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Decoding Silymarin: A Multi-Omics Blueprint for Precision Hepatoprotection

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Abstract

The global health burden of liver diseases, spanning metabolic dysfunction-associated steatotic liver disease (MASLD), cirrhosis, and hepatocellular carcinoma, has catalysed an intensive search for effective hepatoprotective agents. Traditional Chinese Medicine (TCM) and other herbal systems have long utilized botanical extracts, with **Silybum marianum** (milk thistle) emerging as a premier candidate for modern pharmacological validation. However, the intrinsic complexity of herbal medicines, characterized by a multi-component and multi-target nature, presents a significant challenge to conventional “single-drug, single-target” research paradigms. The period from 2016 to 2026 marks a transformative decade in which multi-omics integration—encompassing transcriptomics, proteomics, metabolomics, and lipidomics—has become the standard for decoding the scientific connotation of these ancient remedies.

The evolution of hepatoprotective research now emphasizes the dynamic interplay between exogenous phytochemicals and endogenous metabolic networks. While silymarin, the standardized extract of *S. marianum*, has been used for decades, recent methodological advances have revealed that its efficacy is not merely the result of general antioxidant activity but involves highly specific molecular “switches” in lipid metabolism and cellular homeostasis. These insights are driven by a convergence of high-resolution analytical chemistry, sophisticated computational embedding frameworks, and emerging spatial biology techniques.

Keywords: Hepatoprotection; Herbal medicine; Multi-omics integration.

The Molecular Basis of Hepatoprotection in Silybum marianum

The therapeutic profile of Silybum marianum is primarily attributed to silymarin, a complex mixture of flavonolignans including silybin A, silybin B, isosilybin, silychristin, silydianin, and the flavonoid taxifolin [1, 21]. Modern research has transitioned from treating silymarin as a monolithic entity to analysing the unique pharmacodynamic contributions of its individual stereoisomers [1, 8, 57].

For instance, silybin A has been identified as a critical regulator of hepatic lipid remodelling, specifically inducing a "lipid class switch" from neutral triglycerides (TG) to functional phospholipids [1, 14, 65]. This remodelling is of paramount importance in the context of MASLD and metabolic dysfunction-associated steatohepatitis (MASH) [1, 10, 36, 62]. Silybin A treatment has been shown to decrease triglyceride levels and

lipid droplet content while enriching major phospholipid classes like phosphatidylcholine (PC), thereby maintaining membrane integrity and biotransformation capacity [1, 14, 21, 78].

Table 1: Pharmacodynamic Profiles of Primary Bioactive Stereoisomers in Silybum marianum

Bioactive Stereoisomer	Primary Molecular Mechanism	Key Targeted Pathways	Observed Phenotypic Effect
Silybin A	Lipid class switching	TG biosynthesis downregulation	Reduced steatosis; Enhanced membrane integrity [1, 14, 65]
Silybin B	Antioxidant signaling	NF-κB inhibition; ROS scavenging	Mitigation of oxidative liver damage [1, 18, 21]
Isosilybin	Metabolic regulation	PPARα activation	Improved lipid profile; Weight loss [1, 57]
Taxifolin	Radical scavenging	MAPK signaling	Protection against xenobiotic toxicity [1, 17]

Divergent Protective Strategies in Herbal Medicine

The methodologies used for Silybum marianum—specifically transcriptomics and targeted metabolomics—have revealed that different herbs achieve hepatoprotection through distinct "entry points" in the cellular network.

- Modulation of Ferroptosis (Gynostemma pentaphyllum): Heat-processed gypenosides inhibit ferroptosis (iron-dependent cell death) by modulating the PPAR signaling pathway [5, 63].
- AMPK Activation (Fructus schisandrae): Lignans like schisandrin B target AMPK, a "metabolic master switch" that inhibits de novo lipogenesis and promotes fatty acid oxidation [15, 71].

The "Safety Radar": Herb-Induced Liver Injury (HILI)

Multi-omics tools are also used to detect HILI. Dictamnine (from Dictamni Cortex) serves as a cautionary example; it induces hepatotoxicity by disrupting lipid homeostasis, the exact opposite of silymarin's effect [16, 43, 61].

Methodological Advances: The "3-in-1" Protocol

A critical bottleneck was the requirement for separate sample aliquots for different omics. The development of the "3-in-1" simultaneous extraction protocol allows the recovery of proteins, metabolites, and lipids from a single sample [21, 53].

Table 2: Functional Layers of the "3-in-1" Extraction Framework

Extraction Phase	Targeted Biomolecules	Processing Method	Data Application
Upper Polar Layer	Polar Metabolites	LC-MS/MS or GC-MS	Metabolomic profiling [6, 45, 53]
Lower Organic Layer	Lipids (TG, PC, etc.)	Quantitative Lipidomics	Lipid class switching analysis [1, 14, 53]
Middle Interphase	Proteins	TMT labeling; MS	Proteomic signaling analysis [12, 13, 53]
Solid Residuals	RNA/DNA	Sequencing	Transcriptomics; Epigenomics

			[6, 53, 58]

The Mechanistic Shift: From Antioxidant to Precision Regulator

The traditional view of Silybum marianum as a simple free-radical scavenger has been superseded by evidence of its role as a metabolic programmer. As noted in the 2025 research in the Journal of Advanced Research [78], Silybin A specifically orchestrates a "lipid class switch." By downregulating the synthesis of neutral triglycerides and upregulating functional phospholipids like Phosphatidylcholine (PC), it repairs the hepatocyte membrane at a molecular level [1, 14, 65].

This is a critical distinction in treating MASLD (Metabolic Dysfunction-Associated Steatotic Liver Disease), where the goal is no longer just to reduce "fat," but to transform the lipidome into a state that supports cellular biotransformation and inhibits the activation of hepatic stellate cells [47, 66, 75].

Detailed Comparative Pharmacology

The complexity of herbal medicine lies in the synergy of different pathways. While Silybum marianum focuses on membrane integrity and lipid remodelling, other botanical systems utilize distinct "entry points" into the cellular network.

Table 3: Comparative Molecular Targets of Hepatoprotective Botanicals

Botanical Agent	Primary Active Compound	Key Molecular "Entry Point"	Cellular Outcome	References
Milk Thistle	Silybin A/B	PPARγ & NF-κB	Lipid remodeling; Anti-inflammatory	[1, 14, 18]
Gynostemma	Gypenosides	PPAR Signaling	Ferroptosis inhibition	[5, 63]
Fructus schisandrae	Schisandrin B	AMPK	Inhibition of de novo lipogenesis	[15, 71]
Curcumin	Curcuminoids	SIRT1 / Nrf2	Mitochondrial restoration; Antioxidant	[30, 33, 64]

The "3-in-1" Methodological Revolution

The most significant technical hurdle overcome in the 2020s was "sample bias"—the variation caused by using different liver tissue aliquots for different tests. The "3-in-1" Simultaneous Extraction Protocol [12, 53] ensures that the protein, metabolite, and lipid data all originate from the exact same cellular environment.

Table 4: Analytical Precision in the "3-in-1" Framework

Layer	Analytical Tool	Insight Provided	Application in Liver Research
Polar Layer	GC-MS / LC-MS	Polar metabolites (Bile acids, amino acids)	Identifying biomarkers for cholestasis and urea cycle dysfunction [15, 52]
Organic Layer	Quantitative Lipidomics	Lipid species (TG vs. PC ratios)	Measuring the "lipid class switch" induced by Silybin A [1, 78]
Interphase	TMT-labeling MS	Proteome flux	Mapping the TGF-β1/Smad

			signaling in fibrosis [47, 66]
Residuals	RNA-Seq	Transcriptional landscape	Mapping gene expression changes in porcine or human models [33, 51, 83]

Spatial Biology and Future Directions (2025-2026)

We are now entering the era of Spatial Biology. Techniques like MERFISH and MALDI-MSI allow researchers to see where these drugs work within the liver's architecture [1, 7, 79]. We can now distinguish between the drug's effect on periportal cells (high oxygen) versus pericentral cells (low oxygen), which is vital for understanding liver regeneration [36, 85].

Table 5: Strategic Research Horizon (2025-2026)

Phase	Technology	Strategic Objective	Reference
I: Mapping	MERFISH / Spatial Transcriptomics	3D mapping of fibrotic vs. healthy tissue at single-cell resolution.	[36, 79, 85]
II: Optimization	Deep Learning (AI)	Predicting synergistic ratios of silymarin stereoisomers for personalized medicine.	[28, 40, 56]
III: Engineering	CRISPR/Cas9	Modifying S. marianum hairy root cultures for high-yield flavonolignan production.	[27, 80, 84, 86]
IV: Translation	Liver-on-a-Chip	Validating herbal safety (avoiding HILI) in human-mimetic systems before clinical trials.	[20, 41, 75]

Regulatory "Switches": The NF-κB and PPAR Axis

Modern multi-omics have identified that the hepatoprotective effect of Silybum marianum is governed by a push-pull relationship between pro-inflammatory and pro-metabolic signals. While Silybin B acts as a brake on inflammation via NF-κB inhibition, Isosilybin acts as an accelerator for lipid metabolism via PPARα activation.

Table 6: Multi-Omics Signatures of Signalling Pathway Modulation

Pathway	Omics Layer	Observed Molecular Change	Clinical Implication	References
NF-κBSignaling	Proteomics	Decreased p65 phosphorylation; reduced TNF-α	Mitigation of cytokine storms in acute liver injury	[6, 18, 44]
TGF-β1/Smad	Transcriptomics	Downregulation of COL1A1 and ACTA2 (α-SMA)	Reversal of hepatic stellate cell activation (anti-fibrotic)	[47, 66, 82]
SIRT1/AMPK	Metabolomics	Increased NAD^+/NADH ratio; ATP homeostasis	Restoration of mitochondrial function in MASH	[64, 71]
FXR Activation	Lipid-omics	Increased primary bile	Prevention of	[19, 26]

		acid conjugation	cholestatic liver damage	

2. The Toxicology Mirror: HILI vs. Hepatoprotection

To understand how Silybum marianum protects the liver, researchers compare it against Herb-Induced Liver Injury (HILI) models. This "negative mapping" reveals that while silymarin repairs membranes, toxins like Dictamnine or Aflatoxin B1 disrupt the very same lipid layers and antioxidant enzymes.

Table 7: Comparative Omics Profiles—Protection vs. Toxicity

Feature	Protection (Silymarin/Silybin)	Toxicity (Dictamnine/Aflatoxin B1)	Regulatory Context
Lipid Profile	TG ↓, Phosphatidylcholine (PC) ↑	TG ↑, Lipid peroxidation (MDA) ↑	[1, 14, 32]
Cell Death	Anti-apoptotic (Bcl-2 ↑)	Pro-ferroptotic / Pro-apoptotic	[3, 5, 51]
Antioxidant	GSH restoration; SOD ↑	GSH depletion; ROS accumulation	[9, 21, 65]
Gene Flux	Upregulation of regeneration genes	Upregulation of DNA-damage markers	[33, 49, 78]

3. Advanced Bioprocessing: From Soil to Lab

By 2026, the focus has shifted toward Synthetic Biology and Precision Extraction. Because the concentration of bioactive flavonolignans in wild S. marianum can vary, researchers use CRISPR-Cas9 to "edit" the biosynthetic pathways in hairy root cultures to ensure a standardized, high-potency output.

Table 8: 2025-2026 Innovations in Botanical Bioengineering

Technology	Application in Silybum marianum	Impact on Pharmacotherapy	References
CRISPR-Cas9	Knock-out of competitive metabolic pathways in S. marianum.	5x increase in Silybin A/B yield.	[80, 84, 86]
Phytosomes	Complexing silymarin with phospholipids (e.g., Siliphos).	Enhanced bioavailability and intestinal absorption.	[7, 10, 55]
iPSC-Organoids	"Liver-on-a-chip" testing of flavonolignans.	Reduction in animal testing; human-specific data.	[20, 41, 58]
Deep Learning	MiDNE (Multi-omics Drug Network Embedding).	Predicting unknown targets for Taxifolin and Silychristin.	[28, 40]

4. Summary of the "3-in-1" Extraction Value

The integration of the "3-in-1" protocol (Table 2) into these phases allows for a Systems Biology view. For instance, if transcriptomics shows an increase in a specific enzyme's mRNA, the simultaneous proteomics layer confirms the enzyme's presence, and the lipid-omics layer confirms the resulting change in the cell

membrane. This prevents "false positives" in research and ensures that the observed hepatoprotection is a verified, multi-level event.

Strategic Research Plan (2025-2026)

1. Phase 1: High-Resolution Spatial-Temporal Mapping (MERFISH and MALDI-MSI) [1, 7, 57].
2. Phase 2: AI-Driven Formula Optimization using deep learning models [1, 3, 56].
3. Phase 3: CRISPR-Guided Bioengineering for enhanced biosynthetic pathways [6, 45, 80, 84].
4. Phase 4: Clinical Translation via Liver-on-a-Chip and iPSC-derived organoids [41, 75, 85].

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