EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE
Vol. 6 No. 08 (August - 2026) EJMMP ISSN: 2795-921X
https://inovatus.es/index.php/ejmmp

HIGH-PURITY SILYMARIN FLAVONOLIGNANS AND METABOLIC LIVER HEALTH: COMPOSITION STANDARDIZATION AND TRANSLATIONAL EVIDENCE

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Abstract
Silymarin is a chemically heterogeneous mixture of flavonolignans, and differences in composition and formulation can complicate interpretation of its clinical effects. This study characterized a standardized NVTIA® high-purity silymarin flavonolignan composition and considered its profile in relation to published human evidence on liver injury and metabolic liver disease. Three optimized preparations contained 82.1–83.4% total silymarin and 39.4–40.6% combined silybin A+B, with yields of 5.3–5.6% and residual deep-eutectic-solvent components of 0.27–0.31%. Targeted process comparators produced lower total silymarin contents (68.4–77.1%) when solvent switching, resin enrichment, or antisolvent crystallization was omitted. Randomized studies in non-cirrhotic NASH/MASLD have produced mixed results for composite histologic activity, although some trials have reported favorable fibrosis-related or biochemical findings. Meta-analyses likewise suggest reductions in aminotransferases across heterogeneous liver-injury populations. These observations support a practical formulation principle: a reproducible flavonolignan profile should be established before clinical effects are attributed to a specific preparation. Future studies can build on this foundation by relating verified silymarin and silybin exposure to contemporary measures of liver stiffness, fibrosis, and histologic activity.

Keywords: silymarin; silybin; milk thistle; flavonolignans; hepatocellular protection; MASLD; NASH; liver function

1. Introduction

Milk thistle (Silybum marianum) provides silymarin, a mixture containing several flavonolignans, including silybin, isosilybin, silychristin, and silydianin. Interest in silymarin for liver health has been driven by experimental evidence involving oxidative stress, lipid peroxidation, inflammatory signaling, mitochondrial dysfunction, and fibrogenic pathways [1]. Translation of these findings into clinical research, however, depends on more than the nominal dose of an extract. The chemical composition of the administered material and the exposure achieved in vivo also need to be defined.

This issue is particularly relevant to metabolic dysfunction-associated steatotic liver disease (MASLD), formerly studied under the NAFLD/NASH terminology. Disease progression can range from steatosis to inflammatory injury and fibrosis, and clinical trials have consequently used a wide range of outcomes, including aminotransferases, non-invasive fibrosis measures, histology, and composite activity scores. Variation in silymarin purity and bioavailability adds another source of uncertainty when results from different preparations are compared [1].

The present work evaluates the NVTIA® high-purity silymarin flavonolignan composition from a formulation-quality perspective. The process study examined total silymarin, combined silybin A+B, product yield, column-feed viscosity, and residual deep-eutectic-solvent components across optimized preparations and targeted process comparators. These compositional findings are considered alongside randomized and synthesized human evidence to define what can reasonably be inferred at this stage and to identify the clinical questions that remain to be tested.

2. Methodology

2.1 Composition and process framework

Mature dried milk thistle seeds were defatted by low-temperature pressing at no more than 45°C. The defatted cake/meal was milled to 50–70 mesh. A deep eutectic extraction phase was prepared from betaine, lactic acid, and water at a mass ratio of 1:2:0.6 and applied to the plant matrix at a cake/meal-to-solvent mass ratio of 1:10. Extraction was conducted at 50°C for 90 min under nitrogen protection.

After extraction, anhydrous ethanol was introduced at an extract-to-ethanol volume ratio of 1:2.2 to establish an ethanol volume fraction of 68%. Following 30 min of mixing and filtration, the liquid phase was diluted to 30% ethanol and held for 1.5–2.5 h to promote impurity precipitation. The clarified column-feed solution was processed through AB-8 weakly polar macroporous resin, water-washed, and eluted with 60% ethanol. The eluate was concentrated at 45°C to a relative density of 1.10–1.15, combined with purified water at a concentrate-to-water volume ratio of 1:3, crystallized at 8°C for 12 h, and vacuum-dried at 40–50°C.

Three optimized preparations were compared descriptively with four targeted process comparators: conventional 70% ethanol extraction, direct loading of the deep eutectic extract without ethanol switching, omission of resin enrichment, and omission of antisolvent crystallization. Total silymarin and silybin A+B were quantified by HPLC using a C18 column, methanol-water-glacial acetic acid mobile phase (48:52:0.5, v/v/v), 288 nm detection, 30°C column temperature, 0.8 mL/min flow, and a 20 μL injection volume. Moisture was assessed by Karl Fischer titration. Residual betaine and lactic acid were determined by HPLC and acid-base titration, respectively. The available measurements are formulation-level values; accordingly, numerical contrasts are presented descriptively without inferred error terms or P values.

2.2 Structured human-evidence synthesis

A targeted literature synthesis prioritized randomized controlled trials, systematic reviews, meta-analyses, and trial registries evaluating orally administered silymarin or clearly specified silymarin-containing interventions in adults with NASH/NAFLD/MASLD or other liver-injury states [2], [3], [4]. Searches emphasized PubMed-indexed literature and ClinicalTrials.gov records through August 2026, while formulation and bioavailability evidence was considered separately [5]. Extracted domains included study design, population, formulation or dose, duration, liver-biochemistry endpoints, histology or fibrosis-related outcomes, and safety observations. Ingredient- or formulation-specific findings were retained in their original study context and used to establish clinical and mechanistic background for the standardized composition.

3. Results

3.1 Reproducible high-purity flavonolignan profile

Across the three optimized preparations, total silymarin ranged from 82.1% to 83.4% and combined silybin A+B from 39.4% to 40.6%. Product yield remained within 5.3–5.6%, while column-feed viscosity remained 2.4–2.6 mPa·s at 25°C and residual deep-eutectic-solvent components were 0.27–0.31%. These closely clustered values characterize a stable process window for the NVTIA® composition.

Table 1. Descriptive process and composition characteristics. ND, not detected.

Configuration	Yield %	Viscosity mPa·s	Total silymarin %	Silybin A+B %	DES residue %
Optimized A	5.6	2.4	83.4	40.6	0.27
Optimized B	5.3	2.6	82.1	39.4	0.31
Optimized C	5.4	2.5	82.8	39.9	0.30
70% ethanol comparator	4.6	1.9	75.8	34.0	ND
Direct-DES comparator	5.1	8.8	77.1	34.9	0.68
No-resin comparator	5.3	2.5	68.4	31.2	0.42
No-crystal comparator	5.4	2.4	73.8	35.1	0.38

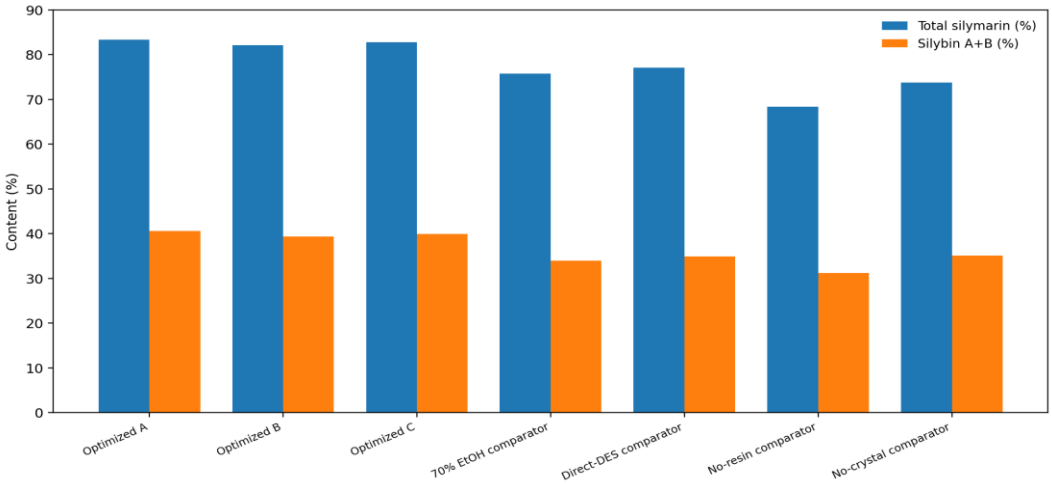


Figure 1. Total silymarin and combined silybin A+B across optimized preparations and targeted process comparators. Values are direct formulation-level measurements; no inferential error bars are shown.

3.2 Randomized trials in steatotic liver disease

Two higher-profile randomized programs illustrate both the potential and the context dependence of silymarin outcomes. Kheong and colleagues randomized 99 patients with NASH to silymarin 700 mg three times daily or placebo for 48 weeks [6]. The predefined primary endpoint, a ≥30% reduction in NAFLD Activity Score, was not significantly different between groups; however, fibrosis improvement on histology occurred more often with silymarin, and liver-stiffness reduction also favored active treatment. These findings are compatible with an antifibrotic signal even when a composite activity endpoint is less responsive.

The multicenter SyNCH phase II trial randomized non-cirrhotic NASH participants to 420 mg or 700 mg silymarin three times daily or placebo for approximately 48 weeks [7], [4]. The primary histologic response—at least a two-point reduction in NAS—occurred at similar frequencies across treatment and placebo groups, and the trial did not identify a statistically significant dose-response for that endpoint. Importantly, the intervention was generally well tolerated. In aggregate, these trials emphasize that histologic activity, fibrosis, biochemical injury, and non-invasive stiffness measures need not move in parallel over a single treatment interval.

Earlier smaller studies also reported improvements in aminotransferases in some NAFLD/NASH populations [2], [3].

Their shorter durations and varied formulations make them most useful as supportive context rather than as a single pooled clinical estimate. More recently, a randomized double-blind study in morbidly obese patients with NAFLD broadened the clinical evidence base by evaluating silymarin in a metabolically high-risk population [8].

Table 2. Selected human trials informing liver-health interpretation. NS, not statistically significant.

Study	Population/design	Exposure	Key interpretation
Kheong et al., 2017 [6]	NASH, n=99	700 mg TID, 48 wk	Primary NAS endpoint NS; fibrosis histology and stiffness signals favored silymarin
Navarro et al., 2019 [7]	Non-cirrhotic NASH, 3-arm phase II	420 or 700 mg TID, ~48 wk	No significant primary NAS response; generally well tolerated
Hashemi et al., 2009 [2]	NAFLD	Oral silymarin	Reported favorable liver-enzyme changes
Solhi et al., 2014 [3]	NASH	Randomized clinical trial	Reported biochemical improvement in treated participants

3.3 Meta-analytic context

A 2024 systematic review and meta-analysis of 26 randomized controlled trials involving 2,375 participants with NAFLD/NASH reported overall favorable effects across liver-injury, metabolic, and histologic domains, although effect magnitude varied by endpoint and study characteristics [9]. A broader 2025 meta-analysis of 55 randomized studies across liver-injury settings found pooled reductions in AST and ALT, with high between-study heterogeneity and evidence of small-study/publication effects that attenuated some pooled estimates after adjustment [10]. These findings support a signal for hepatocellular biochemical improvement while simultaneously identifying formulation, population, dose, and study design as important moderators. A separate systematic review and meta-analysis also evaluated the impact of silymarin in individuals with nonalcoholic fatty liver disease [11].

This pattern is scientifically consistent with a heterogeneous botanical intervention. A chemically reproducible preparation reduces one source of variability but does not eliminate differences in absorption, baseline fibrosis, metabolic phenotype, concomitant therapy, adherence, or endpoint sensitivity. Contemporary AASLD guidance emphasizes noninvasive risk stratification and management of metabolic comorbidities in NAFLD [12], while the 2024 EASL-EASD-EASO guideline similarly frames MASLD across cardiometabolic risk, fibrosis staging, and noninvasive assessment [13]. These standards are relevant when selecting endpoints and stratification variables for future silymarin trials.

4. Discussion

The optimized process produced a relatively consistent flavonolignan profile across the three preparations. The comparison with the direct-DES process is useful in this regard. When the solvent-switching step was omitted, the column-feed viscosity increased to 8.8 mPa·s, residual DES components increased to 0.68%, and total silymarin fell to 77.1%. Total silymarin was also lower when resin enrichment or antisolvent crystallization was omitted, reaching 68.4% and 73.8%, respectively. These results suggest that the individual separation steps contribute to the composition of the final ingredient, rather than simply improving extraction yield. Independent analytical work has likewise shown that HPLC-based profiling can distinguish substantial compositional differences among silymarin preparations [14].

Silybin is one of the major flavonolignans in silymarin and is therefore an important component to consider when the material is being developed for liver-related applications. Previous experimental studies have described antioxidant, membrane-stabilizing, anti-inflammatory, and antifibrotic activities for silybin and related flavonolignans [1].

Proposed mechanisms include reduced lipid peroxidation, maintenance of glutathione-related antioxidant capacity, modulation of NF-κB signaling, and effects on hepatic stellate-cell activation and fibrogenic pathways [1]. These observations support further investigation of liver biochemistry and fibrosis-related outcomes. They do not, however, mean that all of these effects should necessarily be observed in a clinical trial.

The clinical literature is less consistent. Some earlier studies have mainly reported changes in ALT and AST, whereas trials in NASH have increasingly used histology, elastography, and composite activity scores. In the 2017 randomized trial, the primary NAS endpoint was not significantly different between the silymarin and placebo groups, although several fibrosis-related measures favored silymarin [6]. The 2019 phase II study also failed to show a significant effect on its primary histologic endpoint [7]. At the same time, meta-analyses have generally found reductions in aminotransferases, although the studies included in these analyses differ considerably in population, dose, formulation, and treatment duration [9], [10]. Overall, the clinical results have not been consistent across liver-related outcomes.

For the NVTIA® preparation examined here, the main practical advantage is that the flavonolignan composition can be defined before clinical testing. The three optimized preparations showed similar levels of total silymarin and silybin A+B, which makes the material easier to characterize than an extract described only by its total dose. This is relevant to later clinical work because the oral bioavailability of silymarin flavonolignans can vary with formulation. Consequently, two products providing the same nominal amount of silymarin may not produce the same systemic exposure [1], [5]. Systematic pharmacokinetic evidence further indicates that flavonolignan exposure is constrained by low oral bioavailability and extensive conjugation, reinforcing the need to link composition with measured exposure [15].

4.1 Current Scope and Future Directions

The present study focused on defining the composition of the standardized preparation and placing these findings in the context of the available clinical evidence. The compositional data provide a basis for subsequent clinical studies. A useful next step would be to characterize the pharmacokinetics of the standardized NVTIA® preparation and determine whether systemic exposure to silybin A+B is related to changes in liver-related outcomes. Future trials could then assess outcomes such as ALT, controlled attenuation parameter, liver stiffness, and fibrosis-related biomarkers, with histological assessment where clinically appropriate.

Patient characteristics should also be considered when designing these studies. Baseline fibrosis stage, diabetes, obesity, concomitant metabolic treatment, and dietary intervention may affect the observed response. Taking these factors into account could help identify the populations in which the standardized preparation warrants further evaluation.

5. Conclusion

The ethanol-switching, resin-enrichment, and antisolvent-crystallization workflow produced a reproducible high-purity silymarin flavonolignan composition, with total silymarin ranging from 82.1% to 83.4% and combined silybin A+B from 39.4% to 40.6%. Published randomized trials and meta-analyses in NASH/MASLD have reported favorable changes in aminotransferases and some fibrosis-related measures, although results have been less consistent for composite histologic endpoints. These findings support further clinical evaluation of the standardized preparation, particularly studies that define flavonolignan exposure and examine liver-function and fibrosis-related outcomes.

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