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SOLVENT-DEPENDENT SAPONIN RECOVERY AND BIOLOGICAL ACTIVITY POTENTIAL OF BUPLEURUM CHINENSE DC

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Abstract

Background: Bupleurum chinense DC (B. chinense) is widely used in traditional medicine for hepatoprotective and anti-inflammatory purposes, largely attributed to its triterpenoid saponins. This study aimed to investigate the influence of extraction solvent polarity on phytochemical composition and in vitro biological activities of B. chinense extracts.

Methods: Water, 70% ethanol, and 96% ethanol extracts were prepared and evaluated for extraction yield, total saponin content, metabolite profiles, and biological activities. Metabolites were characterized using LC–MS/MS coupled with GNPS molecular networking. Chemometric analyses were employed to assess solvent-dependent metabolite distribution. Antioxidant activity was determined by free-radical scavenging and xanthine oxidase inhibition assays, while nitric oxide (NO) inhibition and cell viability were assessed in vitro.

Results: The highest extraction yield was obtained with 96% ethanol (10.1%), whereas 70% ethanol yielded the highest total saponin content (0.16%). LC–MS/MS analysis identified oleanane-type saikosaponins, flavonoids, coumarins, and phenolic acids. Chemometric analysis revealed distinct clustering of extracts according to solvent polarity, with polar saikosaponins enriched in the water extract and flavonoid aglycones predominating in ethanol extracts, particularly the 96% ethanol extract. The 96% ethanol extract showed the strongest antioxidant and xanthine oxidase inhibitory activities (IC_{50} = 0.79–0.88 mg/mL and 0.80–0.81 mg/mL, respectively). All extracts exhibited weak NO inhibition (IC_{50} > 248 ppm) while maintaining over 80% cell viability.

Conclusion: Extraction polarity significantly modulates the phytochemical composition and bioactivity of B. chinense. The 96% ethanol extract represents a potent antioxidant chemotype, while 70% ethanol is optimal for saponin enrichment, supporting solvent-guided optimization of Bupleurum preparations in phytotherapy.

Keywords: Bupleurum chinense; saikosaponins; extraction solvent; antioxidant activity; metabolomics..

1. Introduction

Chronic liver diseases, particularly viral hepatitis, continue to pose a serious global health challenge. The World Health Organization reported in 2024 that approximately 304 million people were living with hepatitis B or C in 2022, resulting in nearly 1.3 million deaths, equivalent to around 6,000 new infections and 3,500 deaths per day worldwide (Li et al. 2025, Yong et al. 2026) Despite the availability of antiviral therapies, limitations including drug resistance, accessibility issues, and adverse effects have sustained the need for safer and

culturally accepted hepatoprotective interventions (Yong et al. 2026). Currently, medicinal plants used in traditional medicine remain an important resource for discovering therapeutic candidates with multi-target hepatoprotective actions (Lam et al. 2016).

Bupleurum chinense DC. (*Apiaceae*), the source plant of the traditional crude drug *Radix Bupleuri* (Bắc sài hồ), has been widely utilized for more than two millennia in Traditional Chinese and Vietnamese Medicines for the treatment of feverish disorders, alternating chills and fever, menstrual dysfunction, and especially chronic hepatitis, as documented in classical prescriptions such as Xiao Chai Hu Tang (Wu et al. 2010). Modern pharmacological studies have attributed the hepatic and immunomodulating effects of B. *chinense* to its diverse secondary metabolites, particularly triterpenoid saponins (saikosaponins), along with flavonoids, coumarins, phenolic acids, and polysaccharides (Yuan et al. 2017, Sun et al. 2019) Among these, saikosaponins A, C, and D have demonstrated hepatoprotective, anti-inflammatory, antiviral, and autophagy-modulating properties, supporting the traditional medicinal use of the plant (Li et al. 2015, Sun et al. 2019, Wang et al. 2023).

However, accumulating evidence suggests that solvent polarity strongly influences the extractable phytochemical spectrum of B. *chinense*, especially the amphiphilic triterpenoid saponins. Water efficiently extracts highly polar saikosaponins and phenolic acids, whereas ethanol enhances the recovery of less-polar saponins and bioactive flavonoids with strong antioxidant and anti-inflammatory activities (Alam et al 2020, Fordos et al. 2025). A comprehensive understanding of how extraction solvents affect the chemical profiles and bioactivities of B. *chinense* extracts is crucial to improve their therapeutic efficacy rationally.

Therefore, the present study aimed to systematically investigate the effects of different extraction solvents (water, 70% ethanol, and 96% ethanol) on the yield, phytochemical composition, and biological activities of B. *chinense* root extracts. We employed high-resolution LC–MS/MS and chemometric analyses to compare saponin distribution and metabolite patterns, and evaluated key pharmacological properties relevant to liver protection, including DPPH radical scavenging, xanthine oxidase inhibition, and nitric oxide suppression in macrophages. This integrative approach provides new insights into the optimization of saponin-rich preparations of *Radix Bupleuri*, supporting its continued ethnopharmacological use in managing liver-related diseases.

Materials and methods

Materials:

Ethanol, n-butanol (Chemsol, Vietnam) were included in the extracted procedure; DPPH (2,2-diphenyl-1-picrylhydrazyl) (Wako, Japan); Gallic acid, Folin-Ciocalteu reagent, Na_2CO_3, Quercetin, Gress assay (Acors), Vitamin C, acetic acid, NaH_2PO_4, Na_2HPO_4 (Merck, Germany) were included in the characterization of the extracted. Other chemical agents related to assay or cell culture were presented in the experimental section.

Plant materials and extraction procedure

Bupleurum chinense DC was purchased from the National Institute of Medical Materials (Ha Noi, Viet Nam), authenticated by a botanist at Southern Institute of Ecology, washed, and dried at 50 °C to a final moisture content of 11.42%, then pulverized to a fine powder. The powdered material was extracted by ultrasound-assisted extraction (1 h × 3 cycles) using a raw material-to-solvent ratio of 1:9 (w/v). Three extraction solvents were evaluated: distilled water, 70% ethanol, and 96% ethanol. Extracts were combined, filtered, and evaporated at 60 °C under reduced pressure (70–76 cmHg) to obtain dry extracts (moisture content ~5%).

Total polyphenol content (TPC) was quantified by the Folin–Ciocalteu method using gallic acid calibration (0–100 µg/mL). Absorbance was measured at 765 nm and expressed as mg gallic acid equivalents per mL extract (mg GAE/mL). All assays were performed in triplicate.

Isolation and quantification of crude saponins

Crude saponins were isolated using a modified gravimetric procedure as described by Moxime Le Bot [11]. The dried extract was dissolved in water and defatted prior to liquid–liquid partitioning into water-saturated n-butanol. The organic fraction was neutralized, evaporated to dryness, and oven-dried to constant weight. Total saponin content (TSC, mg/g) was calculated using:

$$TSC = (m_1 - m_0) \times (100 - H) / m \times 100$$

where:

m_1 = weight of flask + dried residue (g)

m_0 = weight of empty flask (g)

m = sample weight (g)

H = sample moisture content (%)

Extraction yield and saponin recovery (%) were calculated based on dried extract mass.

UHPLC–QTOF–MS/MS analysis and GNPS molecular networking

Metabolite identification was performed through a multi-step workflow integrating accurate mass matching (≤10 ppm), MS/MS spectral interpretation, and library-based annotation using GNPS. Precursor ions selected under data-dependent acquisition (DDA) were matched against theoretical masses, followed by confirmation via diagnostic fragment ions characteristic of each metabolite class. Spectral similarity and clustering in GNPS molecular networking provided additional confidence for structural assignment. The compounds were annotated through Global Natural Products Social Molecular Networking (GNPS), enabling MS/MS spectral clustering and chemical relationship visualization among detected metabolites.

Chromatographic separation was carried out using an ExionLC™ UHPLC system (AB SCIEX, USA) equipped with an autosampler, binary pump, and column oven. Samples were injected (2 µL) onto a Hypersil GOLD C18 column (150 × 2.1 mm, 3 µm; Thermo Fisher Scientific, USA). The mobile phases consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The optimized gradient elution program was: 0–1 min, 2% B; 1–30 min, linear increase to 98% B; 30–36 min, held at 98% B. The flow rate was maintained at 0.4 mL/min under ambient temperature conditions.

Mass spectrometric detection was performed on an X500R QTOF system (AB SCIEX, USA) equipped with a Turbo V ESI source operated in negative ion mode. Ion source conditions were as follows: ionspray voltage −4.5 kV, source temperature 500 °C, curtain gas 30 psi, GS1 45 psi, and GS2 45 psi. TOF-MS data were acquired over m/z 70–2000 with a declustering potential of −80 V, collision energy (CE) of −20 eV, and collision energy spread (CES) of 10 eV. For TOF–MS/MS acquisition (data-dependent), the mass range was set to m/z 50–1500 with DP −50 V, CE −30 eV, and CES 10 eV.

Data acquisition and processing were performed using SCIEX OS software (v1.2.0.4122). Total ion chromatograms (TICs) were generated from raw data, and metabolite feature tables were further submitted to GNPS for molecular network generation and spectral comparison against public MS libraries. Data matrices were normalized and subjected to multivariate statistical analysis (SIMCA/MetaboAnalyst). Principal component analysis (PCA) and hierarchical clustering analysis (HCA) were conducted to differentiate extracts based on chemical signatures. Heatmaps were generated using Euclidean distance and Ward's linkage. VIP score threshold ≥1 was used to identify discriminant metabolites.

DPPH radical scavenging activity

Antioxidant activity was determined using the DPPH assay. Serial concentrations of each extract were incubated with DPPH solution for 30 min in the dark. Absorbance was recorded at 517 nm. Radical scavenging (%) was calculated relative to untreated controls, and IC_{50} values were obtained from dose–response curves. Ascorbic acid served as a positive control.

Xanthine oxidase assay

XO inhibition was evaluated by measuring uric acid formation from xanthine at 290 nm. Extracts (0.5–5 mg/mL) were preincubated with XO enzyme, followed by substrate addition. Activity and IC_{50} values were calculated relative to enzyme-only controls. All measurements were performed in triplicate.

In vitro anti-inflammatory evaluation

Crude saponin fraction was assessed for anti-inflammatory activity using RAW 264.7 macrophages (ATCC, 6^{th} generation). Cells were cultured in DMEM (Gibco) and stimulated with LPS (Thermo Fisher Scientific) for 24 h in the presence of test samples (16–256 µg/mL). Nitric oxide (NO) production was quantified using the

Griess reagent (570 nm). Cytotoxicity was evaluated using the MTT assay (Thermo Fisher Scientific). Cardamonin (Thermo Fisher Scientific) was used as a reference inhibitor.

Statistical analysis

Results are expressed as mean ± standard deviation (SD) of triplicate measurements (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Results

The yield of extract and the distribution of bioactive compounds

The extraction procedure applied in this study yielded three *Bupleurum chinense* DC extracts: water, 70% ethanol, and 96% ethanol. These extracts were then extracted with n-butanol to obtain the final extracts, namely SAPO_NUOC (water solvent), SAPO70 (ethanol 70%), and SAPO96 (ethanol 96%). Extraction yields of *Bupleurum chinense* DC varied markedly depending on the solvent employed (**Table 1**). A clear increase in recovery was observed as solvent polarity decreased: water extract yielded 2.40 g of dry extract (8.0%), while 70% ethanol and 96% ethanol produced 2.91 g (9.7%) and 3.04 g (10.1%), respectively. This trend demonstrates that organic solvent content facilitates penetration into plant cell matrices and improves the solubilization of both semi-polar and non-polar constituents.

In term of total polyphenol content (TPC), SAPO96 exhibited the highest TPC values (29.34–29.61 mg GAE/mL), SAPO70 showed intermediate levels (25.72–26.15 mg GAE/mL), while SAPO_NUOC contained the lowest (22.13–23.97 mg GAE/mL). This suggests that ethanol facilitates more efficient extraction of both flavonoids and phenolic acids, key contributors to antioxidant capacity and free radical quenching. In term of saponin – the significant feature of *Bupleurum chinense* DC, the total saponin content did not increase proportionally with ethanol strength. The greatest saponin extraction efficiency (0.16%) were obtained from the 70% ethanol extract, compared to 96% ethanol with 0.13% and water extract with 0.086%. This pattern indicates that intermediate solvent polarity is optimal for recovering saponins, consistent with the amphiphilic nature of these glycosylated compounds. Excessively high ethanol percentages favor less-polar aglycones at the expense of more hydrophilic glycosides, causing a decline in total saponin yield.

The constituents in the extracts were identified and characterized using LC-ESI-MS/MS by comparing retention behavior, accurate mass measurements, and MS/MS fragment ions with reference databases in GNPS. Based on retention time, exact mass measurement (m/z), and MS/MS fragmentation pattern matching with reference databases and existing literature reports. The characterized metabolites covered multiple phytochemical classes, including: phenolic acids (e.g., 2,3-dihydroxybenzoic acid; 3,4-dihydroxybenzoic acid), coumarins (e.g., isofraxidin derivatives), flavonoids: both glycosylated and aglycone types, saponins, fatty acid derivatives and oxylipins, terpenoids and sterol-like structures.

The chemometric evaluation further clarified the influence of extraction polarity on the chemical profiles of the *Bupleurum chinense* extracts via the PCA loading plot. As shown in **Figure 1A**, it was clearly separated SAPO_NUOC, SAPO70 and SAPO96 into distinct regions, indicating substantial compositional differences between samples. PC1 (39.06%) and PC2 (28.56%) together explained approximately 68% of total variance, demonstrating that solvent polarity was the primary determinant of metabolite distribution. SAPO_NUOC clustered toward the positive PC1 axis, representing water-soluble phytochemicals, including phenolic acids and coumarins together with several highly polar saikosaponins. SAPO70 grouped toward the moderate-polarity region of the PCA space, reflecting balanced extraction of both saponins and flavonoid glycosides. SAPO96 separated strongly toward the negative PC1 and positive PC2 region, confirming enrichment of less-polar flavonoids and hydrophobic saponins. The correlation heatmap (**Figure 1B**) revealed low similarity between SAPO_NUOC and ethanol extracts ($r < 0.25$), while SAPO70 and SAPO96 exhibited a moderate correlation, reflecting overlap in ethanol-soluble constituents. The Venn diagram (**Figure 1C**) further demonstrated unique metabolic signatures among the extracts: SAPO70 contained the most exclusive features (128), followed by SAPO_NUOC (114) and SAPO96 (102), while only 66 metabolites were shared across all three samples. These data indicate that no single solvent captures the full chemical diversity of B. *chinense.*

The effect of the extracted solvent on the distribution of saponin compounds

The extraction procedure used in this study aimed to concentrate triterpenoid saponins efficiently. Compound assignment was supported by accurate mass measurements of [M+H]+ precursor ions, with mass errors typically within ±10 ppm, confirming high confidence in the elemental composition. Furthermore, MS/MS

spectra exhibited characteristic fragmentation features, such as neutral loss of sugar residues and diagnostic aglycone ions, providing distinctive molecular fingerprints for each saponin subclass. Saikosaponins displayed sequential cleavage of glycosidic moieties attached to an oleanane backbone, consistent with previously reported fragmentation behavior in *Bupleurum* species [8]. These included saikosaponin a, saikosaponin C, and saikosaponin D**,** which demonstrated strong fragment ion similarities to library spectra, confirming their structural identity as major endogenous *Bupleurum* saponins [7]. In contrast, dammarane-type ginsenosides (Rb1, Rb3, Re, Rg1, Rg5 and Rg6) were identified by the presence of low-mass fragment ions at m/z 59, 71, 89 and 101 and successive neutral losses from the dammarane triterpenoid core, which is not common in *Bupleurum chinense* [12-13]. Soyasaponins (Ba, Bb) and glycyr, and Rg6) were identified by the presence of low-mass fragment ions at m/z 59, 71, 89, which were not confirmed as a natural constituent of B. *chinense* previously. Also, in this study, another Licorice-type triterpenoid was observed primarily in ethanol-extracted fractions (**table 2**).

Inspection of the compound-level PCA feature distribution **(Figure 1D**) showed that the majority of saponin-assigned signals clustered with SAPO70 and SAPO96, whereas SAPO_NUOC retained relatively fewer saponin elements, mainly belonging to highly polar saikosaponins. The saponin distribution patterns among the three Bupleurum chinense DC extracts differed markedly according to extraction polarity (**Figure 2A**). Hierarchical clustering of individual saponin intensities (Figure B) revealed statistically distinct chemotypes (ANOVA, $p < 0.05$). SAPO70 formed a separate cluster, indicating a uniquely balanced recovery of both polar and semi-polar saponins, whereas SAPO96 and SAPO_NUOC grouped more closely, reflecting partial overlap in their saponin profiles. Inspection of the heatmap confirmed that these differences were strongly associated with biosynthetic subclass. The water extract (SAPO_NUOC) exhibited the highest abundance of oleanane-type saikosaponins, including saikosaponin C, saikosaponin D, and saikosaponin a, aligning with their high polarity and multiple sugar chains. These compounds are considered the primary endogenous biomarkers of Radix Bupleuri. In contrast, SAPO96 was enriched in dammarane-type ginsenosides (e.g. Rg5, Rg6, Rb3, Re), representing exogenous triterpenoids co-extracted from companion herbal sources during initial material processing. Their strong extraction into high-ethanol solvent reflects their hydrophobic triterpenoid backbones and lower glycosylation levels.

SAPO70 demonstrated a hybrid distribution, recovering both polar oleanane-type and moderately lipophilic dammarane-type saponins at appreciable intensity. This observation is consistent with its extraction performance, where 70% ethanol delivered the highest total saponin yield. The presence of soyasaponins and licorice-derived triterpenoids additionally suggests ethanol-dependent enhancement of botanical diversity, which may contribute to synergistic pharmacological effects.

Thus, depending on the desired saponin chemotype, controlled manipulation of extraction polarity enables tailored phytochemical preparation, supporting both traditional usage and the rational development of standardized formulations. Solvent polarity selectively enriches distinct saponin subclasses, with water favoring highly polar oleanane-type saikosaponins, 70% ethanol extracting both polar and semi-polar saponins, and 96% ethanol concentrating low-polarity dammarane-type ginsenosides. These chemometric outcomes are consistent with the LC–MS/MS identification results, which confirmed the presence of 17 saponins with a polarity-dependent distribution pattern. Taken together, these findings establish saponins as the dominant class of discriminant metabolites that contribute to the separation between extraction treatments and highlight the importance of solvent polarity as a key driver in the selective enrichment of bioactive compounds in *Bupleurum chinense* DC.

When compared with previous phytochemical studies on *B. chinense* and other *Bupleurum* species, the present results are broadly consistent yet provide additional resolution at the extract level. Classical investigations and systematic reviews have shown that *Radix Bupleuri* is rich in oleanane-type saikosaponins, with saikosaponin a, c and d among the predominant constituents, and more than 100 saikosaponins reported across the genus [11-13]. Targeted quantitative work has further confirmed that the relative contents of saikosaponins a, c, d and b2 vary among Bupleurum variueties and cultivation regions, but consistently dominate the saponin profile of *B. chinense* [8]. Our data agree with this pattern by identifying saikosaponin a, C, D and b2 as major components, particularly in the aqueous and 70% ethanol extracts. In contrast, ginsenosides, soyasaponins and glycyrrhizin-type saponins detected here have not been reported as intrinsic metabolites of *B. chinense* or other *Bupleurum* species, where triterpenoid saponins are almost exclusively saikosaponin-type. Thus, this study extends previous reports by showing how a polarity-guided extraction strategy reorganizes not only endogenous *Bupleurum* saikosaponins but also exogenous saponins, yielding distinct saponin-rich chemotypes with potentially different pharmacological profiles. Therefore, controlled manipulation of solvent polarity can

be strategically applied to tailor saponin-rich preparations: water extracts that maximize polar saikosaponins and antioxidant phenolics; 70% ethanol extracts that provide broad-spectrum saponins and flavonoid glycosides; and 96% ethanol extracts that enhance recovery of lipophilic saponins and high-bioactivity flavonoids. This polarity-guided selectivity offers a rational basis for optimizing *Bupleurum*-containing formulations in traditional and modern phytopharmaceutical applications

Integration of metabolite composition

DPPH assay

Figure 2C illustrates the time-dependent DPPH radical scavenging behavior of the three Bupleurum chinense extracts at two test concentrations. The untreated DPPH solution (control) retained a strong purple color throughout the reaction period, confirming the persistence of free radicals in the absence of antioxidants. In contrast, SAPO96 and SAPO70 induced progressive fading in color intensity, reflecting rapid consumption of DPPH radicals, while SAPO_NUOC caused only a minor shift, indicating weaker activity.

The reaction rate and extent of color disappearance were consistent with the quantitative IC_{50} results (**Table 3**). SAPO96 showed the fastest and most pronounced decolorization (yellow endpoint at 5 mg/mL), consistent with its lowest IC_{50} values for DPPH scavenging (0.79–0.88 mg/mL). SAPO70 displayed a moderate fading progression, correlating with its intermediate IC_{50} range (0.87–0.88 mg/mL). Meanwhile, SAPO_NUOC retained a clear purple tone throughout the reaction period, matching its highest IC₵₅₀ values (0.98–0.99 mg/mL), indicative of limited radical quenching. These trends confirm the antioxidant activity order: SAPO96 > SAPO70 >> SAPO_NUOC.

The time-response visualization reinforces that ethanol-rich extracts deliver superior antioxidant performance due to higher abundances of hydrophobic flavonoids and triterpenoid saponins, whereas the water extract—though containing polar phenolics and saikosaponins—exhibits significantly slower antioxidant kinetics [13-14] . Thus, both qualitative kinetics and quantitative IC_{50} evidence demonstrate that solvent polarity plays a critical role in dictating the rate and efficacy of DPPH radical neutralization.

Xanthine oxidase inhibitory activities

The xanthine oxidase (XO) inhibitory assay further demonstrated the strong influence of extraction solvent polarity on the biological performance of *Bupleurum chinense* extracts. As shown in **Table 3**, SAPO96 exhibited the most potent XO inhibition with the lowest measured IC_{50} values (0.80–0.81 mg/mL), followed by SAPO70 with moderate inhibition (IC_{50}: 0.88–0.90 mg/mL), while SAPO_NUOC showed only weak suppression of XO activity (IC_{50}: 0.94–0.96 mg/mL). This potency ranking (SAPO96 > SAPO70 >> SAPO_NUOC) mirrors both the metabolite distribution and antioxidant activity results, supporting a shared biochemical basis for the two bioassays. The superior inhibitory effect of SAPO96 is consistent with its enrichment in hydrophobic flavonoids (particularly isorhamnetin and its aglycones) and dammarane-type saponins, both of which have been previously reported to interfere with XO catalytic activity and uric generation in vitro [15]. SAPO70 retained intermediate levels of these metabolites and accordingly demonstrated moderate XO inhibition, whereas SAPO_NUOC, composed mainly of polar saikosaponins and phenolic acids, displayed limited activity due to lower content of XO-targeting phytochemicals. Collectively, these findings confirm that extracts enriched in less-polar bioactive constituents are more effective at inhibiting XO, highlighting the potential relevance of ethanol-based B. *chinense* extracts in supporting anti-hyperuricemic and gout-related therapeutic applications.

Anti-inflammatory assays

The anti-inflammatory properties of the extracts were evaluated based on inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages, with MTT cytotoxicity testing conducted in parallel to verify biological specificity. All extracts demonstrated a concentration-responsive reduction in NO levels over the range of 15.6–500 ppm; however, the extent of inhibition remained comparatively low, with none of the samples achieving 50% suppression at or below 200 ppm. (**Table 4**) Nonlinear regression analysis yielded high IC_{50} values, indicating weak NO inhibitory effects: 248.22 ± 13.48 ppm for SAPO96, 257.07 ± 10.93 ppm for SAPO70, and 330.67 ± 14.10 ppm for SAPO_NUOC. The ranking of activity (SAPO96 > SAPO70 >> SAPO_NUOC) parallels the distribution of hydrophobic flavonoids and dammarane-type saponins observed through LC–MS/MS analysis, suggesting a modest contribution from these constituents to NO regulation.

Across all conditions, macrophage viability remained above 80%, confirming that the limited inhibition of NO was not associated with cytotoxicity but reflects intrinsic pharmacological behavior of the extracts. This pattern is consistent with known mechanistic profiles of saikosaponins, which primarily modulate inflammatory responses by suppressing NF-κB and MAPK signaling and reducing cytokine overexpression [16] rather than through direct blockade of inducible nitric oxide synthase (iNOS) activity [17]. Therefore, the weak NO suppression observed here does not contradict the traditional anti-inflammatory use of *Bupleurum chinense* but rather supports the concept that its primary immunomodulatory actions are mediated upstream of the NO pathway. In other word, these results demonstrate that *Bupleurum* extracts remain biologically safe at pharmacologically relevant concentrations and possess indirect anti-inflammatory potential via saponin-mediated modulation of immune signaling, even though their direct inhibitory effects on NO production are limited. When integrated with chemometric and antioxidant outcomes, the data highlight that ethanol-based extracts, especially SAPO96, offer the most promising multifunctional bioactivity profile while retaining low cytotoxicity.

Discussion

This study demonstrates that extraction solvent polarity critically shapes both the phytochemical composition and biological functionality of *Bupleurum chinense* DC (**Schematic 1**). Although 96% ethanol achieved the greatest extraction yield, 70% ethanol was most effective for concentrating total saponins—especially oleanane-type saikosaponins, the principal bioactive markers of Radix Bupleuri. Integrated LC–MS/MS profiling and chemometric analysis confirmed a clear polarity-dependent differentiation of saponin subclasses: polar saikosaponins were predominant in the aqueous extract, 70% ethanol afforded a balanced amphiphilic chemotype, and 96% ethanol selectively enhanced recovery of less-polar ginsenosides and flavonoid aglycones. These compositional differences were reflected in the bioassays, with SAPO96 exhibiting the strongest antioxidant and xanthine oxidase inhibitory effects, whereas all extracts displayed weak direct NO suppression but retained high cell viability, suggesting low cytotoxicity and anti-inflammatory mechanisms. While the present work provides a comprehensive solvent–chemotype–bioactivity correlation, further studies are required to strengthen translational applicability. Isolation and structural confirmation of key discriminant saponins—particularly saikosaponins and ethanol-enriched ginsenosides—will help establish causal relationships between individual constituents and therapeutic outcomes. In vivo evaluation in hepatoprotective and anti-hyperuricemic models would also be valuable to validate the biological relevance of the identified chemotypes. Moreover, expanding metabolomic comparisons across different *Bupleurum* species, cultivation sources, and processing methods may better elucidate sources of phytochemical variability, while mechanistic investigations into immune signaling pathways could clarify anti-inflammatory modes of action beyond NO regulation. In conclusion, the polarity-guided extraction approach effectively delineates distinct bioactive chemotypes of B. chinense, identifying SAPO96 as the most promising extract for oxidative stress and hyperuricemia-related applications, and SAPO70 as the preferred preparation for traditional saikosaponin-based therapeutic uses. These findings provide a rational foundation for optimizing *Bupleurum*-containing formulations and tailoring extraction strategies to specific pharmacological objectives in modern phytotherapy.

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Conflict of interest

The authors have declared no conflict of interest..

Table 1: Extraction yield, total saponin content, total polyphenol content of *Bupleurum chinense* DC extracts

Extract	Extraction yield (%)	Total saponins	Total polyphenols (mg GAE/mL)

		(g as-preparated mass / %)	
SAPO_NUOC (Water)	8.0	0.086%	22.13 – 23.97
SAPO70 (70% EtOH)	9.7	0.16%	25.72 – 26.15
SAPO96 (96% EtOH)	10.1	0.13%	29.34 – 29.61

Table 2: The variability in saponin profiles observed among SAPO_NUOC, SAPO70, and SAPO96 based on LC-MS/MS identification results

Saponin	m/z experiment	Aglycone Type (estimated)	Water solvent	SAPO70	SAPO96
Saikosaponins					
Saikosaponin a	804.5487	Oleanane-type	+	+	+
Saikosaponin b2	825.4596	Oleanane-type	+	+	-
Saikosaponin B2	779.4540	Oleanane-type	-	+	-
Saikosaponin C	957.3609	Oleanane-type	+	+	+
Saikosaponin D	806.2901	Oleanane-type	+	-	+
Soyasaponins					
Soyasaponin Ba	943.5208	Soyasapogenol-B	+	-	+
Soyasaponin Bb	927.5267	Soyasapogenol-B	+	-	+
3-Rha(1-2)Gal(1-2)GluA-Soyasaponenol B	~942	Soyasapogenol derivative	-	-	+
Licorice-type					
Licoricesaponin H2	821.3934	Glycyrrhetinic acid-type	+		+
Glycyrrhizin	821.3922	Glycyrrhetinic acid-type	-	+	-
Ginsenosides					
Ginsenoside Rb1	1153.5937	Dammarane	-	-	+
Ginsenoside Rb3	1123.5843	Dammarane	-	+	-
Ginsenoside Re	991.5492	Dammarane	-	-	+
Ginsenoside Rg1	845.4874	Dammarane	+	+	+
Ginsenoside Rg5	811.4800	Protopanaxadiol	-	+	+
Ginsenoside Rg6	811.4807	Protopanaxadiol	-	+	+
Ginsenoside F3	815.4749	Dammarane	-	+	-
Other Triterpenoid Saponins					

Chikusetsusaponin IV	925.4725	Oleanane / triterpenoid-type	-	+	+

Table 3: antioxidant/xanthine oxidase inhibitory activities of *Bupleurum chinense* DC extracts (n= 3)

Extract	DPPH IC_{50} (mg/mL)	XO IC_{50} (mg/mL)
SAPO_NUOC (Water)	0.98 – 0.99	0.94 – 0.96
SAPO70 (70% EtOH)	0.87 – 0.88	0.88 – 0.90
SAPO96 (96% EtOH)	**0.79 – 0.88**	**0.80 – 0.81**

Table 4: The inhibition of nitric oxide from RAW264.7 activated by LPS after incubated with *Bupleurum chinense* DC extracts

Sample	Concentration (ppm)						IC_{50} (ppm)
	15.6125	**31.25**	**62.5**	**125**	**250**	**500**	
SAPO_NUOC	23.09 ± 8.32	23.19± 7.38	26.43± 8.69	29.98± 5.91	45.64± 12.62	56.27± 3.33	330.67 ± 14.1
SAPO70	21.91 ± 7.72	23.47 ± 7.78	30.4 ± 4.68	34.82 ± 6.39	50.44 ± 3.44	64.11 ± 6.43	257.07± 10.93
SAPO96	22.99 ± 7.65	25.86 ± 5.52	29.20 ± 5.89	33.73 ± 6.67	48.79 ± 6.73	57.81 ± 0.12	248.22 ± 13.48

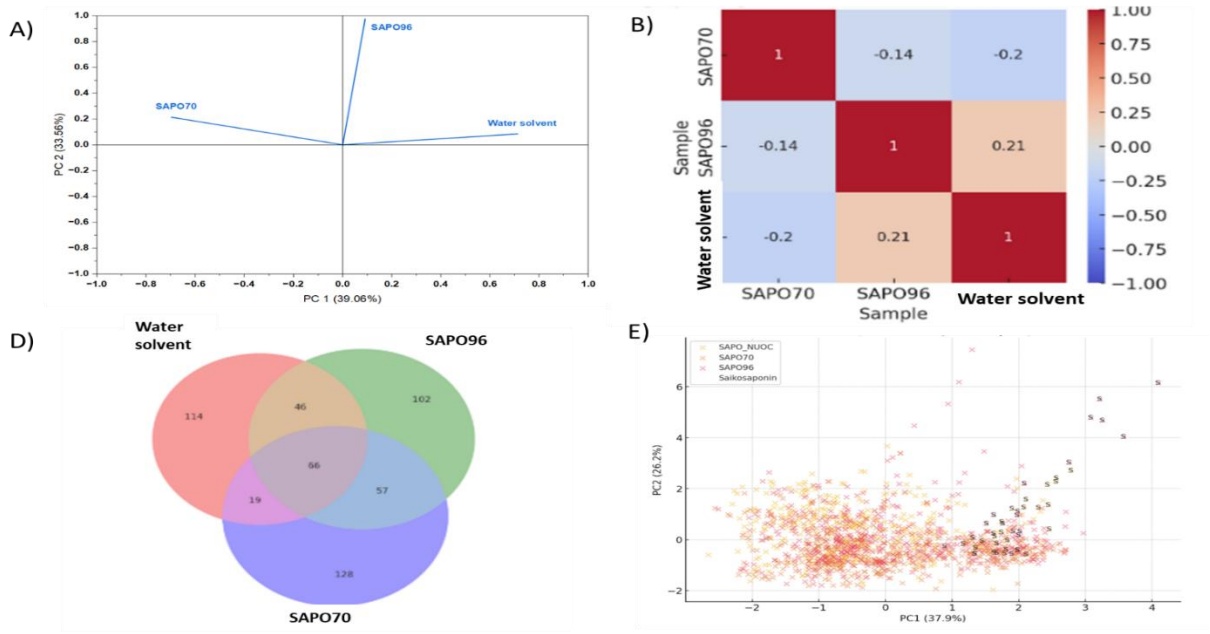


Figure 1. Chemometric characterization of metabolite variation among *Bupleurum chinense* DC extracts prepared using different extraction solvents. A) Principal component analysis (PCA) score plot; B) Correlation heatmap; C) Venn diagram; D) Feature-level PCA scatter plot highlighting the distribution of identified saponins (marked with S).

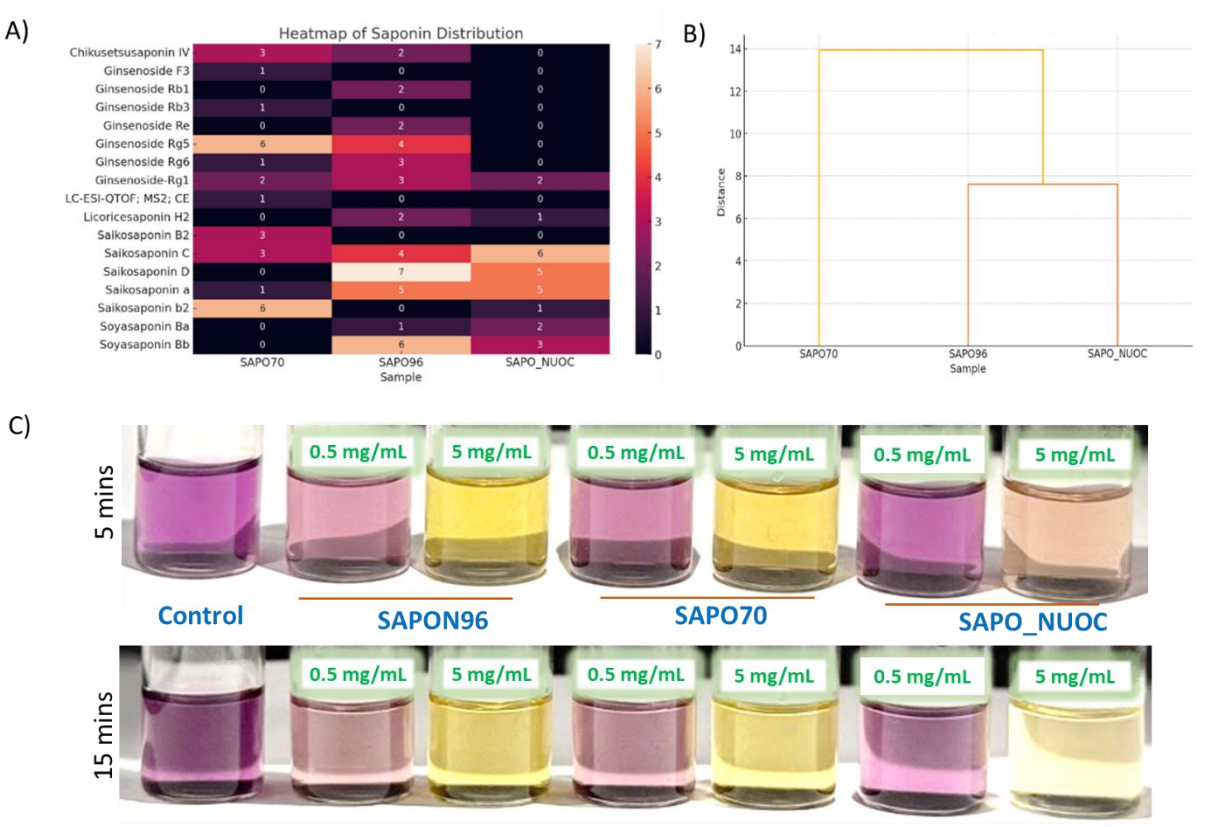
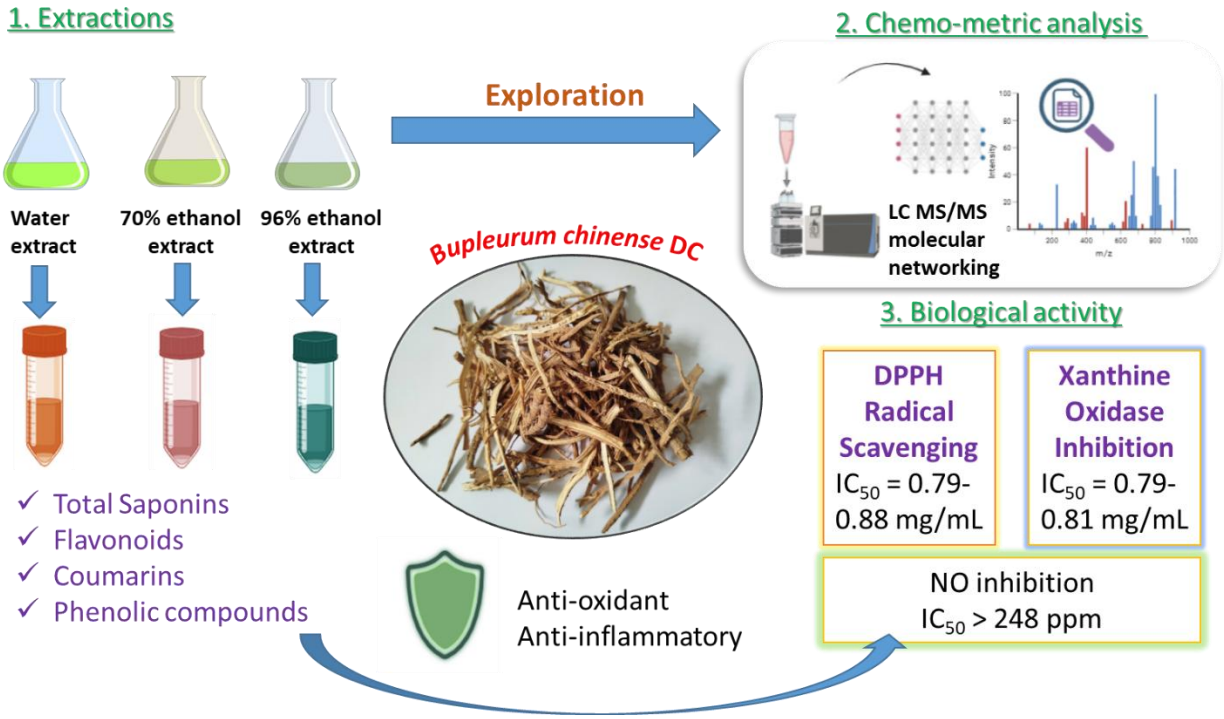


Figure 2: A) Heatmap showing the relative distribution of 17 MS/MS-identified saponins across the three extracts: SAPO70 (70% ethanol), SAPO96 (96% ethanol), and SAPO_NUOC (water extract). B) Hierarchical clustering analysis (HCA) based on saponin abundance demonstrates distinct sample grouping driven by extraction polarity. C) DPPH radical scavenging assay illustrating the time- and concentration-dependent antioxidant performance of the extracts at 0.5 mg/mL and 5 mg/mL



Schematic 1: Solvent dependent saponin recovery and biological activity potential of *Bupleurum chinense* DC

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