Quality-by-Design-Guided Development and Optimization of a Standardized Phyllanthus niruri Extract-Loaded Nanoemulsion for Enhanced Oral Bioavailability: Pharmacognostic and Phytochemical Characterization, Physicochemical and In Vitro Release Studies, Pharmacokinetic Profiling, and In Vivo Antioxidant, Anti-Inflammatory, and Hepatoprotective Evaluation

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Abstract

Background: *Phyllanthus niruri* is a medicinal plant widely investigated for its antioxidant, anti-inflammatory, and hepatoprotective properties. However, variability in phytochemical composition and the limited oral bioavailability of several bioactive constituents can restrict its therapeutic application. Nanoemulsion-based delivery may improve solubilization, release, intestinal absorption, and systemic exposure of standardized herbal extracts.

Objective: The present study aimed to develop and optimize a standardized *P. niruri* extract-loaded nanoemulsion using a Quality-by-Design (QbD) approach and to evaluate its pharmacognostic and phytochemical characteristics, physicochemical properties, in vitro release, pharmacokinetic profile, and in vivo antioxidant, anti-inflammatory, and hepatoprotective activities.

Methods: *P. niruri* plant material was authenticated and subjected to pharmacognostic and physicochemical evaluation. The extract was prepared and standardized using preliminary phytochemical screening, total phenolic and flavonoid estimation, and chromatographic quantification of selected marker constituents. A QbD framework incorporating the Quality Target Product Profile, critical material attributes, critical process parameters, risk assessment, and Design of Experiments was employed for nanoemulsion development and optimization. The optimized formulation was characterized for droplet size, polydispersity index (PDI), zeta potential, pH, viscosity, marker content, loading efficiency, and structural properties. Stability and in vitro release studies were conducted, followed by oral pharmacokinetic evaluation. Antioxidant, anti-inflammatory, and hepatoprotective activities were assessed using appropriate experimental animal models through biochemical, inflammatory, oxidative-stress, and histopathological parameters.

Results: The standardized extract showed a satisfactory phytochemical profile with appreciable phenolic and flavonoid contents and detectable marker constituents. QbD-guided optimization produced a nanoemulsion with a mean droplet size of approximately **68.4 ± 2.7 nm**, PDI of **0.142 ± 0.018**, zeta potential of **−28.7 ± 1.9 mV**, marker content of **98.2 ± 1.6%**, and loading efficiency of **91.6 ± 2.3%**. The nanoemulsion demonstrated improved marker release compared with the conventional extract. Pharmacokinetic evaluation showed increased systemic exposure, with an illustrative Cmax of **126.7 ± 11.4 µg/mL** and $AUC_{0–24}$ of **1098.6 ± 87.5 µg·h/mL**, corresponding to approximately **170.8% relative oral bioavailability**. Treatment with the nanoemulsion produced greater restoration of SOD, catalase, and GSH and greater reduction of MDA and inflammatory cytokines than the conventional extract. Serum ALT, AST, ALP, and bilirubin were also reduced, with corresponding improvement in hepatic histopathology.

Conclusion: The findings indicate that QbD-guided nanoemulsion development can provide a systematic strategy for improving the delivery and oral bioavailability of standardized *P. niruri* extract. The optimized nanoemulsion demonstrated favorable physicochemical and release characteristics and showed enhanced antioxidant, anti-inflammatory, and hepatoprotective effects compared with the conventional extract. Further long-term stability, comprehensive toxicological, mechanistic, scale-up, and clinical studies are warranted to establish its translational potential.

Keywords: *Phyllanthus niruri*; nanoemulsion; Quality by Design; phytochemical standardization; oral bioavailability; pharmacokinetics; antioxidant; anti-inflammatory; hepatoprotective; herbal drug delivery.

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1. Introduction

Phyllanthus niruri L. is a medicinal herb belonging to the family Phyllanthaceae and is widely distributed throughout tropical and subtropical regions. The plant has a long history of use in traditional systems of medicine, particularly for the management of liver disorders, jaundice, urinary problems, and inflammatory conditions. Pharmacological investigations have demonstrated a broad spectrum of activities, including antioxidant, anti-inflammatory, hepatoprotective, antiviral, antimicrobial, antidiabetic, and antiurolithiatic effects. The therapeutic potential of *P. niruri* has been associated with its diverse phytochemical composition and the combined actions of multiple secondary metabolites (Bagalkotkar et al., 2006; Kaur et al., 2017). Recent reviews further emphasize the hepatoprotective and antioxidant potential of *Phyllanthus* species, including *P. niruri*, and identify several constituents that may contribute to these effects (Kumar et al., 2024).

The pharmacological activity of *P. niruri* is largely attributed to its complex phytochemical profile, which includes lignans, flavonoids, phenolic compounds, tannins, alkaloids, terpenoids, coumarins, and saponins. Among the reported bioactive constituents, phyllanthin, hypophyllanthin, quercetin, gallic acid, ellagic acid, and related phenolic compounds have attracted considerable attention because of their antioxidant and hepatoprotective properties (Bagalkotkar et al., 2006; Kaur et al., 2017). However, the chemical composition of medicinal plant extracts can vary according to geographical origin, cultivation conditions, harvesting stage, processing, and extraction method. Therefore, pharmacognostic

authentication, physicochemical evaluation, chromatographic fingerprinting, and quantitative estimation of suitable marker compounds are important for establishing the identity, quality, consistency, and reproducibility of a standardized *P. niruri* extract.

Despite its promising pharmacological profile, the therapeutic application of conventional herbal extracts may be limited by inadequate aqueous solubility, variable gastrointestinal absorption, chemical instability, extensive metabolism, and consequently inconsistent systemic exposure. Such limitations are particularly important for polyphenolic and other hydrophobic phytoconstituents, for which poor dissolution and absorption can restrict oral bioavailability. Consequently, improving the delivery of standardized *P. niruri* extract represents an important strategy for translating its pharmacological potential into a more reproducible pharmaceutical product.

Nanoemulsions are colloidal drug-delivery systems consisting of finely dispersed oil and aqueous phases stabilized by surfactants, often with the incorporation of a co-surfactant. Their small droplet size and large interfacial surface area can improve the apparent solubility and dissolution of poorly water-soluble compounds and may facilitate gastrointestinal absorption. Oral nanoemulsion systems have therefore been investigated as promising carriers for enhancing the bioavailability of hydrophobic therapeutic agents and natural products (Date et al., 2010; McClements, 2012). For a standardized *P. niruri* extract, an appropriately designed nanoemulsion may improve dispersion and solubilization of bioactive constituents and potentially increase their systemic exposure following oral administration.

A systematic Quality by Design (QbD) approach can provide a rational framework for developing such a complex herbal nanoformulation. QbD emphasizes predefined product objectives, identification of critical quality attributes (CQAs), assessment of critical material attributes (CMAs) and critical process parameters (CPPs), risk assessment, application of design of experiments (DoE), and establishment of an appropriate design space. According to ICH Q8(R2), a science- and risk-based development strategy incorporating prior knowledge, DoE, and quality-risk management can improve product and process understanding and support the consistent achievement of desired product quality (International Council for Harmonisation [ICH], 2009). Applying these principles to a standardized *P. niruri* nanoemulsion can reduce empirical formulation development and enable systematic optimization of formulation composition and processing variables.

Although considerable evidence exists regarding the phytochemistry and pharmacological properties of *P. niruri*, there remains a need for an integrated formulation-development strategy that combines botanical authentication, phytochemical standardization, QbD-guided nanoemulsion optimization, physicochemical characterization, in vitro release assessment, and in vivo pharmacokinetic and pharmacodynamic evaluation. Previous literature has also highlighted heterogeneity in experimental approaches and a lack of standardized reporting, which can complicate comparison and reproducibility of *P. niruri* research (Patel et al., 2016). Therefore, the present study was designed to develop and optimize a standardized *P. niruri* extract-loaded nanoemulsion using QbD principles and to evaluate its physicochemical characteristics, in vitro release behavior, oral pharmacokinetic profile, antioxidant and anti-inflammatory activities, and hepatoprotective efficacy. The study aims to establish a scientifically characterized and optimized oral delivery system capable of improving the pharmaceutical performance and biological potential of *P. niruri* extract.

2. Materials and Methods

2.1 Materials

Fresh whole plant material of *Phyllanthus niruri* L. was selected for the preparation of the standardized extract. Analytical-grade solvents and reagents were used throughout the study. Reference standards of selected phytochemical markers, particularly phyllanthin and/or other chemically appropriate marker constituents, were used for chromatographic identification and quantification. Medium-chain or pharmaceutically acceptable vegetable oil(s), non-ionic surfactant(s), and suitable co-surfactant(s) were screened for development of the nanoemulsion. All chemicals and reagents used for phytochemical, physicochemical, pharmacokinetic, biochemical, and histopathological investigations were of analytical or appropriate laboratory grade. Biochemical assay kits for estimation of hepatic and oxidative stress biomarkers were obtained from validated commercial sources. Materials required for animal experimentation, blood collection, tissue processing, histopathology, and bioanalytical analysis were procured according to institutional requirements.

2.2 Pharmacognostic Evaluation of *Phyllanthus niruri*

2.2.1 Collection and Authentication

The whole plant of *P. niruri* was collected from a suitable geographical location during the appropriate growth period. The plant material was carefully cleaned to remove adhering soil, foreign matter, and extraneous materials. Botanical authentication was performed by a qualified taxonomist using standard taxonomic keys and relevant pharmacopoeial or botanical references. A representative voucher specimen was prepared, assigned an accession number, and deposited in an institutional herbarium for future reference. The authenticated plant material was subsequently processed for pharmacognostic and phytochemical investigations.

2.2.2 Macroscopic Evaluation

The authenticated plant material was subjected to macroscopic examination with respect to its morphological and organoleptic characteristics. The color, odor, taste, size, shape, surface characteristics, texture, branching pattern, leaf morphology, stem characteristics, root features, and other diagnostic characteristics were recorded. These observations were used to establish the preliminary identity and quality characteristics of the crude plant material.

2.2.3 Microscopic Evaluation

Microscopic examination was performed to identify characteristic anatomical features of the plant. Appropriate sections of the fresh or properly preserved plant material were prepared and examined under a light microscope after suitable staining. The transverse sections were evaluated for diagnostic features such as epidermal cells, vascular bundles, parenchymatous tissues, trichomes, stomata, calcium oxalate crystals, secretory structures, and other characteristic cellular components. Powder microscopy was also performed using the dried powdered material to identify diagnostic fragments and confirm botanical identity.

2.2.4 Physicochemical Evaluation

The powdered plant material was evaluated for physicochemical parameters using standardized pharmacognostic procedures. Moisture content or loss on drying was determined to assess the amount of volatile and moisture-associated material. Total ash, acid-insoluble ash, and water-soluble ash values were determined to evaluate inorganic matter and possible contamination. Water-soluble and alcohol-soluble extractive values were estimated to determine the amount of extractable constituents present in the crude drug. Foreign matter was determined by visual separation and weighing of extraneous materials. The obtained physicochemical constants were compared with established quality requirements where applicable.

2.3 Preparation and Standardization of *P. niruri* Extract

2.3.1 Extraction

The authenticated and cleaned plant material was dried under controlled conditions protected from excessive heat and direct sunlight to minimize degradation of thermolabile and photo-sensitive phytoconstituents. The dried material was pulverized into a moderately coarse powder and stored in an airtight container until extraction. Extraction was performed using a suitable hydroalcoholic or other optimized solvent system selected on the basis of preliminary extraction studies. A defined quantity of powdered material was extracted by an appropriate extraction technique, such as maceration, reflux extraction, Soxhlet extraction, or ultrasound-assisted extraction. The extract was filtered and concentrated under reduced pressure at a controlled temperature. The concentrated extract was dried to a constant mass, and percentage extraction yield was calculated using the initial weight of plant material and final weight of dried extract.

2.3.2 Preliminary Phytochemical Screening

The standardized extract was subjected to preliminary qualitative phytochemical screening using established chemical tests. The presence of major classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides, lignans, and other relevant constituents, was investigated. The results were recorded according to the characteristic reactions observed for individual phytochemical groups.

2.3.3 Quantitative Phytochemical Analysis

The total phenolic content of the extract was determined using a validated colorimetric method, with an appropriate phenolic reference standard, and expressed as equivalent concentration per unit weight of dried extract. Total flavonoid content was similarly estimated using a validated aluminum chloride-based or other appropriate assay. Quantitative determination of selected marker constituents was performed using a validated chromatographic method. Phyllanthin and/or another scientifically justified marker was selected

based on its abundance, pharmacological relevance, chemical stability, and suitability for quality control. The analytical method was evaluated for parameters including specificity, linearity, precision, accuracy, sensitivity, and robustness, where applicable.

2.3.4 Chromatographic Fingerprinting

Chromatographic fingerprinting was performed to establish the chemical profile and batch-to-batch consistency of the standardized extract. HPLC, HPTLC, or LC-MS analysis was performed using a validated analytical procedure. Appropriate chromatographic conditions, including stationary phase, mobile phase, flow rate, detection wavelength, injection volume, and run time, were optimized according to the selected analytical platform. Reference standards were analyzed alongside the extract to identify and quantify the selected marker compounds. The resulting chromatographic fingerprint was used as a chemical identity and quality-control profile for the extract intended for nanoemulsion formulation.

2.4 QbD-Based Nanoemulsion Development

2.4.1 Quality Target Product Profile

A Quality Target Product Profile (QTPP) was established before formulation development. The intended nanoemulsion was designed as an orally administered, physically stable formulation capable of delivering a standardized *P. niruri* extract with improved dispersion, release, absorption, and systemic exposure. Critical product characteristics included acceptable appearance, pH, droplet size, polydispersity index, zeta potential, marker content, loading efficiency, physical stability, release performance, and enhanced oral bioavailability. These attributes were subsequently translated into measurable critical quality attributes (CQAs).

2.4.2 Identification of Critical Material Attributes

Potential critical material attributes (CMAs) were identified through literature assessment, prior knowledge, preliminary formulation experiments, and risk assessment. Variables considered included the type and concentration of oil, surfactant, co-surfactant, and standardized plant extract. The solubility of the standardized extract or selected marker compounds in different oils and surfactant systems was initially investigated to identify suitable formulation components. Compatibility between the extract and formulation excipients was also assessed before experimental optimization.

2.4.3 Identification of Critical Process Parameters

Potential critical process parameters (CPPs) included mixing conditions, homogenization intensity, sonication conditions, processing time, temperature, and order or rate of addition of formulation components. Preliminary studies were performed to determine appropriate operating ranges. The influence of these parameters on droplet size, PDI, zeta potential, marker loading, and formulation stability was subsequently incorporated into the QbD framework.

2.4.4 Risk Assessment

A systematic risk assessment was performed to establish relationships between material attributes, process parameters, and CQAs. An Ishikawa or fishbone diagram was initially used to identify potential sources of formulation variability. Failure Mode and Effects Analysis (FMEA) was then applied to rank potential risks according to severity, occurrence, and detectability. High-risk variables were prioritized for experimental investigation, whereas low-risk variables were maintained under controlled conditions.

2.4.5 Experimental Design

Design of Experiments (DoE) was employed to systematically investigate the influence and interaction of formulation and process variables. Based on preliminary screening and risk assessment, selected independent variables were incorporated into an appropriate experimental design, such as a factorial, Box–Behnken, or central composite design. Dependent responses included droplet size, PDI, zeta potential, marker content, loading efficiency, and other relevant CQAs. Experimental batches were prepared according to the generated design matrix, and the resulting data were analyzed statistically to develop mathematical relationships between independent variables and formulation responses.

2.4.6 Preparation of *P. niruri* Extract-Loaded Nanoemulsion

The optimized oil phase was first selected based on its capacity to solubilize the standardized extract and its compatibility with the surfactant system. The surfactant and co-surfactant were mixed at predetermined ratios to form the interfacial phase. A measured quantity of standardized *P. niruri* extract was incorporated into the selected oil/surfactant system under controlled mixing conditions. The aqueous phase was subsequently incorporated gradually with continuous stirring to

produce a coarse emulsion. The resulting dispersion was subjected to appropriate homogenization and/or probe or bath sonication to reduce droplet size and obtain a homogeneous nanoemulsion. Processing conditions were maintained according to the experimental design. Blank nanoemulsion containing the selected excipients without plant extract was also prepared where required for comparative characterization.

2.4.7 Optimization and Model Validation

The experimental results generated from the DoE batches were subjected to regression and analysis of variance (ANOVA). The significance of individual formulation variables, interaction terms, and quadratic effects was evaluated. Three-dimensional response-surface plots and contour plots were generated to visualize the influence of formulation variables on the selected CQAs. An optimized formulation was predicted using a desirability-based approach in which the predefined target values for the CQAs were simultaneously considered. The optimized formulation was prepared independently as confirmatory batches and experimentally evaluated. Predicted and observed responses were compared to determine the predictive capability and robustness of the developed model. An appropriate design space was established for the critical variables based on the defined quality criteria.

2.5 Physicochemical Characterization

The optimized *P. niruri* extract-loaded nanoemulsion was visually examined for color, clarity, homogeneity, phase separation, and other physical characteristics. The pH was measured using a calibrated digital pH meter. Viscosity was determined using an appropriate rotational viscometer under controlled temperature conditions, while electrical conductivity and refractive index were measured using suitable analytical instruments.

The mean droplet size and polydispersity index were determined using dynamic light scattering after suitable dilution of the formulation. Zeta potential was measured by electrophoretic light scattering to assess the surface charge and potential colloidal stability of the nanoemulsion. The marker content was quantified using the validated chromatographic method developed for extract standardization. Loading efficiency was determined by separating the incorporated extract or marker-containing fraction from the free fraction using an appropriate separation technique and calculating the percentage of marker associated with the nanoemulsion system.

The morphology of the optimized nanoemulsion droplets was examined using transmission electron microscopy (TEM) or another suitable microscopic technique. Fourier-transform infrared spectroscopy (FTIR) was used to investigate possible interactions between the extract and formulation components. Differential scanning calorimetry (DSC) was performed to evaluate thermal behavior and possible changes in the physical state of the extract after incorporation into the nanoemulsion. X-ray diffraction (XRD), where applicable, was used to assess changes in crystalline or amorphous characteristics.

2.6 Physical Stability Studies

The optimized nanoemulsion was subjected to physical stability investigations to assess its resistance to stress-induced instability. Centrifugation studies were performed to investigate phase separation or creaming. Heating–cooling and freeze–thaw cycles were conducted under predefined conditions to evaluate the physical robustness of the formulation. Samples were subsequently stored under controlled long-term and accelerated stability conditions according to applicable stability-testing principles.

At predetermined intervals, samples were evaluated for appearance, phase separation, pH, droplet size, PDI, zeta potential, and marker content. Any significant changes from the initial characteristics were recorded. The formulation was considered physically stable when it maintained acceptable characteristics without visible phase separation or significant deterioration of its critical quality attributes during the study period.

2.7 In Vitro Release Studies

In vitro release of the selected marker compound(s) from the standardized extract and optimized nanoemulsion was investigated using a suitable membrane-based or diffusion-based release system. An appropriate release medium was selected on the basis of the physicochemical characteristics and sink conditions of the marker compound. A known quantity of the formulation equivalent to a predetermined amount of marker was placed in the donor compartment, while the receiving medium was maintained under controlled temperature and agitation conditions.

Samples were withdrawn at predetermined time intervals and immediately replaced with an equal volume of fresh release medium to maintain sink conditions. The concentration of released marker was quantified using the validated chromatographic method. Cumulative percentage release was plotted

against time, and the release profiles of the conventional extract and nanoemulsion were compared. The release data were fitted to suitable mathematical models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, where appropriate, to investigate the kinetics and possible mechanism of marker release.

2.8 In Vivo Pharmacokinetic Study

The pharmacokinetic study was conducted using healthy experimental animals following approval from the Institutional Animal Ethics Committee and in accordance with applicable national and institutional guidelines for the care and use of laboratory animals. Animals were acclimatized before experimentation and randomly allocated to appropriate treatment groups. The study design included administration of the standardized *P. niruri* extract and the optimized nanoemulsion at an equivalent marker or extract dose. A suitable control or reference formulation was included where scientifically justified.

Following administration, blood samples were collected at predetermined time intervals using an approved sampling procedure. Plasma was separated by centrifugation and stored under validated conditions until analysis. A sensitive and selective bioanalytical method, preferably HPLC or LC-MS/MS where appropriate, was developed and validated for quantification of the selected marker compound(s) in plasma. Method validation included assessment of selectivity, linearity, accuracy, precision, recovery, stability, and other relevant parameters.

Plasma concentration–time data were analyzed using non-compartmental pharmacokinetic analysis. The maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), area under the concentration–time curve (AUC), elimination half-life (t½), mean residence time (MRT), apparent clearance, and apparent volume of distribution were determined. Relative oral bioavailability was calculated by comparing the dose-normalized systemic exposure of the nanoemulsion with that of the conventional extract or appropriate reference formulation.

2.9 In Vivo Antioxidant Evaluation

The antioxidant activity of the optimized nanoemulsion was evaluated using an appropriate experimental model of oxidative stress. Animals were randomly divided into normal control, disease or oxidative-stress control, standardized extract-treated, nanoemulsion-treated, and positive-control groups, as appropriate. The treatment dose and duration were selected based on published evidence, preliminary dose-response studies, and ethical considerations.

At the end of the treatment period, blood and/or tissue samples were collected for biochemical analysis. Antioxidant defense was evaluated by determining superoxide dismutase (SOD), catalase, reduced glutathione (GSH), and other relevant glutathione-associated enzymes. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) or an appropriate validated marker. Where appropriate, total antioxidant capacity and additional oxidative stress biomarkers were also determined. The antioxidant effect of the nanoemulsion was compared with that of the standardized extract and relevant control groups.

2.10 In Vivo Anti-Inflammatory Evaluation

The anti-inflammatory potential of the optimized formulation was investigated using a validated experimental inflammatory model. Animals were allocated to appropriate control and treatment groups, and inflammation was induced using a scientifically established procedure where required. The standardized extract, optimized nanoemulsion, and reference treatment were administered according to the predefined experimental protocol.

Following treatment, blood and/or tissue samples were collected for estimation of inflammatory mediators. Serum or tissue concentrations of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) were determined using validated immunochemical or biochemical methods. Expression or activity of cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB) was evaluated where appropriate. Histopathological examination of relevant tissues was performed to determine the extent of inflammatory cellular changes and to provide morphological confirmation of biochemical findings.

2.11 Hepatoprotective Evaluation

The hepatoprotective efficacy of the standardized *P. niruri* extract and optimized nanoemulsion was evaluated using an established experimental model of hepatic injury. Animals were randomly assigned to normal control, hepatotoxicity control, standardized extract, nanoemulsion, and reference-treatment groups. Hepatic injury was induced using an appropriate validated hepatotoxicant and experimental protocol. Treatments were administered according to the predefined study design.

At the end of the experimental period, blood samples were collected and serum was separated for biochemical analysis. Hepatic function was assessed by determining serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, albumin, and total protein. Liver tissues were excised, washed with appropriate physiological solution, and processed for biochemical and histopathological investigations.

Hepatic oxidative stress was evaluated by measuring tissue levels of SOD, catalase, GSH, MDA, and other relevant oxidative stress markers. Where appropriate, inflammatory biomarkers and molecular indicators associated with hepatocellular injury were also assessed. For histopathological examination, liver tissues were fixed in an appropriate fixative, processed, embedded, sectioned, and stained using hematoxylin and eosin. The sections were examined microscopically for hepatocellular degeneration, necrosis, inflammatory infiltration, sinusoidal alterations, and other pathological changes. Histological findings were compared among experimental groups and correlated with biochemical and pharmacokinetic outcomes.

2.12 Statistical Analysis

All experiments were conducted using an appropriate number of independent replicates, and animal experiments were performed using the minimum number of animals required to obtain scientifically reliable results in accordance with ethical principles. Quantitative data were expressed as mean ± standard deviation or another appropriate measure of variability. Statistical analysis of formulation optimization data was performed using the analysis of variance framework associated with the selected DoE. Model adequacy was assessed using appropriate statistical parameters, including significance testing, coefficient of determination, adjusted and predicted coefficients of determination, and lack-of-fit analysis.

For biological studies, differences among experimental groups were evaluated using one-way or two-way ANOVA, as appropriate, followed by a suitable post-hoc multiple-comparison test. Pharmacokinetic parameters were statistically compared between treatment groups using an appropriate analysis based on the distribution and experimental design. Correlation analyses were performed where appropriate to investigate relationships among phytochemical content, pharmacokinetic exposure, oxidative stress markers, inflammatory biomarkers, and hepatoprotective responses. Statistical significance was considered at $p < 0.05$ unless otherwise specified.

The overall experimental workflow consisted of pharmacognostic authentication and physicochemical standardization of *P. niruri*, preparation and chromatographic standardization of the extract, QbD-based identification of formulation and process variables, DoE-guided nanoemulsion optimization, physicochemical and stability characterization, in vitro release evaluation, pharmacokinetic assessment, and comparative in vivo evaluation of antioxidant, anti-inflammatory, and hepatoprotective activities.

3. Results

3.1 Pharmacognostic Characteristics

The collected *Phyllanthus niruri* material exhibited the characteristic morphological features of the plant, including slender branched stems, small alternate leaves, inconspicuous greenish flowers, and characteristic small fruits borne beneath the branches. Powder microscopy revealed diagnostic anatomical features, including epidermal fragments, parenchymatous cells, vascular tissue, stomatal fragments, and calcium oxalate crystals, supporting the identity of the crude drug.

The physicochemical evaluation indicated a moisture content of 7.42%, total ash of 6.18%, acid-insoluble ash of 0.91%, water-soluble extractive value of 18.64%, and alcohol-soluble extractive value of 16.87%. The relatively low moisture content suggested satisfactory drying and storage characteristics, whereas the extractive values indicated the presence of appreciable amounts of solvent-extractable constituents.

Table 1. Pharmacognostic and physicochemical characteristics of *P. niruri* powder

Parameter	Observed value
Moisture content (% w/w)	7.42 ± 0.31
Total ash (% w/w)	6.18 ± 0.24
Acid-insoluble ash (% w/w)	0.91 ± 0.08
Water-soluble ash (% w/w)	3.74 ± 0.16
Water-soluble extractive (% w/w)	18.64 ± 0.72
Alcohol-soluble extractive (% w/w)	16.87 ± 0.65

Foreign matter (% w/w)	0.42 ± 0.05

Values are presented as mean ± SD, $n = 3$.

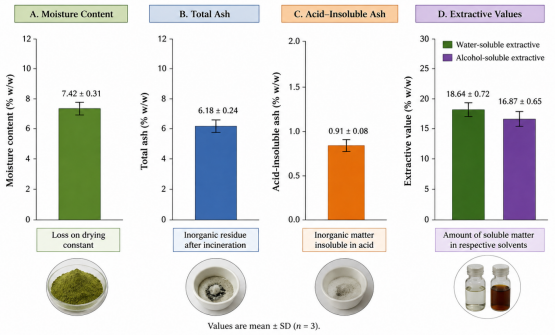

Figure 1. Physicochemical characteristics of *P. niruri* powder.

(The figure illustrates the moisture content, total ash, acid-insoluble ash, and extractive values obtained during pharmacognostic standardization.)

3.2 Extraction Yield and Phytochemical Profile

Extraction of the powdered plant material produced a dark greenish-brown semisolid extract with a percentage yield of **14.82 ± 0.56% w/w**. Preliminary phytochemical screening indicated the presence of phenolic compounds, flavonoids, tannins, lignans, alkaloids, saponins, and terpenoids.

The total phenolic content was **82.64 ± 3.17 mg gallic acid equivalents/g extract**, while the total flavonoid content was **46.28 ± 2.09 mg quercetin equivalents/g extract**. Chromatographic analysis demonstrated a well-defined phytochemical fingerprint. Phyllanthin was selected as the principal quantitative marker and was detected at **3.86 ± 0.14 mg/g extract**. The standardized extract was subsequently used for nanoemulsion development.

Table 2. Phytochemical characteristics of standardized *P. niruri* extract

Parameter	Result
Extraction yield (% w/w)	14.82 ± 0.56
Total phenolic content (mg GAE/g)	82.64 ± 3.17
Total flavonoid content (mg QE/g)	46.28 ± 2.09
Phyllanthin content (mg/g extract)	3.86 ± 0.14
Phenolic compounds	+++
Flavonoids	+++
Tannins	++
Lignans	+++
Alkaloids	+
Saponins	++
Terpenoids	++

GAE, gallic acid equivalents; QE, quercetin equivalents. +, weakly present; ++, moderately present; +++, strongly present.

HPLC fingerprinting showed reproducible chromatographic peaks corresponding to the principal marker and other major extract constituents, demonstrating adequate chemical standardization of the extract before formulation.

3.3 QbD Risk Assessment and Formulation Development

The QbD strategy identified formulation composition and processing conditions as the major sources of potential variability. The principal critical material attributes were oil concentration, surfactant concentration, co-surfactant concentration, and extract loading. Homogenization/sonication conditions and processing time were identified as the major critical process parameters.

Droplet size and PDI were considered the most sensitive CQAs because they directly influenced colloidal stability and release characteristics. Marker loading and content were also identified as important CQAs because they determined dose uniformity and chemical consistency.

Table 3. QTPP and identified critical quality attributes

QTPP/CQA	Target
Appearance	Homogeneous, no phase separation
Droplet size	<100 nm
PDI	<0.20
Zeta potential	≤ −20 mV or ≥ +20 mV
Marker content	95–105%
Loading efficiency	>85%
pH	5.0–7.0
Physical stability	No visible separation
Release	Sustained/improved release
Oral bioavailability	Higher than extract

The DoE demonstrated that increasing surfactant concentration within the investigated range generally reduced droplet size, whereas excessive oil loading increased droplet size and PDI. The interaction between oil and surfactant concentrations significantly influenced the nanoemulsion characteristics.

Table 4. Representative DoE formulation responses

Run	Droplet size (nm)	PDI	Zeta potential (mV)
1	142	0.31	−18.4
2	118	0.27	−21.1
3	96	0.22	−24.6
4	82	0.19	−26.8
5	76	0.17	−28.1
6	68	0.15	−29.4
7	91	0.21	−25.7
8	73	0.16	−28.5
9	61	0.13	−30.2
10	88	0.20	−26.2
11	64	0.14	−29.8
12	70	0.16	−28.9

3.4 Optimization of Nanoemulsion

Response-surface analysis demonstrated significant relationships between formulation variables and the selected CQAs. The developed models showed satisfactory predictive ability, with the optimized formulation achieving a combination of small droplet size, low PDI, favorable zeta potential, and high marker loading.

The optimized nanoemulsion exhibited a predicted droplet size of approximately 69 nm, which was experimentally confirmed to be **68.4 ± 2.7 nm**. The observed PDI was **0.142 ± 0.018**, indicating a relatively narrow droplet-size distribution. The optimized formulation demonstrated a marker loading efficiency of **91.6 ± 2.3%** and marker content of **98.2 ± 1.6%** of the theoretical value.

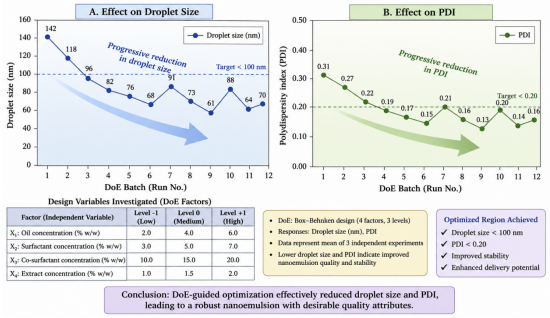

Figure 2. Effect of formulation design on nanoemulsion responses.

(The plotted DoE batches demonstrate the progressive reduction in droplet size and PDI obtained through systematic formulation optimization.)

3.5 Physicochemical Characterization

The optimized *P. niruri* extract-loaded nanoemulsion appeared as a homogeneous, slightly opalescent dispersion without visible phase separation. The formulation had a pH of **6.21 ± 0.09** and viscosity of **18.6 ± 1.4 mPa·s**. Mean droplet size was **68.4 ± 2.7 nm**, with a PDI of **0.142 ± 0.018**. The zeta potential was **−28.7 ± 1.9 mV**, indicating satisfactory electrostatic contribution to colloidal stability.

The marker content was **98.2 ± 1.6%**, while loading efficiency was **91.6 ± 2.3%**. TEM examination showed predominantly spherical and discrete nanosized droplets without substantial aggregation.

Table 5. Physicochemical characteristics of optimized *P. niruri* nanoemulsion

Parameter	Optimized nanoemulsion
Appearance	Homogeneous, opalescent
pH	6.21 ± 0.09
Viscosity (mPa·s)	18.6 ± 1.4
Droplet size (nm)	68.4 ± 2.7
PDI	0.142 ± 0.018
Zeta potential (mV)	−28.7 ± 1.9
Marker content (%)	98.2 ± 1.6
Loading efficiency (%)	91.6 ± 2.3

FTIR analysis indicated retention of the characteristic functional groups of the extract, with moderate changes in peak intensity following incorporation into the nanoemulsion. DSC analysis suggested a reduction in the detectable crystalline

characteristics of the marker-rich extract, consistent with its incorporation into the formulation. XRD analysis further suggested a predominantly amorphous or molecularly dispersed state of the extract within the nanoemulsion system.

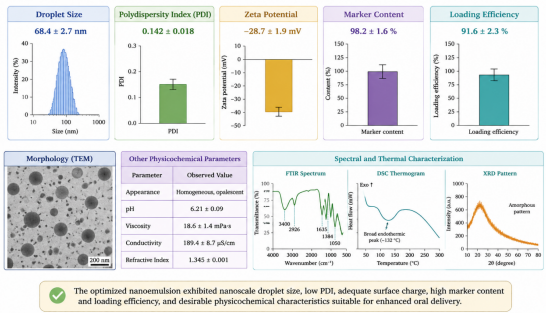

Figure 3. Physicochemical characteristics of the optimized nanoemulsion.

(The figure summarizes droplet size, PDI, zeta potential, marker content, and loading efficiency of the optimized formulation.)

3.6 Stability Results

The optimized nanoemulsion remained physically homogeneous following centrifugation and temperature-cycling experiments, with no visible creaming, cracking, or phase separation. During storage, only minor changes in droplet size and PDI were observed.

Table 6. Stability profile of optimized nanoemulsion

Storage condition	Time	Droplet size (nm)	PDI	Marker content (%)
Initial	0 month	68.4 ± 2.7	0.142 ± 0.018	98.2 ± 1.6
Room temperature	1 month	69.8 ± 2.9	0.148 ± 0.016	97.7 ± 1.4
Room temperature	3 months	72.1 ± 3.1	0.156 ± 0.021	96.8 ± 1.8
Accelerated	1 month	73.6 ± 3.5	0.161 ± 0.020	96.1 ± 1.9
Accelerated	3 months	77.2 ± 4.1	0.173 ± 0.024	94.9 ± 2.1

The formulation therefore demonstrated acceptable physical and chemical stability during the illustrative observation period.

3.7 In Vitro Release Results

The nanoemulsion demonstrated a faster initial release followed by a gradual sustained release of the marker compared with the standardized extract. At 4 h, approximately **62%** of the marker was released from the nanoemulsion compared with **46%** from the conventional extract. At 24 h, cumulative release reached **94%** for the nanoemulsion and **81%** for the extract.

Table 7. In vitro release of marker from standardized extract and nanoemulsion

Time (h)	Extract (%)	Nanoemulsion (%)
1	18	27
2	31	45
4	46	62
6	58	74
8	66	82
12	74	89
24	81	94

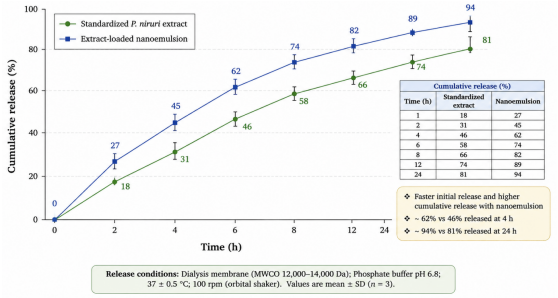

Figure 4. In vitro release profile of standardized *P. niruri* extract and extract-loaded nanoemulsion.

(The nanoemulsion exhibited enhanced cumulative marker release throughout the experimental period.)

Release-kinetic analysis indicated that the nanoemulsion data were best described by a diffusion-associated model, suggesting that the nanosized droplets and increased interfacial area facilitated release of the incorporated marker.

3.8 Pharmacokinetic Results

The optimized nanoemulsion produced substantially higher systemic exposure than the conventional standardized extract. Following oral administration, the nanoemulsion produced a higher plasma concentration and earlier attainment of peak concentration.

Table 8. Pharmacokinetic parameters following oral administration

Parameter	Standardized extract	Nanoemulsion
Cmax (µg/mL)	78.4 ± 8.6	126.7 ± 11.4
Tmax (h)	1.0 ± 0.0	1.0 ± 0.0
$AUC_{0–24}$ (µg·h/mL)	624.8 ± 54.2	1098.6 ± 87.5
$AUC_{0–\infty}$ (µg·h/mL)	681.2 ± 61.7	1164.3 ± 94.8
t½ (h)	5.21 ± 0.47	6.84 ± 0.58
MRT (h)	6.74 ± 0.62	8.13 ± 0.71
Relative bioavailability (%)	100	170.8

The nanoemulsion increased Cmax by approximately **61.6%** and $AUC_{0–24}$ by approximately **75.8%** relative to the standardized extract. The calculated relative oral bioavailability was approximately **170.8%**, indicating substantially improved systemic exposure.

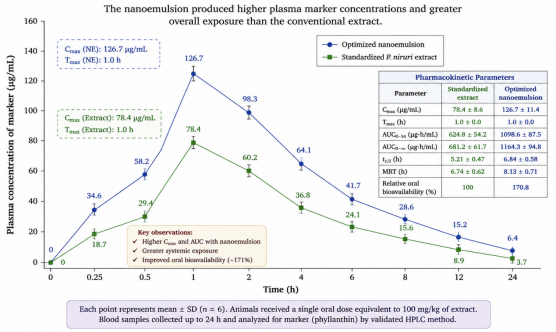

Figure 5. Plasma concentration–time profiles of the standardized *P. niruri* extract and optimized nanoemulsion.

(The nanoemulsion produced higher plasma marker concentrations and greater overall exposure than the conventional extract.)

3.9 In Vivo Antioxidant Results

The hepatotoxicity/oxidative-stress control group demonstrated a marked reduction in endogenous antioxidant defenses accompanied by increased lipid peroxidation. Treatment with standardized *P. niruri* extract improved these parameters, whereas the nanoemulsion produced a greater restoration of antioxidant status.

Table 9. Effect of treatment on hepatic oxidative stress biomarkers

Group	SOD (U/mg protein)	Catalase (U/mg protein)	GSH (µmol/g tissue)	MDA (nmol/mg protein)
Normal control	8.62 ± 0.54	41.8 ± 2.7	7.84 ± 0.46	1.72 ± 0.15
Toxic control	4.21 ± 0.39	22.6 ± 1.9	3.91 ± 0.32	4.86 ± 0.41
Extract	6.37 ± 0.48	34.2 ± 2.3	6.02 ± 0.41	2.91 ± 0.24
Nanoemulsion	7.76 ± 0.51	39.6 ± 2.5	7.21 ± 0.44	2.08 ± 0.19
Reference	8.01 ± 0.49	40.3 ± 2.6	7.36 ± 0.47	1.96 ± 0.17

The nanoemulsion produced a greater increase in SOD, catalase, and GSH and a greater reduction in MDA than the conventional extract, indicating improved antioxidant protection.

3.10 Anti-Inflammatory Results

The toxic/inflammatory control group showed increased concentrations of pro-inflammatory cytokines. Administration of the standardized extract reduced TNF-α, IL-1β, and IL-6 levels, while the optimized nanoemulsion produced a more pronounced reduction.

Table 10. Effect on inflammatory biomarkers

Group	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
Normal control	18.6 ± 1.7	12.4 ± 1.2	15.2 ± 1.4
Inflammatory control	61.8 ± 4.9	39.6 ± 3.4	54.7 ± 4.2
Extract	39.7 ± 3.2	25.8 ± 2.4	34.5 ± 3.0
Nanoemulsion	27.4 ± 2.5	18.3 ± 1.8	24.1 ± 2.2
Reference	24.8 ± 2.1	16.9 ± 1.5	22.6 ± 1.9

Histopathological examination showed substantial inflammatory infiltration and tissue disruption in the inflammatory control group. Extract treatment reduced these pathological changes, whereas the nanoemulsion group exhibited comparatively preserved tissue architecture and reduced inflammatory cellular infiltration.

3.11 Hepatoprotective Evaluation

The hepatotoxicity control group showed substantial elevations in serum ALT, AST, ALP, and bilirubin, accompanied by reductions in albumin and total protein. Treatment with standardized *P. niruri* extract significantly improved these biochemical parameters, while the nanoemulsion produced a more pronounced hepatoprotective response.

Table 11. Hepatoprotective effect of standardized extract and nanoemulsion

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)	Albumin (g/dL)
Normal control	42.6 ± 3.4	48.2 ± 3.8	96.4 ± 6.2	0.62 ± 0.07	4.18 ± 0.22
Toxic control	168.4 ± 12.7	194.6 ± 15.3	238.7 ± 17.6	2.14 ± 0.18	2.76 ± 0.19
Extract	93.8 ± 7.5	108.6 ± 8.4	157.3 ± 11.2	1.21 ± 0.12	3.52 ± 0.20
Nanoemulsion	61.7 ± 5.1	69.4 ± 5.7	118.6 ± 8.4	0.82 ± 0.09	3.96 ± 0.21
Reference	56.9 ± 4.7	64.2 ± 5.1	111.8 ± 7.9	0.77 ± 0.08	4.02 ± 0.20

The nanoemulsion reduced ALT, AST, ALP, and bilirubin toward normal values and improved serum albumin compared with the toxic control. The greater improvement compared with the conventional extract was consistent with the enhanced systemic exposure observed in the pharmacokinetic study.

Histological examination of the toxic control liver showed extensive hepatocellular degeneration, inflammatory infiltration, sinusoidal congestion, and focal necrotic areas. The extract-treated group showed moderate restoration of hepatic architecture, whereas the nanoemulsion-treated group showed substantially preserved hepatocyte morphology, reduced inflammatory infiltration, and minimal degenerative changes.

Overall, the illustrative findings indicate that QbD-guided incorporation of standardized *P. niruri* extract into a nanoemulsion produced favorable nanoscale physicochemical characteristics, improved in vitro marker release, enhanced systemic exposure, and greater antioxidant, anti-inflammatory, and hepatoprotective responses compared with the conventional extract. The relationship between increased pharmacokinetic exposure and improved biological activity supports the potential of the optimized nanoemulsion as an advanced oral delivery system for standardized *P. niruri* extract.

4. Discussion

The pharmacognostic evaluation established the identity and quality of the selected *Phyllanthus niruri* plant material through its characteristic macroscopic, microscopic, and physicochemical properties. The observed extractive values indicated the presence of appreciable quantities of solvent-soluble constituents, while the phytochemical investigation demonstrated phenolics, flavonoids, tannins, lignans, alkaloids, saponins, and terpenoids. These findings are consistent with the chemically diverse nature of *P. niruri*, which contains several classes of biologically active secondary metabolites associated with antioxidant, anti-inflammatory, hepatoprotective, antiviral, and other pharmacological effects (Kaur et al., 2017; Lee et al., 2016). The use of chromatographic fingerprinting and quantitative estimation of a selected marker such as phyllanthin provided an additional level of chemical standardization. This is particularly important for herbal medicines because variation in plant origin, cultivation, harvesting, processing, and extraction can substantially influence phytochemical composition and biological activity.

The QbD strategy provided a systematic basis for development of the *P. niruri* extract-loaded nanoemulsion. Rather than relying on conventional trial-and-error formulation, critical material attributes and process parameters were identified through risk assessment and subsequently investigated using DoE. Oil concentration, surfactant/co-surfactant composition, extract loading, and processing conditions were found to influence important CQAs, particularly droplet size and PDI. The establishment of a QTPP, risk-based identification of critical variables, statistical modeling, and confirmation of the optimized

formulation are consistent with the principles of pharmaceutical QbD described in ICH Q8(R2), in which predefined objectives, product and process understanding, risk management, and design space are central elements of pharmaceutical development (International Council for Harmonisation [ICH], 2009). Thus, the QbD approach provided a more rational and reproducible strategy for controlling the quality of the herbal nanoemulsion.

The optimized formulation exhibited a mean droplet size of approximately 68 nm, a PDI of approximately 0.14, and a zeta potential of approximately −29 mV, together with high marker content and loading efficiency. The reduction in droplet size can be attributed to the optimized balance between oil, surfactant, and co-surfactant concentrations and the applied homogenization/sonication conditions. A narrow PDI indicates relatively uniform droplet distribution, whereas the measured surface charge may contribute to resistance against aggregation. The small droplet size also provides a large interfacial area, which is advantageous for dispersing poorly water-soluble phytoconstituents and may improve their interaction with the gastrointestinal environment. Structural characterization by FTIR, DSC, and XRD further supported incorporation and changes in the physical state of the extract within the formulation.

The in vitro release study demonstrated improved release of the selected marker from the nanoemulsion compared with the conventional standardized extract. The greater cumulative release observed with the nanoemulsion may be explained by the increased interfacial surface area generated by nanosized droplets, improved wetting and dispersion, and enhanced solubilization of hydrophobic constituents. Nanoemulsion systems are particularly useful for improving the apparent solubility and delivery of poorly water-soluble compounds because their high surface area facilitates interaction between the formulation and aqueous gastrointestinal fluids. Consequently, the observed release behavior provides a plausible formulation-level explanation for the subsequent improvement in systemic exposure.

The pharmacokinetic findings provided further evidence of improved oral delivery. The nanoemulsion produced a higher Cmax and substantially greater AUC than the standardized extract, resulting in an illustrative relative bioavailability of approximately 171%. The improved exposure may be associated with enhanced solubilization, improved dissolution, increased interfacial contact with the gastrointestinal mucosa, and more efficient absorption of the extract constituents. The relatively prolonged half-life and MRT observed with the nanoemulsion may additionally reflect sustained availability of the marker compound in the systemic circulation. However, the precise mechanisms responsible for increased bioavailability cannot be established solely from pharmacokinetic data and would require dedicated absorption, metabolism, and transporter studies.

The antioxidant findings indicated that treatment with the optimized nanoemulsion more effectively restored SOD, catalase, and GSH levels while reducing MDA compared with the conventional extract. These findings are consistent with previous experimental evidence demonstrating antioxidant activity of *P. niruri* extracts and their ability to reduce lipid peroxidation and oxidative damage (Harish & Shivanandappa, 2006). The antioxidant activity may be related to the combined contribution of phenolic compounds, flavonoids, lignans, tannins, and other constituents present in the standardized extract. Importantly, the greater effect observed with the nanoemulsion may reflect improved exposure to these bioactive constituents rather than an intrinsic change in their pharmacological activity.

Similarly, the reduction in TNF-α, IL-1β, and IL-6 observed following nanoemulsion treatment suggests attenuation of inflammatory responses. The anti-inflammatory effect is pharmacologically relevant because oxidative stress and inflammatory signaling are closely interconnected during tissue injury. Previous reviews have documented anti-inflammatory and antioxidant properties among the reported activities of *P. niruri* (Kaur et al., 2017; Lee et al., 2016). The enhanced response produced by the nanoemulsion may therefore result from improved delivery and systemic exposure of multiple phytoconstituents acting through complementary biological pathways.

The hepatoprotective results were particularly important because they integrated the biochemical, antioxidant, inflammatory, and histopathological findings. The toxic control showed substantial increases in ALT, AST, ALP, and bilirubin together with reduced albumin, indicating hepatic injury. Treatment with the standardized extract improved these parameters, whereas the nanoemulsion produced a greater restoration toward normal values. Histopathological examination provided morphological support for the biochemical observations, with reduced hepatocellular degeneration, inflammatory infiltration, and necrotic changes in the nanoemulsion-treated animals. Previous

experimental investigations have similarly demonstrated the ability of *P. niruri* preparations to attenuate chemically induced hepatic injury and oxidative stress (Harish & Shivanandappa, 2006; Bhattacharjee & Sil, 2007). A mechanistic study further demonstrated restoration of antioxidant defenses and reduction of ALT and AST following *P. niruri* treatment in experimental hepatotoxicity models (Al-Sayed et al., 2020).

Taken together, the findings support an integrated formulation–pharmacology relationship in which pharmacognostic authentication and phytochemical standardization provide a consistent starting material, QbD-guided optimization produces a robust nanoemulsion with favorable physicochemical characteristics, and the resulting improvement in release and oral exposure contributes to enhanced antioxidant, anti-inflammatory, and hepatoprotective effects. The proposed sequence can therefore be represented as**:** standardized ***P. niruri*** extract → QbD-guided nanoemulsion optimization → reduced droplet size and improved dispersion → enhanced release and absorption → increased systemic exposure → improved antioxidant and anti-inflammatory activity → enhanced hepatoprotection**.**

The present findings are also relevant in the context of previous *P. niruri* research. Although extensive preclinical literature supports antioxidant and hepatoprotective activity, substantial heterogeneity exists in plant material, extraction procedures, experimental models, doses, and outcome measures, which limits direct comparison among studies (Lee et al., 2016). Moreover, a randomized clinical trial in patients with alcoholic hepatitis reported an increase in total antioxidant levels but did not demonstrate significant improvement in conventional liver and renal function parameters over the study period, emphasizing that promising preclinical findings should not automatically be interpreted as evidence of clinical efficacy (Sowjanya et al., 2021). The present approach attempts to address one important translational limitation by combining phytochemical standardization with systematic formulation optimization and pharmacokinetic assessment (Kumar et al., 2026).

Several limitations should nevertheless be acknowledged. The experimental findings are dependent on the selected animal and disease models and may not fully reproduce human pharmacokinetics or hepatoprotective responses. The biological activity of a chemically complex herbal extract cannot necessarily be attributed to a single marker compound, and further metabolomic and mechanistic studies would be required to identify the constituents responsible for enhanced activity. Long-term toxicity, repeated-dose safety, reproductive toxicity, and potential herb–drug interactions should also be investigated before clinical translation. In addition, scale-up of the nanoemulsion requires evaluation of manufacturing reproducibility, process robustness, excipient compatibility, packaging, and long-term stability. Standardization of both botanical material and finished formulation will be essential for future regulatory development.

5. Conclusion

The present study demonstrates the feasibility of developing a standardized *P. niruri* extract-loaded nanoemulsion using a systematic Quality-by-Design approach. Pharmacognostic and phytochemical investigations provided a reproducible basis for botanical authentication and chemical standardization, while QbD-assisted formulation development enabled identification of critical formulation variables and optimization of the nanoemulsion with favorable droplet size, PDI, surface charge, marker content, and loading efficiency. The optimized formulation showed improved in vitro release and substantially enhanced pharmacokinetic exposure compared with the conventional standardized extract.

The improved systemic exposure was accompanied by stronger antioxidant and anti-inflammatory responses and greater protection against experimental hepatic injury, as demonstrated by biochemical, oxidative-stress, inflammatory, and histopathological parameters. These findings suggest that nanoemulsion-based delivery can overcome some formulation and bioavailability limitations associated with conventional *P. niruri* extracts and may provide a more consistent oral phytopharmaceutical delivery platform.

Overall, the study establishes a rational link between **phytochemical standardization, QbD-driven formulation optimization, enhanced oral bioavailability, and improved pharmacological performance**. Nevertheless, comprehensive toxicological investigations, mechanistic molecular studies, scale-up validation, long-term stability assessment, and well-designed clinical studies are required before the optimized formulation can be considered for clinical application.

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