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Genetic Diversity, Germplasm Conservation and Breeding Strategies for Medicinal Plant Species with Therapeutic Potential against Liver Diseases

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ABSTRACT

Liver diseases impose a large and rising global burden, and metabolic dysfunction-associated steatotic liver disease (MASLD) is now among the most common causes [1,2]. Medicinal plants are widely used for liver disorders, but their sustainable use depends on the genetic resources behind them, which are threatened by over-harvesting, habitat loss and genetic erosion. This narrative review examines four Indian medicinal species with hepatoprotective relevance (*Boerhavia diffusa*, *Eclipta prostrata*, *Phyllanthus amarus* and *Curcuma longa*) from a genetic-resource perspective. We justify the choice of species, compare the molecular marker systems used to assess diversity, tabulate target bioactive compounds and their analytical methods, summarise in vitro and in vivo hepatoprotective models with their controls, discuss breeding strategies matched to each species' reproductive biology, and outline in situ and ex situ conservation including seed viability and long-term storage. We also examine how metabolomic profiling and molecular docking can connect genotype, chemotype and bioactivity. The literature shows marked accession-level variation in DNA profiles and marker-compound content, yet few studies combine diversity, chemistry and hepatoprotective testing in the same accessions. We propose an integrated framework and research priorities.

Keywords: Hepatoprotective Plants; Genetic Diversity; Germplasm Conservation; Medicinal Plant Breeding; Molecular Markers; Metabolomics; Molecular Docking; MASLD

INTRODUCTION

Chronic liver diseases, including viral hepatitis, alcohol-related liver disease, cirrhosis, liver cancer and metabolic liver disease, are a major and often neglected public health problem [3]. Recent analyses describe their epidemiology, causes and projected trends worldwide [1]. Non-alcoholic fatty liver disease (NAFLD) has been renamed MASLD under a multisociety consensus; because several studies cited here pre-date this change, the original terms are retained when quoting them. Global prevalence is high in overweight and obese populations, the projected disease burden is rising, and MASLD is closely linked with type 2 diabetes [2-6].

Medicinal plants have long been used in traditional systems of medicine for jaundice, fatty liver and cirrhosis. Their hepatoprotective effects are commonly linked to antioxidant, anti-inflammatory and anti-fibrotic mechanisms. Milk thistle (*Silybum marianum*) is the best-known example [7], and Indian species such as turmeric (*Curcuma longa*) [8], Bhumyamalaki (*Phyllanthus amarus*) [9], Punarnava (*Boerhavia diffusa*) [10] and Bhringraj (*Eclipta prostrata*) [11] contain compounds that protect liver cells in experimental systems. However, the concentration of these compounds varies among species, populations and genotypes, and the resource base is under pressure. Systematic germplasm collection, genetic characterisation, breeding and conservation are therefore essential for sustainable use [12].

Most reviews of hepatoprotective plants focus on pharmacology. Far fewer connect pharmacology to genetic diversity, propagation, breeding and conservation. This review addresses that gap. Its objectives are to (i) justify the selection of four representative species, (ii) compare approaches to measuring genetic and chemical diversity, (iii) summarise hepatoprotective test systems and controls, (iv) discuss breeding and conservation strategies, and (v) propose how metabolomics and molecular docking can be integrated with these components.

LITERATURE SEARCH METHODOLOGY

This is a narrative review prepared with reference to the SANRA quality criteria [13].

- **Databases:** PubMed, Scopus, Web of Science and Google Scholar.
- **Period covered:** Recent publications were emphasised; older studies were retained when they are the primary source for a species or method.
- **Search terms (combined with AND/OR):** species names and synonyms (e.g., *Boerhavia diffusa/Boerhaavia diffusa*, *Eclipta prostrata/Eclipta alba*, *Phyllanthus amarus*, *Curcuma longa*) with "genetic diversity", "RAPD", "ISSR", "SSR", "germplasm", "conservation", "cryopreservation", "breeding", "hepatoprotective", "HPLC", "metabolomics" and "molecular docking".
- **Inclusion criteria:** peer-reviewed articles in English on the four species addressing genetic or morphological diversity, marker-compound quantification, hepatoprotective activity, breeding or conservation.
- **Exclusion criteria:** conference abstracts without data, studies without species authentication, and studies on other *Phyllanthus* or *Boerhavia* species unless they inform methodology.
- **Synthesis:** Findings were synthesised narratively because study designs and endpoints differ too widely for meta-analysis.

Selection of Species and Accessions

The four species were selected against five criteria:

- documented traditional use for liver disorders;
- experimental evidence of hepatoprotective activity in vitro or in vivo;
- defined marker compounds that can be quantified and used for selection;
- availability of germplasm or genetic-diversity studies from India; and
- contrasting biology, so that different conservation and breeding challenges are represented: an annual herb (*E. prostrata*), a cross-pollinated weedy herb (*P. amarus*), a widely distributed herb with a medicinally valuable root (*B. diffusa*) and a triploid, vegetatively propagated crop (*C. longa*).

In this review, an "accession" means a distinct germplasm entry collected from a defined location, as used in the cited studies, for example 28 *Boerhavia* accessions from different Indian regions [14], 25 *Eclipta* accessions from natural habitats in Kerala [15] and 152 high-curcumin *Curcuma* accessions [16]. *Silybum marianum* is discussed only as a benchmark for hepatoprotective activity [7] and is not a study species.

Genetic Diversity and Molecular Characterisation

Boerhavia diffusa

RAPD analysis of 28 accessions with 18 primers (nine highly informative) showed higher polymorphism among accessions from Uttar Pradesh than among those from other states, lower polymorphism around Lucknow, and greater similarity between neighbouring regions than between distant ones; some markers were associated with leaf shape and flower colour [14]. This indicates geographic structuring but used only one dominant marker type and a small sample.

Eclipta prostrata

Twenty-five accessions from natural habitats in Kerala showed highly significant variation in twelve growth characters, but the authors warned that wetland filling and conversion of paddy fields threaten the species [15]. A recent evaluation recorded 35 morphological, yield and quality traits and identified nine elite genotypes, with fresh whole-plant yield positively correlated with plant height, internodal length, leaf size and inflorescence diameter; GC-MS profiling was used for quality assessment

Species (family; common name)	Principal bioactive compounds	Hepatoprotective evidence (examples)	Rationale
Boerhavia diffusa (Nyctaginaceae; Punarnava)	*Punarnavine, rotenoids (boeravinones),* flavonoids, phenolics [17]	Root and leaf extracts protective in rodent liver-injury models [18,19]; reviewed in [17,20]	Wide Indian distribution; accession variation shown by RAPD [14]
Eclipta prostrata (Asteraceae; Bhringraj)	Wedelolactone, demethylwedelolactone [21]	Wedelolactone described as hepatoprotective [21]	Annual herb with field variability [15] and elite genotypes identified [22]
Phyllanthus amarus (Phyllanthaceae; Bhumyamalaki)	*Lignans (phyllanthin, hypophyllanthin);* ellagitannins (geraniin, amariin) [9,23]	Phyllanthin protects HepG2 cells and hepatocytes [24,25]; geraniin and amariin protect liver cells [26]	South India a genetic hot spot; species admixture in trade [23]
Curcuma longa (Zingiberaceae; Turmeric)	*Curcumin, demethoxycurcumin,* bisdemethoxycurcumin [27]	Hepatoprotective mechanisms reviewed in several toxicity models [28]; meta-analysis of rodent studies supports protection [29]	Major crop; curcuminoid content varies among germplasm [27]

Table 1: Species Reviewed, Key Compounds, Hepatoprotective Evidence and Rationale for Inclusion

[22]. A review notes that research on this species has concentrated on pharmacology, with biodiversity and cultivation less studied [21].

Phyllanthus amarus

Across *P. amarus*, *P. debilis* and *P. virgatus*, RAPD and ISSR markers revealed average polymorphism of about 68–70%, and RAPD analysis of *P. amarus* from different locations in India showed high intrapopulation variation in southern India, attributed to cross-pollination and weedy growth with little human intervention; southern India has been described as a genetic hot spot [23]. Species identification is a practical concern because morphologically similar herbs grow together and are admixed in the raw-drug trade; SCAR markers, ISSR-based diagnostic markers and *psbA-trnH* barcoding have been used to resolve them [23]. Another study combined RAPD with HPLC quantification of phyllanthin and hypophyllanthin across three habitats, assessing genetic relatedness, chemical content and environmental factors together [30].

Curcuma longa

C. longa is triploid and vegetatively propagated [31]. DAMD and ISSR markers distinguished Indian turmeric germplasm and were considered reliable for diversity assessment [32]. In a germplasm set evaluated for rhizome yield traits and curcuminoids, curcumin ranged from 0.41% to 2.17% and total curcuminoids from 1.26% to 4.55% [27]. Thirty-nine SSR primers produced 163 alleles (100% polymorphism) across 152 high-curcumin accessions from North-East India, and AMOVA attributed 87% of variation to within-population differences [16]. SSR analysis of Brazilian turmeric found that most diversity lay within states, while differences between countries were large [31].

Sampling designs in published studies and a recommended design

Table 2 summarises the sampling locations and sample sizes of the diversity studies cited above. Sample sizes range from 25 to 152 accessions, and the sources accessed did not consistently report biological replication, which limits comparison.

For primary studies we recommend: sampling across ecological zones with GPS coordinates and habitat recorded for every collection; enough individuals per population to represent local variation, with the number justified from pilot data; sampling individuals far enough apart to avoid clonal duplicates (essential for vegetatively propagated *C. longa*); morphological and agronomic trials in a randomised block design with at least three replications in more than one environment; and chemical analyses in triplicate on independent extractions. Voucher specimens should be deposited in a herbarium.

Choosing markers and analysing diversity

Marker choice should follow the question asked (Table 3). Dominant markers (RAPD, ISSR) are inexpensive and need no prior sequence information, which is why they dominate the older literature for these species, but they have limited reproducibility and cannot separate heterozygotes. Co-dominant SSRs are more informative, although scoring in polyploids such as triploid turmeric needs care [16,31]. DNA barcoding and SCAR markers serve authentication rather than diversity [23]. For analysis, we recommend reporting the number of polymorphic loci, polymorphism information content (PIC), Shannon's index, genetic distance/similarity,

Species	Sampling locations	Sample size	Markers / traits	Reference
B. diffusa	Accessions from different Indian regions (Uttar Pradesh, other states, and around Lucknow)	28 accessions	18 RAPD primers (9 most informative)	[14]
E. prostrata	Natural habitats in Kerala	25 accessions	12 growth characters	[15]
E. prostrata	Field experiment, 2023–24	Not reported	35 traits (16 qualitative, 19 quantitative); GC-MS	[22]
P. amarus	Three habitats differing in soil	Not reported	RAPD; HPLC of lignans; soil and microbial factors	[30]
C. longa	North-East India (high-curcumin lines)	152 accessions	39 SSR primers	[16]
C. longa	Brazilian germplasm collection plus genotypes from India and Puerto Rico	39 + 18 genotypes	17 SSR markers	[31]

Table 2: Sampling Locations, Sample Size and Measured Traits in Cited Diversity Studies

Marker	Type	Strengths and limitations	Use in the reviewed species
RAPD	Dominant, PCR-based	Low cost, no sequence needed; limited reproducibility	*B. diffusa* [14]; Phyllanthus [23,30]; C. longa in vitro germplasm [33]
ISSR	Dominant, PCR-based	Higher reproducibility than RAPD; dominant scoring	*Phyllanthus* [23]; *C. longa* (with DAMD) [32]
SSR	Co-dominant	Highly informative; needs development; scoring complex in polyploids	*C. longa* [16,31]
SCAR / DNA barcoding	Sequence-based	Species authentication and adulterant detection; not for population diversity	Phyllanthus admixture [23]
Morpho-biochemical descriptors	Phenotypic	Direct link to yield and quality; influenced by environment	*E. prostrata* [22]; *C. longa* [27]

Table 3: Molecular Marker Systems Used for the Four Species and their Suitability

AMOVA, cluster analysis or PCoA, and a Mantel test of genetic versus geographic distance, as already applied in several cited studies [14,16].

Phytochemical Variation: Targets, Quantification and Standards

Table 4 lists the target compounds proposed for each species with suitable analytical methods. Variation in these compounds is large: curcuminoids differ several-fold among turmeric germplasm [27], and phyllanthin and hypophyllanthin content in *P. amarus* varies with species, geographic origin and habitat [30,34]. Extraction solvent also matters; in an HPTLC study of *P. amarus* leaves, methanol gave the highest recovery of both lignans among the solvents tested [34].

Methods should be validated for linearity, specificity, precision, accuracy (recovery), and limits of detection and quantification following ICH Q2(R1) [35]; the HPTLC/HPLC study on *Phyllanthus* lignans reported precision and recovery data as an example [34]. Quantification should use reference standards of documented purity, calibration curves prepared on each run, replicate extractions per accession, and results expressed as mg/g dry weight with the harvest stage, plant part and drying method recorded, since these affect content. Antioxidant assays (DPPH, ABTS, FRAP) are useful complementary screens but do not replace compound-specific quantification.

A single marker compound may not predict hepatoprotective activity. Ellagitannins such as geraniin and amariin contribute to the hepatoprotective effect of *P. amarus* [26], and other *Phyllanthus* species that lack phyllanthin are also reported to be hepatoprotective [23]. This supports profiling several compounds, or the whole metabolome (Section 9), rather than relying on one marker.

Species	Target compounds	Plant part	Quantification method	Standards
B. diffusa	*Punarnavine; rotenoids (boeravinones)* [17]	Root	HPLC/UPLC with DAD; LC-MS for rotenoids	Authentic reference compounds
E. prostrata	Wedelolactone, demethylwedelolactone [21]	Aerial parts	HPLC-DAD or HPTLC; GC-MS for volatile profile [22]	Authentic reference compounds
P. amarus	*Phyllanthin, hypophyllanthin* [30,34]	Leaf / aerial parts	HPLC or HPTLC [30,34]	External standards of phyllanthin and hypophyllanthin
C. longa	*Curcumin, demethoxycurcumin,* bisdemethoxycurcumin [27]	Rhizome	HPLC-PDA [27]	Authentic curcuminoid standards

Table 4: Target Bioactive Compounds, Plant Part and Analytical Approach

Level	Model / injury inducer	Typical endpoints	Examples
In vitro (cell line)	HepG2 with CCl_4 or acetaminophen	Cell viability (MTT), LDH/ ALT/AST leakage, oxidative-stress markers (MDA, GSH, SOD, CAT)	[24,36]
In vitro (primary cells)	Rat hepatocytes with ethanol	Viability, enzyme leakage, antioxidant markers	[25,26]
In vivo (rodent)	Paracetamol or CCl_4 injury	Serum ALT, AST, ALP, bilirubin; liver antioxidant markers; histopathology	[18,19]

Table 5: Experimental Models, Controls and Endpoints for Hepatoprotective Evaluation

Hepatoprotective Evaluation: Models, Controls and Procedures

Reported studies use a range of in vitro and in vivo systems (Table 5). Human hepatoma HepG2 cells are widely used because they resemble normal hepatocytes in many respects and are practical for screening [17]; examples include phyllanthin against CCl4-induced toxicity in HepG2 cells [24] and acetaminophen-induced injury in HepG2 cells [36]. Primary rat hepatocytes treated with ethanol [25] and liver cells exposed to ethanol [26] have also been used. In vivo, paracetamol (acetaminophen)-induced liver damage in rats has been used for *B. diffusa* leaf extract [19].

Controls and design. Every experiment should include a vehicle (untreated) control, a toxicant-only control and a positive control treated with a reference hepatoprotective agent, most commonly silymarin [7]. Extracts should be tested at no fewer than three concentrations, prepared from standardised extracts whose marker-compound content is known (Section 5), after a pilot range-finding and cytotoxicity test (for example, MTT) so that protection is not confused with toxicity. Doses and concentrations in the literature vary widely between studies, which limits comparison; we therefore recommend expressing dose on a standardised-extract basis and reporting both extract and marker-compound amounts. Cell experiments should have at least three independent biological replicates, each with technical triplicates. As a benchmark for animal dosing, a meta-analysis of preclinical curcumin studies reported hepatoprotective effects at 100–400 mg/kg for 4–10 weeks [29]; doses for the other species must be justified from their own literature and pilot work.

Animal studies. Any in vivo work requires prior approval from the Institutional Animal Ethics Committee under CPCSEA rules in India, randomisation, blinding where feasible, an a priori sample-size justification, and reporting according to the ARRIVE 2.0 guidelines [37]. Evidence from cell and rodent models is preclinical: it supports prioritisation of accessions but does not establish clinical efficacy. For curcumin, for example, trial results on liver enzymes in fatty liver disease remain inconsistent [38].

Breeding Strategies and Selection Criteria

Breeding approaches must match each species' reproductive biology. Selection criteria should combine (i) yield (herb, root or rhizome biomass), (ii) quality (content of the marker compounds in Table 4), (iii) stability across environments, tested in multi-location trials, (iv) authenticity and freedom from adulterants, and (v) bioactivity in the assays of Section 6. Marker-assisted selection (MAS) and genomics are increasingly proposed to speed improvement of secondary-metabolite content in medicinal plants [39–41], but these tools depend on adequate marker-trait information, which is still limited for the species reviewed here.

Micropropagation can multiply elite material, but genetic fidelity must be checked; RAPD profiling of in vitro conserved *C. longa* germplasm is an example [33]. Genome editing is discussed as a future tool for medicinal plants but is not yet established for these species and carries regulatory considerations.

Germplasm Conservation

In situ and ex situ approaches are complementary [12,42]. Conservation planning should start from threat assessment and the diversity data described in Section 4, so that collections capture the range of genetic variation rather than a convenient sample.

In situ conservation

In situ measures include protected areas, designated medicinal-plant conservation areas, and on-farm or community management of cultivated landraces. They preserve evolutionary processes and are the only option that conserves species in their ecological context [12]. For these species the main threats are habitat conversion (for example, filling of wetlands and conversion of paddy fields for *E. prostrata* [15]) and over-collection from weedy populations of *Phyllanthus* [23]. Monitoring should record population size, habitat condition and harvest pressure at each conserved site.

Ex situ conservation

Ex situ options include seed banks, field gene banks, botanical gardens, in vitro collections and cryopreservation [12,42]. Seed banks suit seed-propagated species (*E. prostrata*, *P. amarus*, *B.*

Species	Relevant biology and evidence	Suggested selection approach
Boerhavia diffusa	Accessions differ in DNA profile and leaf/flower traits [14].	Accession evaluation followed by progeny selection for root biomass and punarnavine/rotenoid content (proposed).
Eclipta prostrata	Annual herb; nine elite genotypes identified through yield-trait correlations [22].	Pure-line or mass selection from elite accessions; multi-location testing; yield and wedelolactone as joint criteria.
Phyllanthus amarus	High intrapopulation variation and cross-pollination [23].	Population improvement and recurrent selection for lignan and tannin content; barcode-based authentication of selections.
Curcuma longa	Triploid; hybridisation often unsuccessful, so improvement largely depends on germplasm selection [27,31].	Clonal selection of high-curcuminoid lines [16]; mutation breeding or somaclonal variation as options; marker-assisted selection (MAS) where associations exist.

Table 6: Breeding Considerations and Suggested Approaches by Species

diffusa), whereas *C. longa*, which is vegetatively propagated, is maintained in field gene banks and in vitro, with periodic genetic-fidelity checks [33] (Table 7). Each accession should carry passport data (collection site, GPS coordinates, date, collector, habitat), a herbarium voucher, and linked morphological, molecular and chemical data.

Seed viability and long-term storage

For seed collections, international genebank standards recommend drying seeds, sealing them in airtight containers, and storing base collections at about −18 °C, with viability measured at the start and re-tested at defined intervals, and regeneration when viability falls below the set threshold [43,44]. Storage behaviour (orthodox, intermediate or recalcitrant) should be established experimentally for each species before banking. Genetic integrity should be checked with markers after regeneration, to detect drift and contamination.

Integrating Metabolomics and Molecular Docking

Targeted quantification captures only a few compounds, whereas untargeted LC-MS or GC-MS metabolomics can profile many metabolites per accession, and molecular docking can propose which of them interact with hepatic targets. Network pharmacology, docking and cell-based validation have been combined to study compounds against acetaminophen-induced injury in HepG2 cells, for example confusoside, where PI3K and caspase-3 were predicted as targets and then tested experimentally [36]. We propose the following workflow, which uses the same accessions as the diversity, chemistry and bioassay components above:

- Collect and authenticate accessions; record genotype (Section 4) and growth conditions.
- Prepare standardised extracts and run untargeted LC-MS/GC-MS together with the targeted assays of Section 5.
- Process data and apply multivariate statistics (PCA, PLS-DA/OPLS-DA) to separate accessions by chemotype, and compare chemotype groups with DNA-marker clusters.
- Annotate discriminating features, stating the identification confidence level.
- Predict targets and perform docking for annotated compounds against hepatic-injury-related proteins, using validated protocols and reporting binding energies with controls (for example, known ligands).

Species	Main propagule	Ex situ strategy	Monitoring
Boerhavia diffusa	Seed; vegetative material	Seed bank plus field collection; in vitro back-up	Initial and periodic germination tests; marker check after regeneration
Eclipta prostrata	Seed (annual)	Seed bank as primary method; field regeneration	Germination tests at set intervals; regeneration schedule
Phyllanthus amarus	Seed	Seed bank; field collection for lignan-rich lines	Germination tests; authentication by barcode
Curcuma longa	Rhizome	Field gene bank; in vitro cultures [33]	Periodic fidelity check; disease indexing

Table 7: Suggested Conservation Plan by Species

- Test the top-ranked accessions and compounds in the cell models of Section 6; correlate metabolite abundance with bioactivity.

Docking is hypothesis-generating: a favourable score does not prove activity, and predictions must be validated experimentally, as in the HepG2 study cited above [36]. Placing these steps inside the same workflow, rather than as an isolated add-on, is what links genotype, chemotype and bioactivity.

RESEARCH GAPS AND FUTURE DIRECTIONS

- Few studies measure DNA diversity, marker-compound content and hepatoprotective activity in the *same* accessions; this is the central gap.
- Dominant markers and small accession numbers dominate the older literature; larger, geographically structured collections with co-dominant markers are needed.
- Genotype-by-environment interaction is rarely separated from genetic effects on compound content [30].
- Seed-storage behaviour and long-term viability data for several of these species are sparse in the literature screened.
- Hepatoprotective studies differ in model, dose and extract standardisation, limiting comparison; standardised reporting is needed.
- Evidence is preclinical; clinical efficacy has not been established by the studies reviewed.

CONCLUSION

B. diffusa, *E. prostrata*, *P. amarus* and *C. longa* show measurable genetic and chemical variation that is relevant to liver-protective potential, yet their genetic resources are not systematically characterised or conserved. Matching marker systems, analytical methods, test models, breeding approaches and conservation methods to each species' biology, and combining them with metabolomics and docking in one workflow, offers a route from traditional use towards evidence-based, sustainable resource development.

AUTHOR CONTRIBUTIONS

Susmita Sahoo: conceptualisation, supervision, review and editing. Janki Mahida: literature review, drafting of the manuscript.

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