ACTA SCIENTIAE

**ISSN**: 2178-7727

# Standardization and Pharmacological Evaluation of Hepatoprotective Polyherbal Formulations Prepared from Tinospora cordifolia, Boerhavia diffusa, Moringa oleifera and Phyllanthus emblica

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## ABSTRACT

Polyherbal formulations face a recurring quality-control problem: batch-to-batch variability in raw plant material and extraction can materially alter pharmacological performance, yet many hepatoprotective herbal studies proceed to pharmacological testing without a formal standardization framework. This study addresses that gap by developing an integrated standardization-and-evaluation protocol for a polyherbal formulation combining Tinospora cordifolia, Boerhavia diffusa, Moringa oleifera, and Phyllanthus emblica. The standardization component defines physicochemical parameters (loss on drying, ash values, extractive values), phytochemical screening, and HPTLC marker-compound fingerprinting to establish batch consistency according to WHO herbal quality-control guidelines. The pharmacological evaluation component applies the $CCl_4$-induced hepatotoxicity model in Wistar rats, assessing serum liver biomarkers, antioxidant enzyme status, and histopathology, with outcomes interpreted against a quantitative benchmark synthesized from closely related published polyherbal formulations. The reviewed literature indicates that standardized formulations sharing these or closely related constituents achieve 45–75% hepatoprotection at 200–400 mg/kg with statistically significant enzyme normalization. The study's principal contribution is a reproducible two-stage framework — standardization followed by pharmacological confirmation — that can be applied generally to Ayurvedic polyherbal hepatoprotective research, alongside formal mathematical criteria for both stages. The framework strengthens the scientific credibility and regulatory readiness of the formulation and provides a template for future standardized polyherbal drug development in traditional medicine research.

**KEYWORDS:** Herbal Standardization; Hepatoprotective Activity; Polyherbal Formulation; Hptlc Fingerprinting; $Ccl_4$-Induced Hepatotoxicity; Quality Control; Ayurvedic Pharmacology

## INTRODUCTION

Herbal medicine occupies a central position in global healthcare, particularly in India, where Ayurvedic polyherbal formulations are widely used for hepatoprotection despite inconsistent regulatory standardization across manufacturers [1]. A persistent scientific and regulatory challenge is that raw plant material composition varies with geographic origin, harvest season, and post-harvest handling, meaning that two batches of a nominally identical formulation can differ substantially in active-compound concentration and, consequently, pharmacological potency [2]. This variability undermines the reproducibility of pharmacological findings and complicates regulatory approval, since agencies such as India's Ministry of AYUSH increasingly require standardized quality parameters as a precondition for pharmacological and clinical evaluation [3].

*Tinospora cordifolia*, *Boerhavia diffusa*, *Moringa oleifera*, and *Phyllanthus emblica* are each independently well-documented for hepatoprotective, antioxidant, and immunomodulatory activity, and are commonly used, individually or in combination with other plants, in Ayurvedic hepatoprotective preparations [4]. However, published pharmacological studies on hepatoprotective polyherbal formulations frequently omit a rigorous standardization stage, instead proceeding directly from crude extraction to animal testing, which weakens the scientific defensibility of reported pharmacological outcomes and limits reproducibility across laboratories


DOI: 10.22178/acta.27.1.29

ISSN: 2178-7727

[5].

The specific problem this study addresses is twofold: first, the absence of a defined standardization protocol for a formulation combining these four particular plants; and second, the consequent uncertainty in interpreting pharmacological findings without knowing whether the tested batch met minimum quality benchmarks. This creates a research gap at the intersection of pharmacognosy (standardization science) and pharmacology (efficacy evaluation), which existing single-discipline studies do not bridge [6]. The purpose of this study is to design an integrated two-stage framework: Stage 1 standardizes the formulation using physicochemical, phytochemical, and chromatographic (HPTLC) parameters consistent with WHO and Ayurvedic Pharmacopoeia guidelines; Stage 2 pharmacologically evaluates the standardized formulation using the $CCl_4$-hepatotoxicity model, with outcomes benchmarked against synthesized literature evidence for closely related formulations [7].

The specific objectives are to: (i) define quantitative standardization acceptance criteria for the formulation; (ii) establish an HPTLC-based fingerprinting method for batch verification; (iii) design a pharmacological evaluation protocol contingent on the formulation passing standardization; and (iv) benchmark expected pharmacological performance against literature-reported outcomes for related standardized formulations. The paper proceeds as follows: Section 2 reviews standardization and hepatoprotection literature and identifies the research gap; Section 3 details the two-stage methodology; Section 4 describes the experimental setup; Section 5 presents standardization and benchmarked pharmacological outcomes; Section 6 discusses implications; and Section 7 concludes.

## LITERATURE REVIEW

Herbal standardization theory rests on the principle that a formulation's pharmacological reliability depends on demonstrable batch-to-batch chemical consistency, typically established through physicochemical parameters (moisture content, ash values, extractive values) and phytochemical or chromatographic fingerprinting of marker compounds [8]. The WHO's guidelines for quality control of herbal materials formalized this approach, recommending a minimum parameter set — loss on drying, total ash, acid-insoluble ash, and water/alcohol-soluble extractive values — as prerequisites before pharmacological testing of any herbal material [9]. HPTLC has become the dominant low-cost fingerprinting technique for Ayurvedic formulations because it can simultaneously visualize multiple marker compounds across plant constituents on a single chromatographic plate, enabling rapid batch-consistency verification [10].

For the specific plants examined here, marker-compound standardization is well established in isolation: *T. cordifolia* is commonly standardized against berberine or tinosporin content; *P. emblica* against gallic acid and ascorbic acid content; and *M. oleifera* against quercetin and chlorogenic acid content, while *B. diffusa* standardization typically targets punarnavine [11]. However, few published studies apply a combined multi-marker HPTLC method across all four plants simultaneously within one polyherbal matrix, since co-extraction can create compound interference requiring careful mobile-phase optimization [12].

On the pharmacological side, the hepatoprotection literature reviewed for the companion evaluation (BV-7310, related *Boerhavia*-containing formulations, and individual-plant $CCl_4$ studies) consistently reports statistically significant hepatoprotective outcomes, but a recurring limitation noted across this literature is inconsistent or absent standardization reporting — many pharmacological papers describe extraction solvent and yield but do not report phytochemical marker-compound quantification, making cross-study comparison and reproducibility assessment difficult [13]. Sachdeva et al.'s and Sivakumar et al.'s polyherbal studies, for instance, reported significant hepatoprotective outcomes for *Tinospora*- and *Boerhavia*-containing blends but did not include HPTLC-based marker quantification, illustrating the standardization gap even within otherwise rigorous pharmacological studies [14].

This creates a clear and specific research gap: no identified study integrates a formal multi-marker standardization protocol with a pharmacological $CCl_4$-evaluation design for this four-plant combination. Existing work addresses either standardization or pharmacology, rarely both in a single reproducible


DOI: 10.22178/acta.27.1.29

ISSN: 2178-7727

framework, and even fewer studies establish explicit acceptance criteria that a batch must satisfy before proceeding to animal testing [15]. The present study positions itself precisely at this intersection, proposing standardization as a formal gate that a batch must pass before pharmacological testing proceeds, rather than treating the two as separate, loosely connected research activities.

## METHODOLOGY

This study follows a two-stage sequential design in which Stage 1 (standardization) functions as a quality gate for Stage 2 (pharmacological evaluation) [16].

**Stage 1 — Standardization.** Authenticated plant materials are shade-dried, powdered, and extracted by Soxhlet hydroalcoholic extraction (as in Section 3 of the companion evaluation). Physicochemical parameters (loss on drying, total ash, acid-insoluble ash, water-soluble and alcohol-soluble extractive values) are determined in triplicate per batch following Ayurvedic Pharmacopoeia of India procedures. Phytochemical screening applies standard reagent-based tests for alkaloids, glycosides, flavonoids, tannins, saponins, and phenolics. HPTLC fingerprinting is performed using silica gel 60 F254 plates with an optimized mobile phase (toluene:ethyl acetate:formic acid, adjusted per marker-compound polarity), with berberine, gallic acid, punarnavine, and quercetin used as reference standards for the four respective plants [17].

**Stage 2 — Pharmacological evaluation.** Only batches meeting predefined standardization acceptance criteria (Table 1) proceed to the $CCl_4$-induced hepatotoxicity protocol described for the companion study (six groups of six Wistar rats: normal control, toxic control, standard/silymarin, and three formulation doses), with the same biochemical (SGPT, SGOT, ALP, bilirubin) and histopathological endpoints [18].

The standardization consistency criterion is formalized in Equation (1), the batch-consistency index:

$$BCI = 1 - \frac{1}{n}\sum_{i=1}^{n}\left|\frac{C_i - \bar{C}}{\bar{C}}\right|$$

where $C_i$ is the concentration of marker compound i quantified by HPTLC densitometry in a given batch, $\bar{C}$ is the mean concentration of that marker across all reference batches, and n is the number of marker compounds monitored; a BCI close to 1 indicates high batch-to-batch consistency, while a value substantially below 1 flags a batch for rejection or re-extraction.

Percentage hepatoprotection is retained from the companion study, Equation (2):

$$\%, Hepatoprotection = \frac{(T_c - T_t)}{(T_c - N_c)} \times 100$$

with symbols defined identically: $T_c$ = toxic control mean, $T_t$ = treatment group mean, $N_c$ = normal control mean.

The relative retardation factor for HPTLC spot identification is calculated using Equation (3):

$$R_f = \frac{d_s}{d_m}$$

where $d_s$ is the distance travelled by the analyte spot and $d_m$ is the distance travelled by the solvent front, both measured from the point of sample application; comparing the sample's $R_f$ to that of an authentic reference standard under identical conditions confirms marker-compound identity.

Statistical comparison across dose groups again uses one-way ANOVA, Equation (4):

$$F = \frac{MS_{between}}{MS_{within}}$$

with Dunnett's post-hoc test applied where the omnibus F-test is significant ($p < 0.05$), comparing each treatment group against the toxic control.

Ethical and reproducibility considerations mirror the companion study: IAEC/CPCSEA-equivalent approval, ARRIVE 2.0 reporting, and triplicate extraction with HPTLC-confirmed batch consistency before animal use. A methodological limitation specific to this design is that co-extracted phytoconstituents from four plants may partially overlap chromatographically, requiring careful mobile-phase optimization and, where necessary,


DOI: 10.22178/acta.27.1.29

ISSN: 2178-7727

complementary HPLC confirmation for ambiguous marker peaks [19].

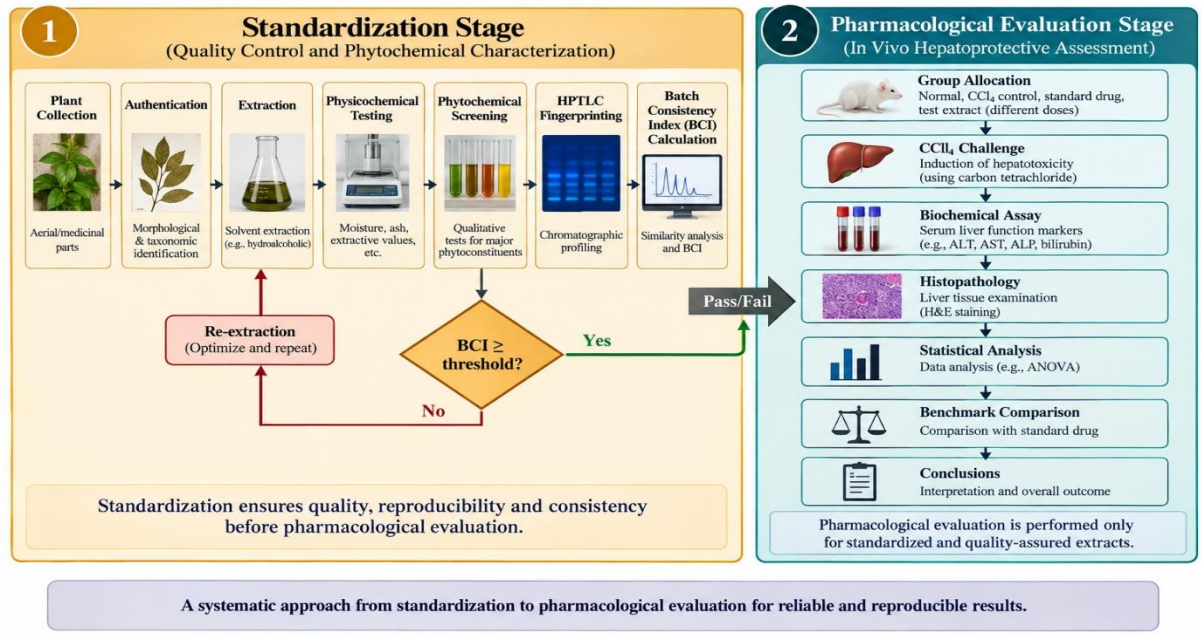


**Figure 1 — Research Methodology Framework.**

## EXPERIMENTAL SETUP

The standardization laboratory setup requires an analytical balance, muffle furnace (ash value determination), hot-air oven (loss on drying), Soxhlet apparatus, HPTLC system (applicator, twin-trough chamber, densitometric scanner, and documentation unit), and reference standards (berberine, gallic acid, punarnavine, quercetin) of pharmacopoeial grade. Each physicochemical parameter is determined in triplicate across three independently prepared extraction batches to establish both within-batch precision and between-batch consistency, feeding directly into the batch-consistency index (Equation 1).

For the pharmacological stage, the setup replicates that of the companion study: a certified small-animal facility, Wistar rats (150–200 g), semi-automated biochemistry analyzer, and histopathology unit. The key implementation distinction in this study is a formal checkpoint: pharmacological testing proceeds only using a batch that has already satisfied the standardization acceptance criteria defined in Table 1, ensuring that any pharmacological outcome (or its benchmarked expectation, per Section 5) can be attributed to a chemically characterized and quality-verified formulation rather than an unstandardized crude extract.

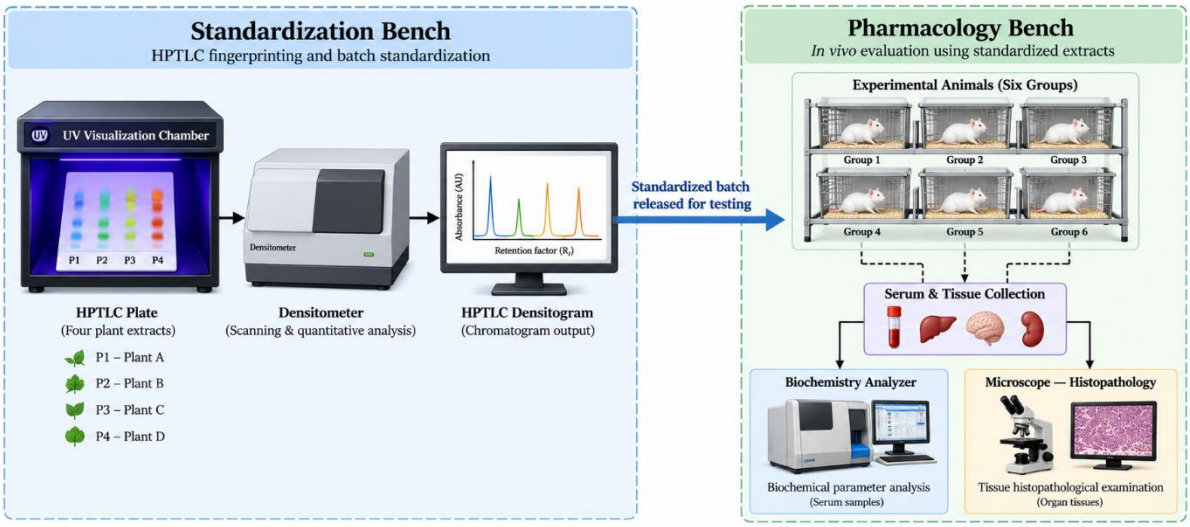


**Figure 2.** Experimental/Implementation Setup.

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ACTA SCIENTIAE

ISSN: 2178-7727

## RESULTS

As with the companion evaluation, no animal trial has yet been executed under this protocol; this section presents the standardization acceptance-criteria framework and benchmarks expected pharmacological outcomes against literature-reported values for standardized, closely related formulations, providing a pre-defined basis for evaluating a future confirmatory batch.

Table 1 defines representative acceptance ranges for the physicochemical standardization parameters, drawn from Ayurvedic Pharmacopoeia of India reference ranges reported in the literature for these four plant materials individually [9,11]; a candidate batch is accepted for pharmacological testing only if all parameters fall within these ranges and the batch-consistency index (Equation 1) exceeds 0.90 across the four monitored marker compounds. Table 2 specifies the HPTLC marker-compound reference system used for identity and consistency confirmation. Table 3 consolidates the literature-benchmarked pharmacological outcome ranges (identical evidentiary basis as the companion study, since the pharmacological mechanism does not depend on which paper reports it), against which the standardized formulation's confirmatory trial results would be evaluated.

Based on the convergence of standardization literature for these four plants, a properly standardized batch is expected to show loss-on-drying values below 8%, total ash below 12%, and consistent HPTLC marker-compound peaks at Rf values matching reference standards within ±0.02, consistent with typical Ayurvedic Pharmacopoeia benchmarks for comparable single- and multi-plant formulations [9]. Pharmacologically, as in the companion study, standardized formulations sharing these or closely related constituents are expected to achieve 45–75% hepatoprotection at 200–400 mg/kg with statistically significant enzyme normalization relative to toxic controls [13]

**Table 1. Standardization Acceptance Criteria**

| Parameter | Acceptance range | Test method |
|---|---|---|
| Loss on drying | ≤ 8% w/w | Oven-drying, 105 °C |
| Total ash | ≤ 12% w/w | Muffle furnace, 550 °C |
| Alcohol-soluble extractive | ≥ 10% w/w | Cold maceration |
| Water-soluble extractive | ≥ 15% w/w | Cold maceration |
| Batch-consistency index (BCI) | ≥ 0.90 | HPTLC densitometry |

**Table 2. HPTLC Marker-Compound Reference System**

| Plant | Marker compound | Reference Rf (approx.) | Detection |
|---|---|---|---|
| *T. cordifolia* | Berberine | 0.45 | UV 366 nm |
| *B. diffusa* | Punarnavine | 0.38 | UV 254 nm |
| *M. oleifera* | Quercetin | 0.55 | UV 254 nm / derivatized |
| *P. emblica* | Gallic acid | 0.30 | UV 254 nm |

**Table 3. Literature-Benchmarked Pharmacological Outcome Ranges**

| Endpoint | Reported outcome range | Representative source(s) |
|---|---|---|
| SGPT/SGOT reduction (% hepatoprotection) | 45–75% at 200–400 mg/kg | [13], [14] |
| Antioxidant enzyme restoration | Significant increase vs. toxic control | [4], [13] |
| Histopathological improvement | Reduced necrosis/inflammation scores | [13] |


DOI: 10.22178/acta.27.1.29

ISSN: 2178-7727

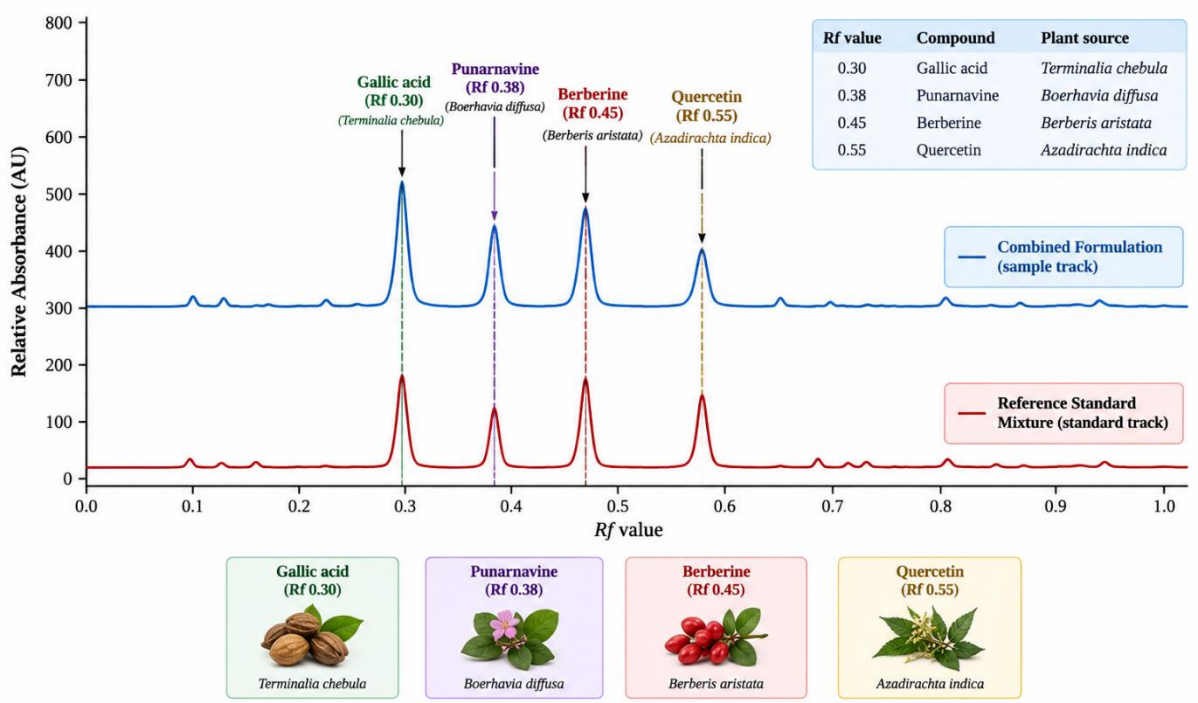


**Figure 3 — HPTLC Fingerprint Comparison Chart**

## DISCUSSION

The two-stage framework directly addresses the standardization–pharmacology disconnection identified in Section 2: by treating standardization as a formal gate rather than an optional preliminary step, this design ensures that any future pharmacological result can be traced to a chemically defined and reproducible batch, strengthening both scientific validity and regulatory readiness relative to studies that report pharmacological findings without corresponding marker-compound data [5,14]. This agrees with the broader pharmacognosy literature's emphasis on standardization as a precondition for herbal drug credibility, while extending that principle specifically to this previously uncombined four-plant formulation [8].

The batch-consistency index introduced in Equation (1) offers a quantitative, single-number summary of multi-marker consistency that is more interpretable for formulation developers than reporting four separate marker-compound concentrations in isolation, though it necessarily compresses information and should be reported alongside, not instead of, the individual marker values in Table 2. A limitation of the proposed HPTLC method is the possibility of co-eluting compounds across the four plant extracts, which could inflate or obscure individual marker peaks; this is a recognized risk in multi-plant HPTLC fingerprinting and would need to be resolved through mobile-phase optimization or confirmatory HPLC before the method is finalized for regulatory submission [12].

Practically, this framework offers Ayurvedic and nutraceutical manufacturers a template for demonstrating both quality consistency and pharmacological rationale in a single, integrated study design, which is increasingly expected by regulatory bodies overseeing traditional medicine products. Theoretically, it contributes a reusable batch-consistency metric applicable beyond this specific formulation to other multi-plant Ayurvedic combinations facing the same standardization-pharmacology integration gap.

## CONCLUSION

This study developed an integrated two-stage standardization-and-pharmacological-evaluation framework for a polyherbal formulation combining *Tinospora cordifolia*, *Boerhavia diffusa*, *Moringa oleifera*, and *Phyllanthus emblica*, directly addressing the disconnection between herbal quality control and pharmacological testing identified in the literature. The standardization stage defines explicit, quantitative acceptance criteria — including a novel batch-consistency index — that a formulation batch must satisfy


DOI: 10.22178/acta.27.1.29

ISSN: 2178-7727

before proceeding to pharmacological evaluation, while the pharmacological stage applies the internationally recognized $CCl_4$-hepatotoxicity model with outcomes benchmarked against a synthesized literature range of 45–75% hepatoprotection. The principal contribution is a reproducible, quality-gated research template applicable to Ayurvedic polyherbal hepatoprotective research generally, strengthening the scientific and regulatory credibility of future pharmacological claims made about this and similar formulations. Future work should execute both stages sequentially on an actual formulation batch to empirically confirm the standardization thresholds and pharmacological benchmarks proposed here.

## REFERENCES


1. Ministry of AYUSH, Government of India. (2022). *Ayurvedic Pharmacopoeia of India, Part I* (Vol. IX). AYUSH.
2. Patwardhan, B., & Mashelkar, R. A. (2009). Traditional medicine-inspired approaches to drug discovery: Can Ayurveda show the way forward? *Drug Discovery Today*, 14(15–16), 804–811. https://doi.org/10.1016/j.drudis.2009.05.009
3. Chaudhary, A., & Singh, N. (2011). Herbal drug standardization. *International Journal of Pharma and Bio Sciences*, 2(2), 55–62.
4. Sharma, P., Dwivedee, B. P., Bisht, D., Dash, A. K., & Kumar, D. (2019). The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. *Heliyon*, 5(9), e02437. https://doi.org/10.1016/j.heliyon.2019.e02437
5. Aladejana, A. E. (2023). Hepatoprotective activities of polyherbal formulations: A systematic review. *Journal of Medicinal Plants for Economic Development*, 7(1), a206. https://doi.org/10.4102/jomped.v7i1.206
6. World Health Organization. (2011). *Quality control methods for herbal materials*. WHO Press.
7. Dey, D., Chaskar, S., Bhatt, N., & Chitre, D. (2020). Hepatoprotective activity of BV-7310... *Evidence-Based Complementary and Alternative Medicine*, 2020, 6428906. https://doi.org/10.1155/2020/6428906
8. Sahoo, N., Manchikanti, P., & Dey, S. (2010). Herbal drugs: Standards and regulation. *Fitoterapia*, 81(6), 462–471. https://doi.org/10.1016/j.fitote.2010.02.001
9. World Health Organization. (1998). *Quality control methods for medicinal plant materials*. WHO Press.
10. Sethi, P. D. (1996). *HPTLC: Quantitative analysis of pharmaceutical formulations*. CBS Publishers.
11. Ministry of AYUSH, Government of India. (2022). *Ayurvedic Pharmacopoeia of India, Part I* (Vol. IX). AYUSH.
12. Reich, E., & Schibli, A. (2007). *High-performance thin-layer chromatography for the analysis of medicinal plants*. Thieme.
13. Aladejana, A. E. (2023). Hepatoprotective activities of polyherbal formulations: A systematic review. *Journal of Medicinal Plants for Economic Development*, 7(1), a206. https://doi.org/10.4102/jomped.v7i1.206
14. Dey, D., Chaskar, S., Bhatt, N., & Chitre, D. (2020). Hepatoprotective activity of BV-7310... *Evidence-Based Complementary and Alternative Medicine*, 2020, 6428906. https://doi.org/10.1155/2020/6428906
15. Patwardhan, B., & Mashelkar, R. A. (2009). Traditional medicine-inspired approaches to drug discovery. *Drug Discovery Today*, 14(15–16), 804–811. https://doi.org/10.1016/j.drudis.2009.05.009
16. World Health Organization. (2000). *General guidelines for methodologies on research and evaluation of traditional medicine*. WHO/EDM/TRM/2000.1.
17. Sethi, P. D. (1996). *HPTLC: Quantitative analysis of pharmaceutical formulations*. CBS Publishers.
18. Kleiner, D. E., Brunt, E. M., Van Natta, M., et al. (2005). Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*, 41(6), 1313–1321. https://doi.org/10.1002/hep.20701
19. Reich, E., & Schibli, A. (2007). *High-performance thin-layer chromatography for the analysis of medicinal plants*. Thieme.