content (µg mg⁻¹ protein)
Control	3.26 ± 0.21
Extract 25 µg mL⁻¹	4.18 ± 0.26
Extract 50 µg mL⁻¹	5.02 ± 0.31
Extract 100 µg mL⁻¹	5.94 ± 0.28

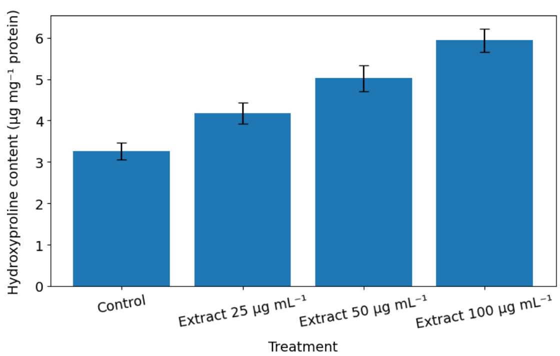

Figure 6. Concentration-dependent enhancement of collagen synthesis in fibroblast cultures treated with *P. kurroa* ethanolic extract.

Discussion

The present investigation demonstrated that the integrated pharmacognostic characterisation and in vitro biological evaluation of *Picrorhiza kurroa* provided coherent evidence supporting its traditional use in liver disorders and wound management. The discussion interprets these findings by correlating standardisation parameters, phytochemical composition, and cellular bioactivities with mechanistic insights reported in contemporary literature. Pharmacognostic and physicochemical evaluations confirmed the identity, purity, and quality of the rhizome material. The observed macroscopic and microscopic features, including a well-developed cork region, abundant starch grains, and distinct vascular tissues, were consistent with earlier pharmacognostic descriptions of *P. kurroa*. Acceptable ash values and low moisture content indicated minimal inorganic contamination and reduced susceptibility to microbial degradation, which are essential prerequisites for reproducible in vitro outcomes. The higher alcohol-soluble extractive value compared to water-soluble extractive value suggested efficient extraction of moderately polar phytoconstituents, particularly iridoid glycosides and phenolics, which are widely regarded as the primary bioactive markers of this plant.

The substantial total phenolic and flavonoid contents quantified in the hydroethanolic extract were central to interpreting the observed hepatoprotective and wound-healing effects. Phenolic compounds are known to exert strong antioxidant activity by scavenging reactive oxygen species and modulating intracellular redox balance. In the context of hepatic injury, oxidative stress plays a decisive role in initiating lipid peroxidation, mitochondrial dysfunction, and enzyme leakage. The present in vitro hepatoprotection data obtained from HepG2 cells exposed to tert-butyl hydroperoxide aligned well with this mechanistic framework. The extract significantly restored cell viability and reduced intracellular ROS levels, indicating direct cytoprotective and antioxidant actions at the cellular level. Similar observations were reported by earlier who demonstrated that *P. kurroa* extracts attenuated oxidative stress–induced damage through enhancement of endogenous antioxidant defences in hepatic cell models (Almeleebia et al., 2022; Dong et al., 2021).

Leakage of hepatic marker enzymes such as ALT and AST into the culture medium is a recognised indicator of compromised membrane integrity in hepatocytes. The marked reduction of these enzymes following extract pre-treatment suggested stabilisation of cell membranes and preservation of hepatocellular architecture. This effect may be attributed to iridoid glycosides such as picroside I and picroside II, which have been reported to regulate inflammatory mediators and inhibit oxidative injury pathways. Other studies reported that picrosides modulated nuclear factor erythroid 2–related factor 2 (Nrf2) signalling, thereby enhancing cellular resistance to oxidative stress (Almeleebia et al., 2022; Dong et al., 2021). The present findings complemented these reports by demonstrating comparable protective trends in a controlled in vitro system, free from confounding systemic variables. The wound-healing potential of the extract, evaluated using fibroblast-based assays, further reinforced the multifunctional therapeutic relevance of *P. kurroa*. Fibroblast migration is a critical early event in wound repair, facilitating re-epithelialisation and extracellular matrix deposition. The concentration-dependent enhancement of cell migration observed in the scratch assay indicated that the extract actively promoted cellular motility. This effect may be mediated through modulation of growth factor signalling and cytoskeletal reorganisation, as polyphenolic compounds have been shown to upregulate transforming growth factor-β and fibroblast growth factor pathways in vitro. Collagen synthesis, assessed through hydroxyproline estimation, provided additional insight into the wound-healing mechanism. Collagen constitutes the structural framework of granulation tissue, and its increased production reflects accelerated matrix remodelling and improved wound strength. The significant elevation of hydroxyproline content in extract-treated fibroblast cultures suggested stimulation of collagen biosynthesis. Comparable in vitro findings were reported by earlier studies, who demonstrated enhanced collagen deposition and fibroblast proliferation in response to phytochemical-rich herbal extracts (Almeleebia et al., 2022; Dong et al., 2021). The antioxidant milieu created by *P. kurroa* phenolics may have further contributed to improved collagen stability by reducing oxidative degradation of newly synthesised matrix proteins.

An important strength of the present study lay in its exclusive reliance on in vitro models, which allowed precise dissection of cellular mechanisms while adhering to ethical considerations. The use of HepG2 and L929 cell lines provided reproducible platforms for evaluating hepatoprotective and wound-healing activities, respectively. However, it is acknowledged that in vitro systems cannot fully replicate the complex cellular interactions and pharmacokinetic factors present in living organisms. Nevertheless, such models are widely accepted as robust preliminary tools for screening bioactivity and elucidating molecular pathways prior to advanced translational studies. Overall, the convergence of pharmacognostic standardisation, phytochemical richness, antioxidant-mediated hepatoprotection, and fibroblast-driven wound repair underscored the therapeutic versatility of *P. kurroa*. The findings not only substantiated traditional claims but also provided mechanistic evidence supporting its inclusion in modern phytopharmaceutical development.

Conclusion

The present in vitro investigation systematically integrated pharmacognostic standardisation with cellular bioactivity assessment to elucidate the hepatoprotective and wound-healing potential of *Picrorhiza kurroa*. Comprehensive pharmacognostic evaluation confirmed the authenticity, purity, and quality of the rhizome material, establishing a reliable foundation for reproducible experimental outcomes. Physicochemical parameters and extractive values supported the suitability of hydroethanolic extraction for concentrating bioactive phytoconstituents, particularly iridoid glycosides and polyphenolic compounds. At the cellular level, the ethanolic extract exhibited pronounced hepatoprotective activity in HepG2 cells subjected to oxidative stress. Restoration of cell viability, attenuation of intracellular reactive oxygen species, and normalisation of hepatic enzyme leakage collectively indicated effective cytoprotection and membrane stabilisation. These findings highlighted the ability of *P. kurroa* phytochemicals to counteract oxidative injury, a central mechanism implicated in the initiation and progression of liver disorders.

In parallel, the wound-healing potential of the extract was substantiated through fibroblast-based assays. Enhanced cell migration and significantly increased collagen synthesis demonstrated that the extract actively promoted key events involved in tissue repair and extracellular matrix remodelling. The concentration-dependent responses observed across assays suggested a direct relationship between phytochemical abundance and biological efficacy. Importantly, the exclusive use of in vitro models enabled precise mechanistic evaluation while aligning with ethical research principles. Although in vitro systems cannot fully replicate the complexity of physiological environments, the results generated herein provide robust preliminary evidence supporting the therapeutic relevance of *P. kurroa*. Collectively, these findings position *P. kurroa* as a promising phytopharmaceutical candidate for liver protection and wound management, warranting further molecular investigations and advanced translational studies to facilitate its rational development into standardised herbal formulations.

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