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Cornuside, a core bioactive of *Cornus officinalis*, alleviates oxidative stress by directly binding CDK2 to activate NRF2/NQO1

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ABSTRACT: *Cornus officinalis* Sieb. et Zucc. is a widely utilized medicinal and edible plant, yet its principal antioxidant compounds and underlying molecular mechanisms remain largely elusive. To address this, an integrated screening strategy was developed. By combining spectrum-effect relationship analysis, target component knockout, metabolomics, and network pharmacology, several core antioxidant candidates were identified, and CDK2 was predicted as a key target. Subsequent screening in an H₂O₂-induced HepG2 cell model pinpointed cornuside as the most potent antioxidant compound. Efficacy evaluations demonstrated that cornuside effectively alleviates oxidative stress *in vitro* and in an AAPH-induced zebrafish model *in vivo* by restoring endogenous antioxidant enzyme reserves and mitigating apoptosis. Mechanistically, molecular docking and Cellular Thermal Shift Assays (CETSA) explicitly verified that cornuside directly binds to the CDK2 protein, which robustly activates the downstream NRF2/NQO1 signaling pathway. Furthermore, specific siRNA-mediated silencing of CDK2 in HepG2 cells completely abolished the antioxidant protection and NRF2/NQO1 activation mediated by cornuside, confirming its absolute target dependency. Through this systematic screening and validation pipeline, cornuside was conclusively identified as the key antioxidant constituent of *C. officinalis*. It mitigates oxidative stress and apoptosis by directly targeting CDK2 to activate the NRF2/NQO1 pathway, providing a robust pharmacological basis for its functional application.

Keywords: *Cornus officinalis*, Cornuside, Antioxidant activity, CDK2 target, NRF2/NQO1 pathway, Target identification

1. Introduction

Oxidative stress arises from a systemic imbalance between the production of reactive oxygen species (ROS) and the cellular capacity to eliminate them. This excessive ROS accumulation inflicts irreversible oxidative damage on essential biomacromolecules (including lipids, proteins, and DNA) and disrupts key intracellular signaling pathways, ultimately compromising cellular function and integrity[1]. Mounting evidence indicates that oxidative stress serves as a central pathological driver in aging and the progression of

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various chronic diseases, such as neurodegenerative disorders, cardiovascular diseases, and metabolic syndromes. Under physiological conditions, ROS levels are tightly regulated by endogenous antioxidant defense systems, particularly the NRF2-driven transcription of downstream antioxidant enzymes (e.g., superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]). However, when ROS generation overwhelms these protective mechanisms, the resultant redox disruption triggers severe cellular injury and apoptosis. Therefore, identifying natural, highly efficient exogenous antioxidants capable of restoring redox homeostasis and halting apoptosis represents a compelling therapeutic strategy.

Cornus officinalis Sieb. et Zucc., a well-known medicinal and edible plant, has been traditionally utilized for its diverse health-benefiting properties. The fruit pulp is rich in bioactive phytochemicals, including iridoid glycosides, triterpenoids, and polyphenols, which exhibit broad-spectrum pharmacological activities, such as hypoglycemic, neuroprotective, and anti-inflammatory effects [2, 3]. Recent studies have increasingly highlighted the profound antioxidant potential of *Cornus officinalis*. For instance, iridoid glycosides and low-molecular-weight polyphenols from *Cornus officinalis* were shown to exert renal protective effects in diabetic models by downregulating oxidative and inflammatory markers. However, existing research regarding its antioxidant properties remains largely phenomenological. Most studies have been confined to the evaluation of crude extracts or macroscopic free radical scavenging capacities (e.g., ABTS and DPPH assays) [4], [5–8]. The principal monomeric constituent responsible for this antioxidant efficacy, along with its direct molecular binding targets within the cellular signaling network, remains entirely elusive. This lack of mechanistic depth significantly hinders the targeted clinical application and functional product development of *Cornus officinalis*.

To systematically bridge this knowledge gap, we developed an integrated discovery and validation pipeline to pinpoint the active constituents and elucidate their precise molecular mechanisms. In this study, we first established an HPLC fingerprint of *Cornus officinalis* and constructed a spectrum-effect relationship model to screen potential antioxidant candidates. Integrating non-targeted metabolomics with network pharmacology, we comprehensively predicted the core bioactive components and their corresponding intracellular targets. Crucially, moving beyond *in silico* predictions, we evaluated the antioxidant and anti-apoptotic efficacy of the optimized constituent, cornuside, both *in vitro* utilizing an H₂O₂-challenged HepG2 cell model and *in vivo* using an AAPH-induced zebrafish developmental model. Furthermore, Cellular Thermal Shift Assay (CETSA) and siRNA-mediated gene silencing were employed to unequivocally confirm the direct engagement of cornuside with its specific target protein (CDK2) and the subsequent activation of the NRF2/NQO1 signaling axis. This study provides a robust pharmacological and molecular foundation for elucidating the material basis of *Cornus officinalis* against oxidative damage.

2. Materials and methods

2.1 Sample Preparation and Extraction

Fruit samples of *Cornus officinalis* (S1–S13) were collected from Luoyang City, Henan Province, China, and authenticated as dried ripe pulp. Voucher specimens were deposited in the Herbarium of Henan

University. Samples were extracted thrice with 75% ethanol (1:10, w/v) for 48 h. The combined extracts were filtered, concentrated under reduced pressure, and lyophilized. For assays, 10 mg/mL stock solutions of the lyophilized powder were prepared in methanol, sonicated, and filtered through 0.22 µm membranes.

2.2 Identification of Antioxidant Components in *Cornus officinalis*

2.2.1 HPLC and Partial Least Squares (PLS) Analysis

HPLC analysis was performed on an Agilent Eclipse XDB-C18 column (4.6 × 250 mm, 5 µm) at 35°C, with a detection wavelength of 250 nm and a flow rate of 0.8 mL/min. The mobile phase comprised acetonitrile (A) and 0.2% aqueous formic acid (B) using a gradient elution: 0-15 min, 2-11% A; 15-50 min, 11-30% A; 50-70 min, 30-50% A. Fingerprint data were standardized using the Similarity Evaluation System for Chromatographic Fingerprint of TCM (2012). PLS regression (DPS 7.05 software) was used to correlate peak areas with ABTS radical scavenging rates to pinpoint bioactive peaks.

2.2.2 Target Component Knockout

According to the established HPLC protocol, the target peak solution (designated as Px⁺) and the remaining eluate (designated as Px⁻) were prepared. Both solutions were concentrated under reduced pressure and reconstituted in methanol for subsequent analysis.

2.2.3 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Analysis Methods

Chromatographic separation was performed using mobile phase A (5 mmol/L ammonium formate in water) and B (acetonitrile) at a flow rate of 0.25 mL/min with an injection volume of 2 µL. The gradient program was as follows: 0.0-1.0 min, 5% B; 1.0-1.6 min, 5-20% B; 1.6-2.5 min, 20-50% B; 2.5-12.0 min, 50-95% B; 12.0-17.0 min, 95% B; 17.0-17.1 min, 95-5% B; 17.1-20.0 min, 5% B[9].

2.2.5 Assessment of Antioxidant Activity

ABTS radical scavenging capacity was evaluated as described[10]. The sample was mixed with the ABTS⁺ working solution, incubated for 30 min, and the absorbance was measured at 405 nm to calculate the inhibition rate. The ABTS radical scavenging rate was calculated using the following formula:

$$\begin{aligned} & \text{ABTS radical scavenging rate (\%)} \\ & = [1 - (A_{\text{sample}} - A_{\text{control}}) \div A_{\text{control}}] \times 100\% \end{aligned}$$

where A_{control} and A_{sample} represent the absorbance of the control and sample, respectively.

2.3 Non-targeted Metabolomics and Network Pharmacology

2.3.1 Non-targeted Metabolomics Analysis (LC-MS/MS)

Metabolite profiling was performed using a Waters 2D UPLC system coupled to a Q-Exactive high-resolution mass spectrometer in both positive and negative ionization modes. Data were processed using Compound Discoverer 3.3 (Thermo Fisher Scientific, USA).

2.3.2 Screening of Bioactive Components and Target Prediction

Bioactive components in *Cornus officinalis* extract were initially screened using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and the Swiss ADME database (<http://www.swissadme.ch/>). The screening criteria for TCMSP were oral bioavailability (OB) $\geq$ 30% and drug-likeness (DL) $\geq$ 0.18. The Swiss ADME screening criteria included: (1) high gastrointestinal absorption; and (2) fulfillment of at least three out of five drug-likeness rules (Lipinski, Ghose, Veber, Egan, and Muegge). Compounds meeting both criteria sets were selected for further analysis. Compounds meeting both sets of criteria are ultimately selected.

Potential targets of the identified bioactive components were predicted using both TCMSP (<https://tcmsp.w.com/tcmsp.php>) and Swiss Target Prediction (<http://www.swisstargetprediction.ch/>) databases. The probability threshold for Swiss Target Prediction was set to > 0.1 . The resulting targets were standardized using the UniProt database (<https://www.uniprot.org/>).

2.3.3 Construction and Analysis of the PPI Network

The intersection between the targets of active components and the oxidative stress-related targets was identified as the potential therapeutic targets of RO-EE against oxidative stress. These overlapping targets were imported into the STRING database (<https://www.string-db.org/>) to construct a protein-protein interaction (PPI) network[11]. Topological analysis was then performed based on the interaction data.

2.3.4 Molecular Docking Analysis

Molecular docking of cornuside with CDK2 was performed using AutoDock Vina (version 1.1.2) with default parameters. The docking results were visualized using Discovery Studio 4.5 and Pymol to generate two- and three-dimensional interaction diagrams.

2.4 Cell Culture and Viability Assay

HepG2 cells were cultured in high-glucose DMEM (10% FBS, 1% penicillin-streptomycin) at 37°C with 5% CO₂. Cells (5×10³/well) were seeded in 96-well plates, cultured for 24 h, and treated with test compounds (12.5-50.0 μmol/L) for 24 h. Viability was assessed using the MTT assay (0.5 mg/mL), dissolving formazan in DMSO and measuring absorbance at 570 nm.

2.5 Measurement of intracellular ROS

Intracellular ROS levels were quantified using the DCFH-DA probe via flow cytometry, strictly according to the protocol detailed in our previous study[12].

2.6 Assessment of Oxidative Stress Markers

After treatment, protein concentration was determined using a BCA kit (Solarbio). The levels of MDA and activities of SOD, CAT, and GSH in cell lysates were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's protocols.

2.7 Zebrafish Maintenance, Treatment, and Survival Rate

Wild-type AB zebrafish maintenance, embryo collection, and ethical approvals were conducted according to our previous protocol[10]. Healthy larvae at 3 days post-fertilization (dpf) were randomly

divided into six groups (n = 30 per group): Control, Model (12.5 mM AAPH), Positive (50 μ M Vitamin C), and cornuside treatment groups (Low, Medium, and High concentrations). The larvae were co-treated with 12.5 mM AAPH and the respective compounds for 18 h. Following the treatment period, the number of live larvae was recorded to calculate the survival rate for each group.

2.8 Assessment of Heart Beat Rate and Yolk Sac Area

At 72 hours post-fertilization (hpf), the treated larvae were anesthetized using 0.2 mg/mL tricaine on ice to evaluate physiological and developmental markers. The heart beats of the larvae were visually counted under a stereomicroscope for 1 min to determine the heart beat rate. Subsequently, the larvae were positioned and photographed under a stereomicroscope to capture the yolk sac morphology. The yolk sac area was quantified using ImageJ software, and the yolk sac area ratio was calculated relative to the control group.

2.9 In Vivo Imaging of ROS and Cell Death

The levels of intracellular ROS and cell death were evaluated using DCFH-DA and Acridine Orange (AO) staining, respectively. The staining procedures and image acquisition were performed following the detailed methods described in our previous study[10].

2.10 Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted and qRT-PCR was performed on a Quant Studio 3 system (Applied Biosystems, USA) using β -actin as an internal control[13]. The mRNA expression levels of *NRF2*, *HSP90AA1*, *HSPA8*, *CDK2*, *NQO1*, *CASP3*, *CAT*, *GPX1*, *SOD1*, and *SOD2* were quantified. Primer sequences are listed in Table S1.

2.11 Western Blot

The Western blot procedure followed a previously published protocol from our laboratory[14, 15].

2.12 Small Interfering RNA (siRNA) Transfection

CDK2-specific siRNA (designed and synthesized by Genema, Shanghai) was used to knock down CDK2 expression. The siRNA sequences were: sense, 5'-GGAGCUGUUAUCGCAAAUTT-3'; antisense, 5'-AUUUGCGAUAACAAGUCCTT-3'. HepG2 cells were seeded in 6-well plates and cultured for 24 h. At 50% confluence, cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions for 24 h. CDK2 knockdown efficiency was verified by Western blotting and RT-qPCR.

2.13 Cellular Thermal Shift Assay (CETSA)

The CETSA procedure followed the experimental protocol published in the literature[16, 17].

2.14 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0. Prior to statistical analyses, normality was assessed using the Shapiro–Wilk test and homogeneity of variances was assessed using Levene's test.

One-way ANOVA followed by Tukey's honest significant difference (HSD) post-hoc test was used for multiple group comparisons, and data are presented as mean $\pm$ SD. Differences with $P < 0.05$ were considered statistically significant. Significance levels are denoted as $*P < 0.05$, $**P < 0.01$, $***P < 0.005$, and $****P < 0.001$.

3. Results

3.1 Screening and Identification of Key Antioxidant Components in *Cornus officinalis* via Spectrum-effect Relationship Analysis

To systematically evaluate the consistency and stability of 13 batches of *Cornus officinalis* samples, we first established HPLC fingerprint profiles and performed similarity analysis using the Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2012 edition). HPLC analysis revealed 12 common peaks, designated F1-F12 (Fig. 1A). All chromatograms showed high similarity (>0.96), indicating consistent chemical composition among batches. We performed hierarchical cluster analysis (HCA) and principal component analysis (PCA) on the Z-score normalized peak areas to assess compositional differences. HCA grouped the 13 batches into three distinct clusters (Fig. S1A), indicating notable variation in chemical profiles. PCA further confirmed this trend, with the first two principal components (PC1 and PC2) explaining 82.14% of the total variance (Fig. S1B, Table S2). The loadings of each peak on these components are detailed in Table S3.

To identify the active antioxidant constituents of *Cornus officinalis*, we first profiled the ABTS radical scavenging activity of 13 extract batches (Fig. 1B). Batch S12 (from Hexiang Village) demonstrated the most potent activity, as evidenced by the lowest IC_{50} value (Fig. 1C), and was thus selected for further analysis. Partial least squares (PLS) regression of its HPLC fingerprint revealed significant positive correlations ($R > 0.1$) between ABTS scavenging activity and seven peaks (F2, F3, F4, F7, F9, F10, F12), while five peaks (F1, F5, F6, F8, F11) showed negative correlations (Fig. 1D). We subsequently isolated nine target peaks (F1, F2, F3, F5, F6, F7, F9, F10, F12) via preparative HPLC (Fig. S2). By analyzing MS/MS fragmentation patterns, molecular masses, and chromatographic behavior, and comparing with literature data, we identified peaks F2, F3, F5, F6, F7, F9, and F12 as kingiside, cornin, sarracenin, loganic acid, morroniside, loganin, and cornuside. The MS/MS spectrum and fragmentation pathway of cornuside are shown in Figure 1E and 1F, respectively. Spectra of the remaining compounds are provided in Figure 2[18-20]. We further evaluated the ABTS radical scavenging activity of the knocked-out peaks and their corresponding negative controls (Fig. 1G). The negative controls (eluates after knockout of F2, F3, or F12) each exhibited higher ABTS radical scavenging activity than the original extract, while the corresponding knocked-out components showed significantly reduced activity, confirming the critical contribution of these peaks to the overall antioxidant capacity. This result suggests potential synergistic interactions between these target components and the remaining constituents in the negative controls. In summary, kingiside, cornin, and cornuside are identified as key bioactive constituents responsible for the antioxidant properties of *Cornus officinalis*.

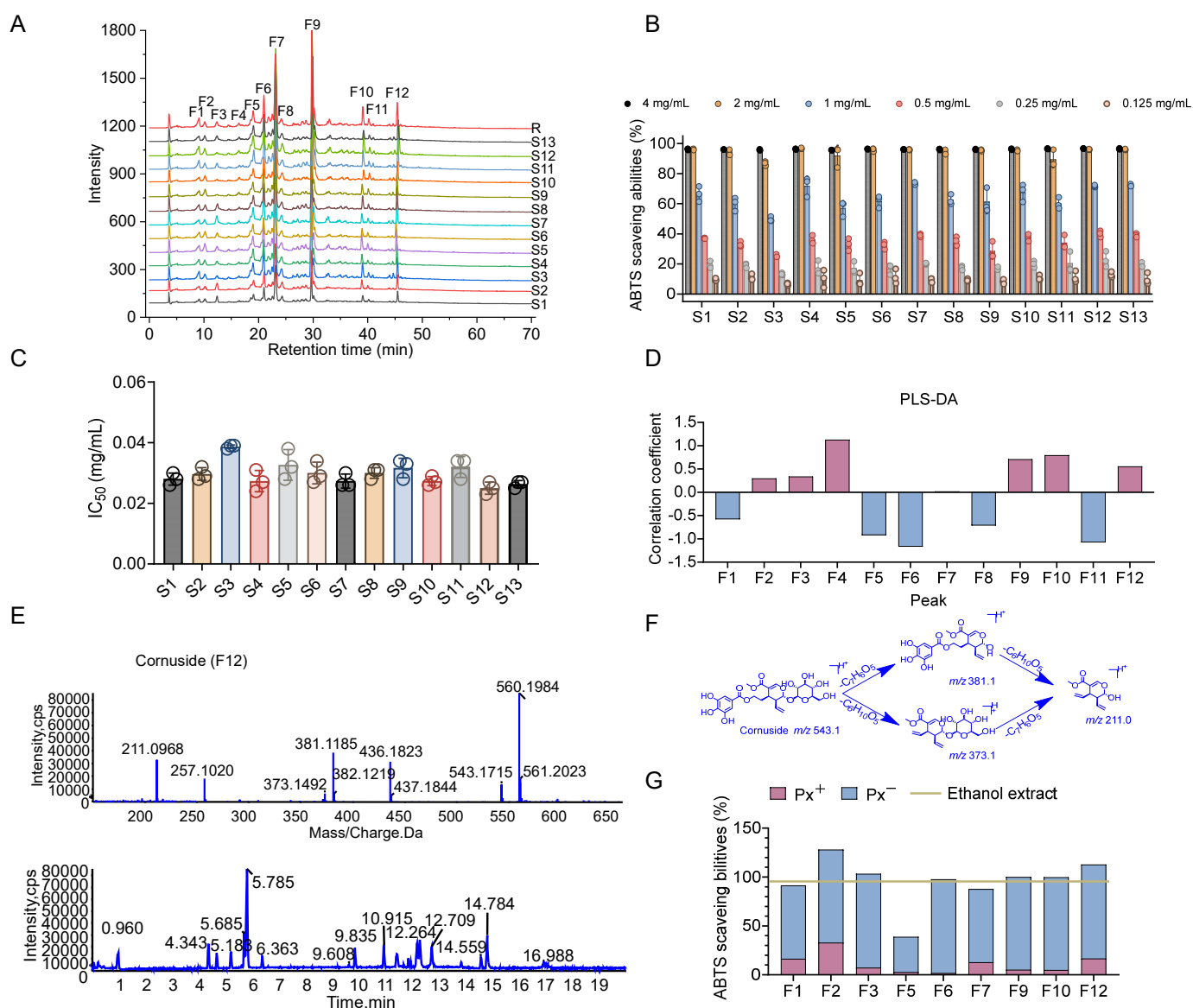


Figure 1. Chemical profiling and identification of antioxidant components in *Cornus officinalis*. (A) Representative HPLC fingerprint of 13 batches of *Cornus officinalis* extracts and its reference fingerprint (R). (B) ABTS assay on 13 batches of *Cornus officinalis*. (C) IC_{50} values for 13 batches of *Cornus officinalis*. (D) PLSR analysis correlating common peaks with ABTS radical scavenging activity. (E) The MS/MS spectrum of cornuside. (F) MS and MS/MS spectra of cornuside. (G) The ABTS radical scavenging activity of knock-out components and negative samples cornuside (F12).

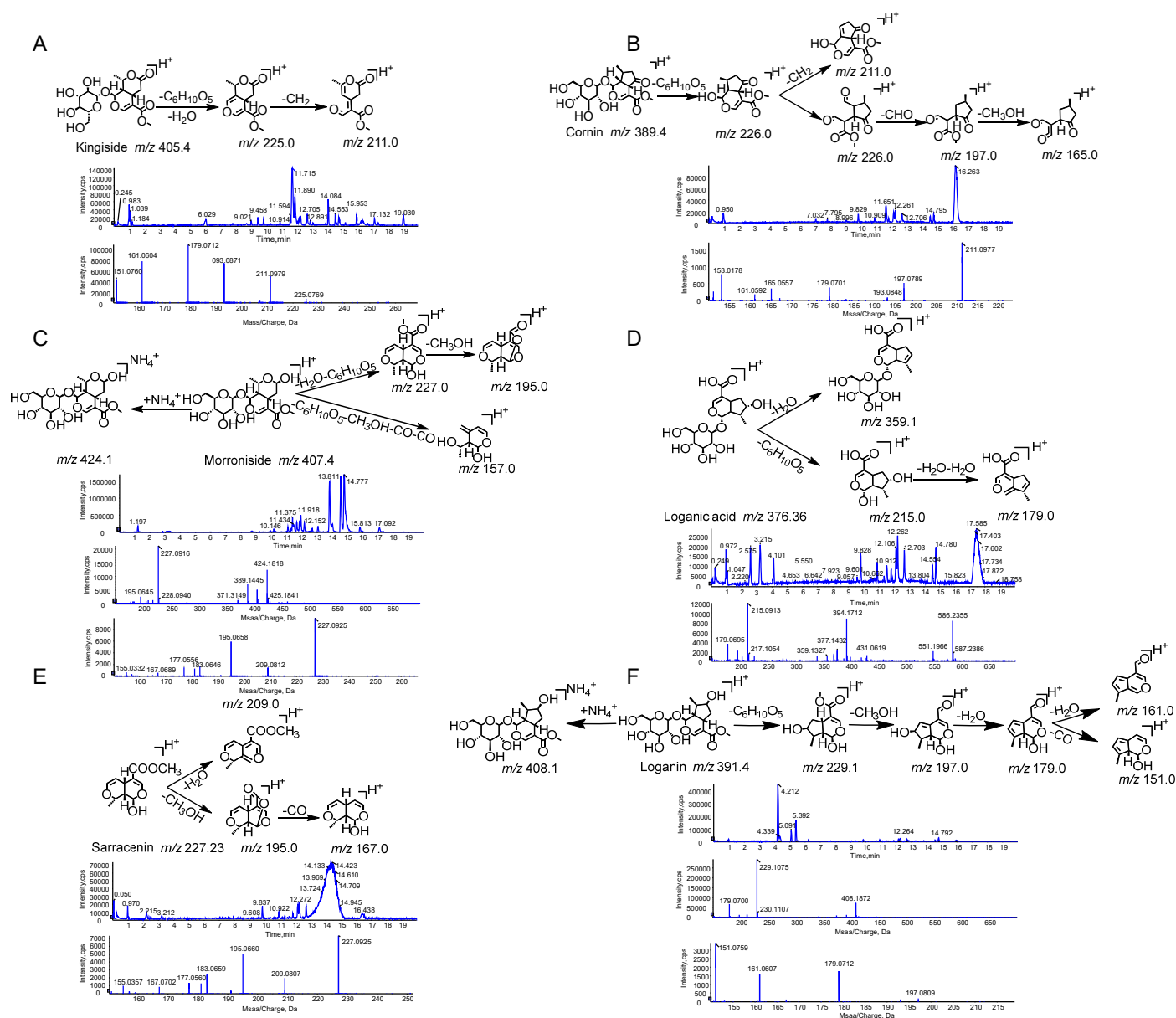


Figure 2. The MS/MS fragmentation pathway and the MS/MS spectrum of (A) F2, (B) F3, (C) F7, (D) F6, (E) F5, and (F) F9.

3.2 Identification of Antioxidant Components and Key Targets in *Cornus officinalis* via Integrated Metabolomics and Network Pharmacology

To elucidate the molecular targets through which *Cornus officinalis* mitigates oxidative stress, we initiated our investigation with comprehensive chemical characterization of the extract. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), we identified 82 distinct compounds (Tables S4, S5). Subsequent evaluation of drug-like properties through TCMSP and SwissADME databases led to the selection of 55 compounds meeting criteria of oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 , indicating favorable pharmacokinetic properties. Target prediction for these bioactive compounds, following identifier standardization and duplicate removal, revealed 406 unique human targets. The overall screening strategy is presented in Figure 3A. Notably, kaempferol and cornuside displayed the most extensive target interactions among all identified compounds (Table S6). Integration of five public databases (GeneCards, OMIM, PharmGKB, TTD, and DrugBank) identified 7,905 unique oxidative stress-related targets (Fig. 3B).

Comparative analysis between these oxidative stress-related targets and those linked to *Cornus officinalis* compounds identified 362 potential key targets for antioxidant mechanisms (Fig. 3C). We constructed a compound-target interaction network comprising 55 compounds and 362 targets (Fig. 3D). Through protein-protein interaction (PPI) network analysis combined with topological screening, we refined our target list to 13 core proteins: PGR, TNF, MAPK1, CDK2, ESR1, GSK3B, ESR2, HSPA8, HSP90AA1, CASP3, SRC, PPARG, and EGFR (Fig. 3E). Among these candidates, CDK2 emerged as particularly promising, with its corresponding interacting compounds detailed in Figure 3F. Gene Ontology (GO) and KEGG enrichment analyses revealed significant enrichment in biological processes including reactive oxygen species metabolic process and cellular response to oxidative stress, as well as key pathways such as the AGE-RAGE signaling pathway in diabetic complications (Fig. S3A, S3B). These findings suggest that *Cornus officinalis* modulates oxidative stress through coordinated regulation of glucose metabolism and reactive oxygen species homeostasis.

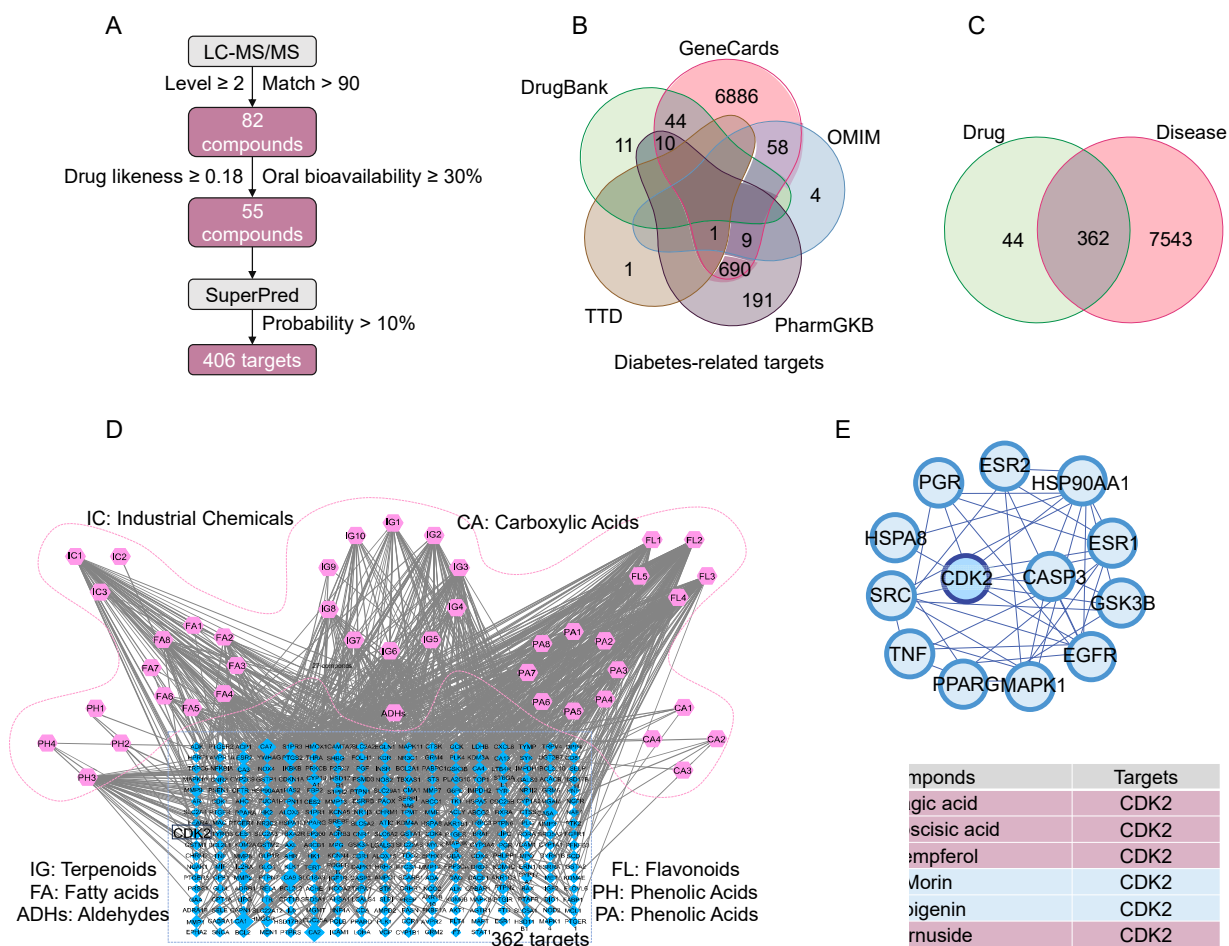


Figure 3. CDK2 is the primary active target through which *Cornus officinalis* ameliorates oxidative stress. (A) Target Screening Workflow. (B) Intersection of oxidative stress targets. (C) Venn diagram showing the intersection between *Cornus officinalis* targets and oxidative stress-related targets. (D) *Cornus officinalis* "active ingredient-target" network modeling. (E) The core targets of active components in *Cornus officinalis* in alleviating oxidative stress. (F) Compounds that bind to CDK2.

3.3 Cornuside Attenuates Oxidative Stress in HepG2 Cells

To precisely identify the most crucial antioxidant compounds within *Cornus officinalis*, we first integrated key findings from spectrum-effect relationships, metabolomics, and network pharmacology.

Subsequently, by combining existing literature data, we identified cornin, loganic acid, loganin, and cornuside as core compounds for further investigation. We first established non-toxic doses (12.5–50.0 $\mu\text{mol/L}$) using the MTT assay in HepG2 cells (Fig. 4A–D). Subsequently, we evaluated their efficacy via a hydrogen peroxide-induced oxidative stress model in HepG2 cells. As shown in Figures 4E–T, all four compounds improved oxidative damage markers (MDA, SOD, CAT, GSH). Notably, cornuside exhibited potent antioxidant activity even at the lowest concentration (12.5 μM), indicating it is the most effective antioxidant component in *Cornus officinalis*.

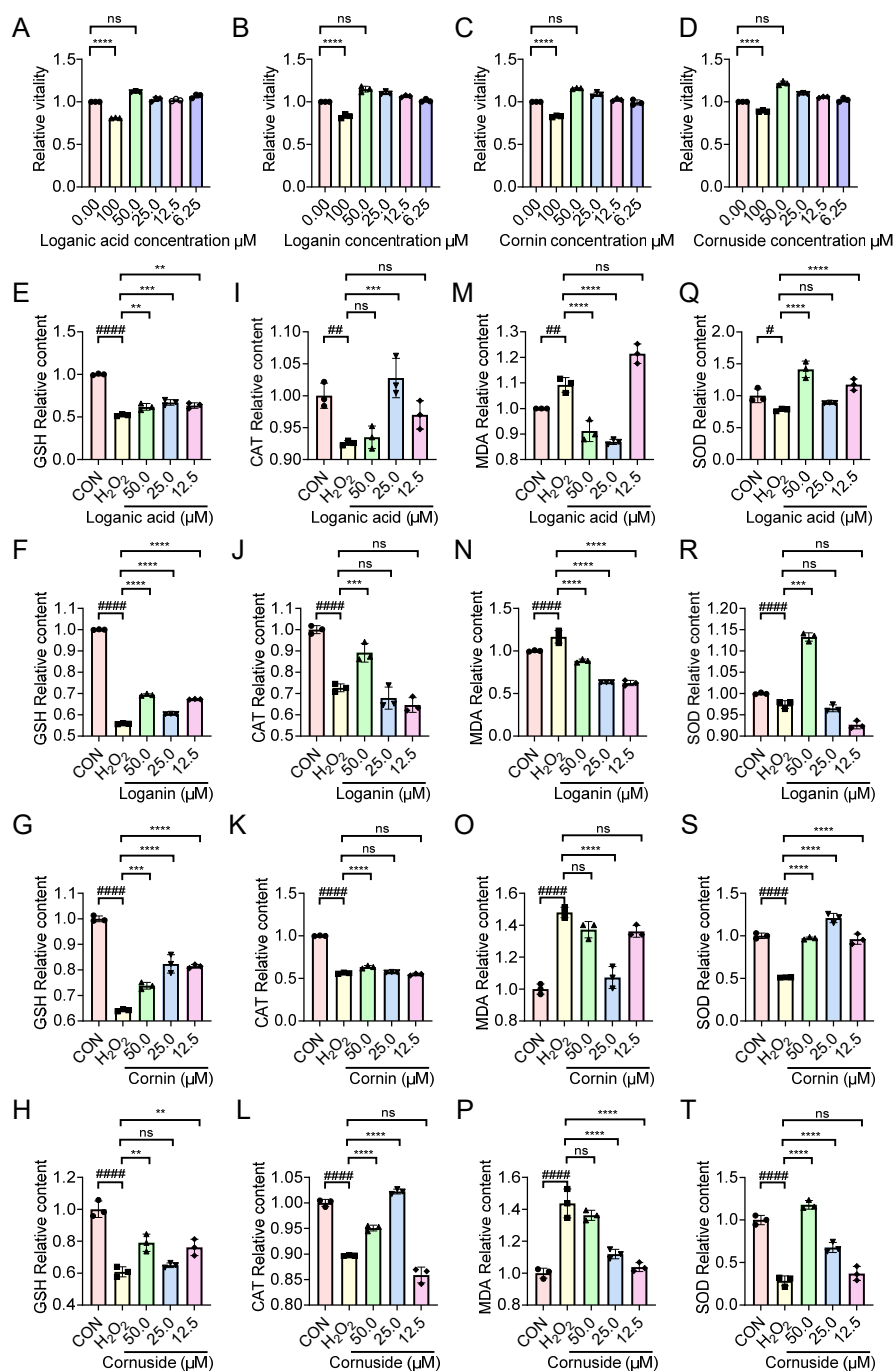
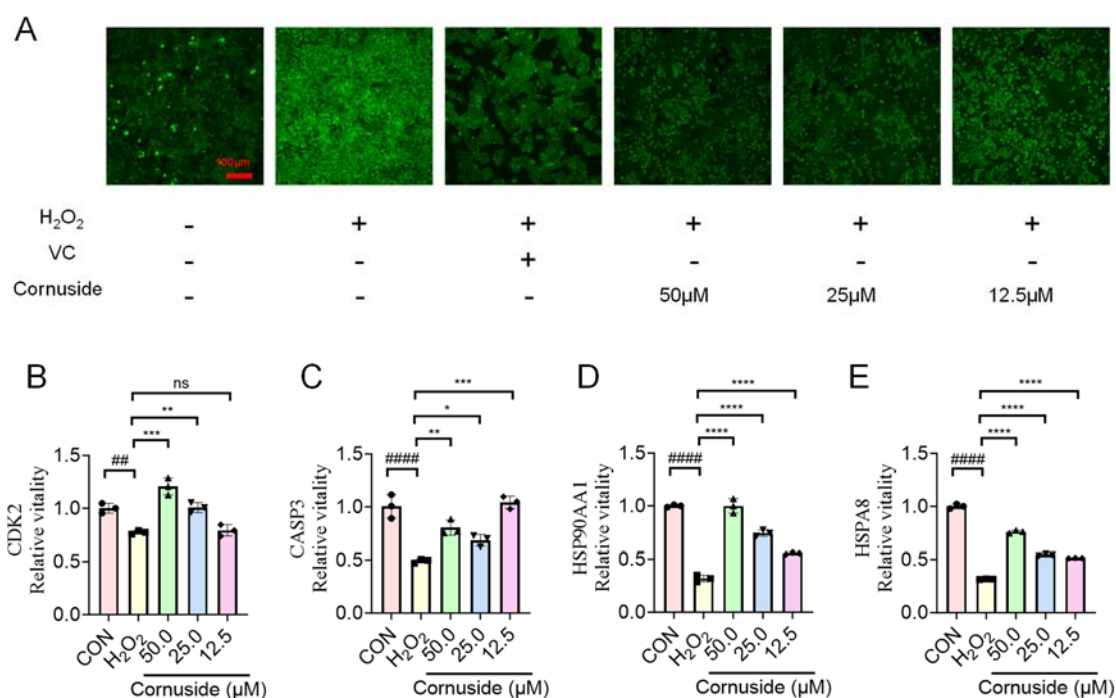


Figure 4. Evaluation of cytotoxicity and antioxidant effects of four core compounds in HepG2 cells. (A) Cell viability was assessed by MTT assay after 24 h treatment with cornin, loganic acid, loganin, and cornuside with different concentrations. Effects of the four compounds on oxidative stress markers in H_2O_2 -induced HepG2 cells: (E–H) GSH content, (I–L) CAT activity, (M–P) MDA activity, and (Q–T) SOD level. Data represent mean $\pm$ SEM. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.005$, #### $P < 0.001$ vs CON; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$ vs H_2O_2 .

ROS serve as key indicators of intracellular oxidative stress, and their cellular levels directly reflect the redox status[21]. We used DCFH-DA staining combined with fluorescence microscopy to assess intracellular ROS levels. The results demonstrated that, compared to the model group, the VC-positive drug group significantly reduced ROS accumulation in HepG2 cells, confirming successful modeling. Furthermore, cornuside treatment dose-dependently decreased intracellular ROS levels (Fig. 5A). To determine whether the antioxidant effects of cornuside involve key oxidative stress targets, we first analyzed the mRNA expression of relevant genes using RT-qPCR. As shown in Figures 5B-E, H₂O₂ treatment significantly downregulated the transcriptional levels of CDK2, CASP3, HSP90AA1, and HSPA8 compared to the control group. Cornuside treatment effectively reversed this H₂O₂-induced downregulation. We next performed Western blot analysis to evaluate CDK2 protein expression and CASP3 cleavage. The results revealed that, compared to the control group, the H₂O₂-treated model group exhibited significantly reduced CDK2 protein expression and an elevated cleaved-CASP3/CASP3 ratio (Fig. 5F, G), confirming altered oxidative stress-related protein expression and successful model establishment. Cornuside treatment significantly increased CDK2 expression and decreased the cleaved-CASP3/CASP3 ratio compared to the model group. Collectively, these findings indicate that cornuside alleviates oxidative stress by promoting cell proliferation via CDK2 upregulation and suppressing apoptosis through modulation of CASP3 activation.



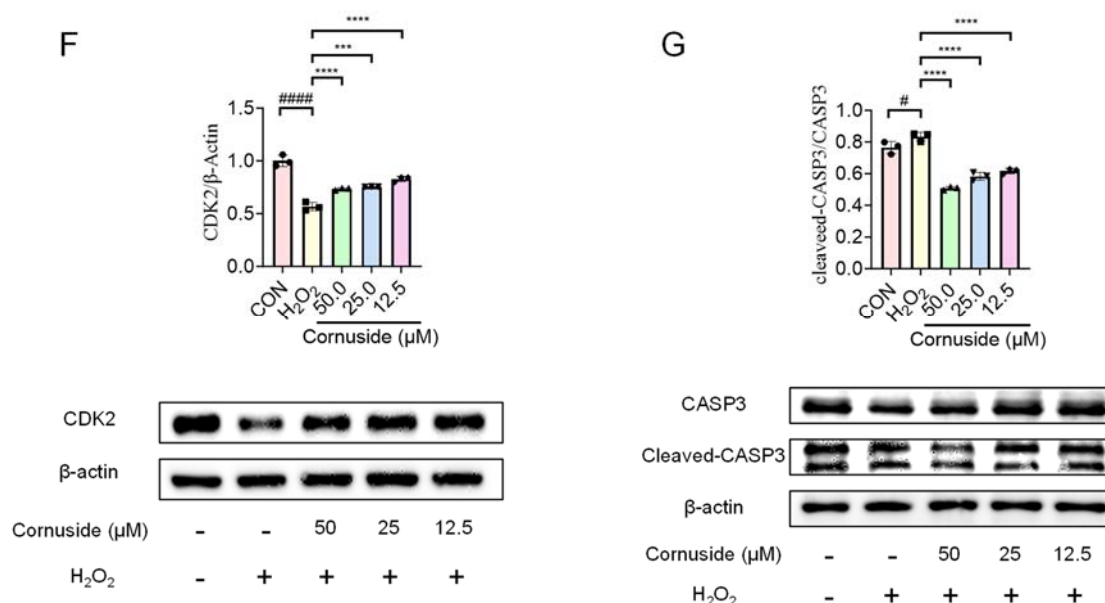


Figure 5. Cornuside reduces intracellular ROS and modulates the expression of key targets at transcriptional and translational levels in H₂O₂-induced HepG2 cells. (A) Representative fluorescence images of intracellular ROS levels detected by DCFH-DA probe. mRNA expression levels of (B) *CDK2*, (C) *CASP3*, (D) *HSP90AA1*, and (E) *HSPA8* were determined by RT-qPCR. Protein expression levels of (F) *CDK2* and (G) cleaved-*CASP3*/*CASP3* were determined by Western blot analysis. Data represent mean $\pm$ SEM. #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.005, ####*P* < 0.001 vs CON; **P* < 0.05, ***P* < 0.01, ****P* < 0.005, *****P* < 0.001 vs H₂O₂.

3.4 In Vivo Protective Effects of Cornuside against AAPH-induced Oxidative Stress in Zebrafish

Prior to evaluating in vivo efficacy, the developmental toxicity of cornuside was assessed to establish a safe pharmacological dosage range. Exposure to 200 μM cornuside severely inhibited embryonic development and significantly reduced zebrafish survival (*P* < 0.001, Fig. 6A). At 100 μM, an elevated heart beat rate was observed, indicating potential mild cardiotoxicity (Fig. 6B). However, intervention concentrations between 12.5 and 50 μM did not adversely alter the survival rate, heart beat, or yolk sac area (Fig. 6C, D), thereby confirming an optimal and safe dosage window for subsequent antioxidant evaluations. To visualize the in vivo antioxidant capacity of cornuside, an AAPH-induced oxidative stress model was employed. DCFH-DA fluorescence imaging revealed that 12.5 mM AAPH exposure provoked massive intracellular ROS accumulation in the larvae, evidenced by intense green fluorescence. Treatment with either the positive control (Vitamin C) or cornuside (12.5–50 μM) markedly attenuated this fluorescence intensity, confirming the restoration of redox homeostasis (Fig. 6E). Consistent with the ROS scavenging profile, Acridine Orange (AO) staining demonstrated widespread cell death triggered by AAPH. Cornuside pretreatment effectively suppressed this apoptotic response in a dose-dependent manner, with the 50 μM cohort exhibiting protective efficacy comparable to Vitamin C (Fig. 6F). At the transcriptional level, qPCR analysis corroborated these phenotypic observations. Compared to the AAPH-challenged model group, cornuside administration (12.5–50 μM) significantly upregulated the mRNA expression of primary antioxidant enzymes, specifically *cat*, *gpx1*, *sod1*, and *sod2* (Fig. 6G–J). Concurrently, cornuside modulated the expression of *cdk2* and *caspase3* (Fig. 6K, L). Together, these in vivo physiological and molecular findings firmly establish that cornuside mitigates oxidative damage and prevents apoptosis in zebrafish.

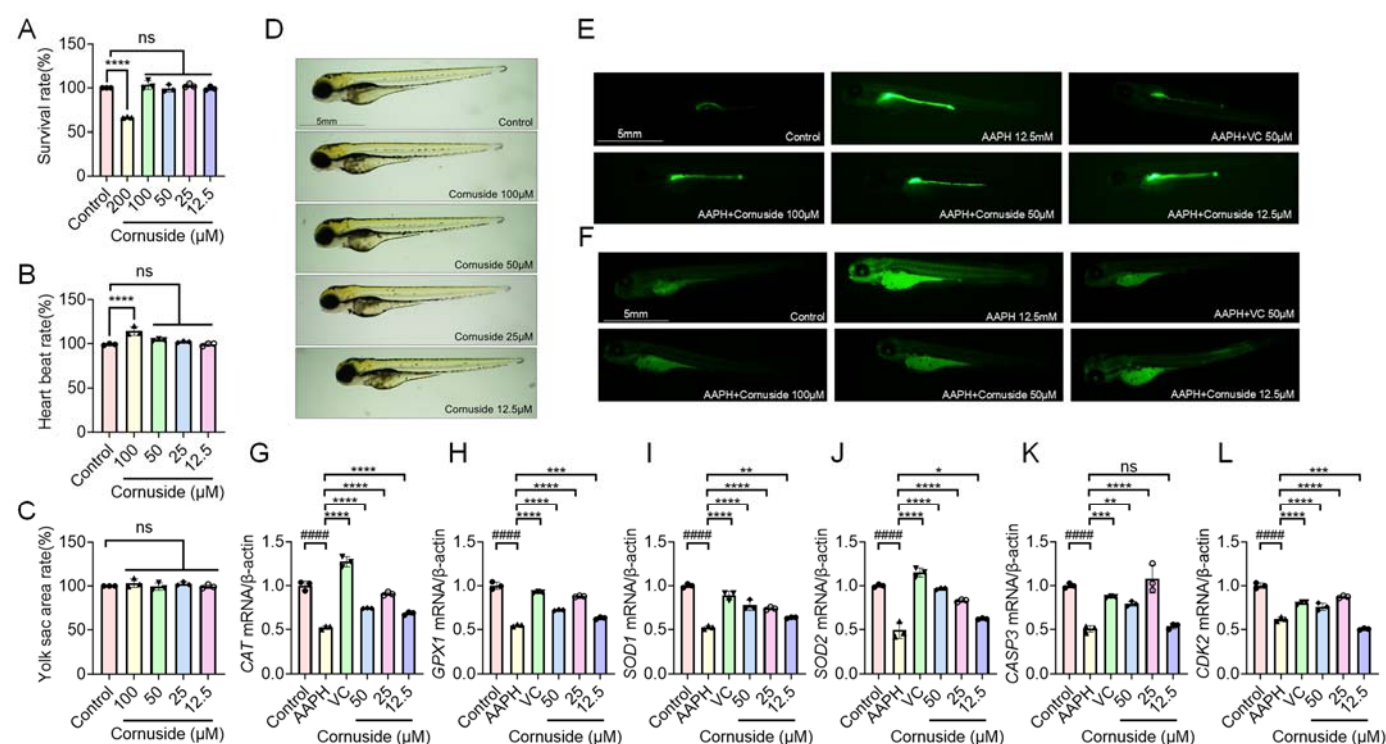


Figure 6. In vivo protective effects of cornuside against AAPH-induced oxidative stress and apoptosis in a zebrafish model. Quantitative analysis of the (A) survival rate and (B) heart beat rate of zebrafish larvae across different treatment groups. (C-D) Representative morphological images and yolk sac area of zebrafish larvae. (E) Representative fluorescence images of reactive oxygen species (ROS) accumulation in zebrafish larvae, evaluated by DCFH-DA staining. (F) Representative fluorescence images in vivo, assessed by Acridine Orange (AO) staining. (G) Relative mRNA expression levels of antioxidant genes (*sod1*, *sod2*, *cat*, *gp1*, *caspase3*, *cdk2*) in zebrafish, as determined by qRT-PCR. Data represent mean $\pm$ SEM. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.005$, #### $P < 0.001$ vs CON; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$ vs H_2O_2 .

3.5 Cornuside Alleviates Oxidative Stress by Targeting to CDK2 and Regulating The NRF2-NQO1 Pathway

NRF2 serves as a central regulator of antioxidant response, with KEAP1 as its primary repressor and NQO1 as a key downstream effector. The mRNA expression of *NRF2* and its downstream target *NQO1* was quantified via RT-qPCR. In HepG2 cells, H_2O_2 exposure significantly suppressed the transcription of *NRF2* and *NQO1* compared to the control (Fig. 7A, B). However, cornuside treatment effectively reversed this inhibition, restoring their mRNA levels in a dose-dependent manner. Consistently, this regulatory effect was mirrored in vivo; cornuside significantly upregulated the mRNA levels of *nrf2* and *nqo1* in the AAPH-challenged zebrafish model (Fig. 7C, D). Western blot analysis was performed to examine NRF2 and NQO1 protein expression and elucidate the mechanism underlying cornuside's antioxidant effects. As shown in Figures 7E and F, H_2O_2 treatment significantly reduced NRF2 and NQO1 protein levels compared to controls, indicating impaired antioxidant signaling and successful model establishment. Cornuside treatment significantly upregulated NRF2 and NQO1 protein expression compared to the model group, demonstrating its antioxidant effects are mediated through NRF2-NQO1 pathway activation.

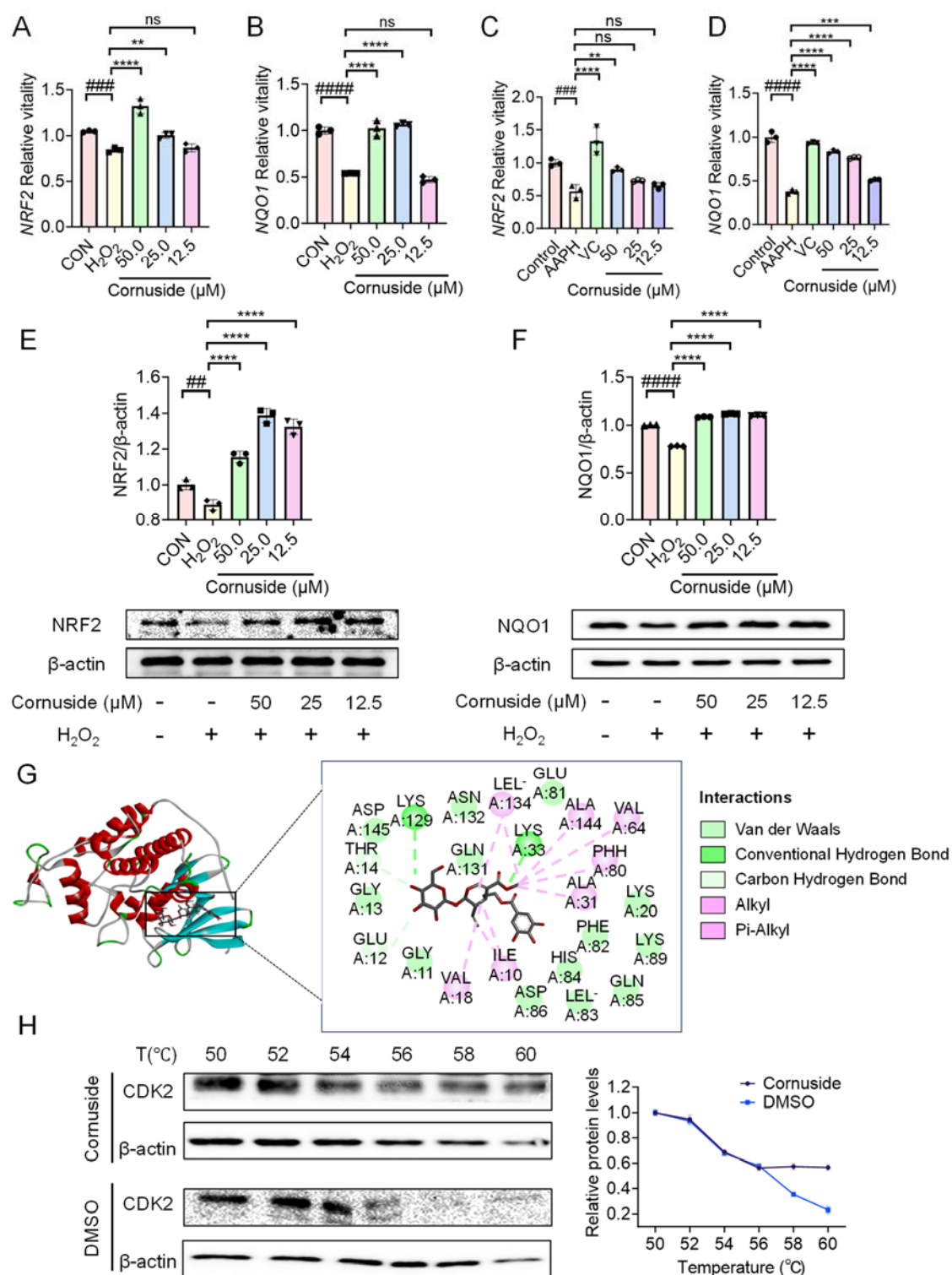


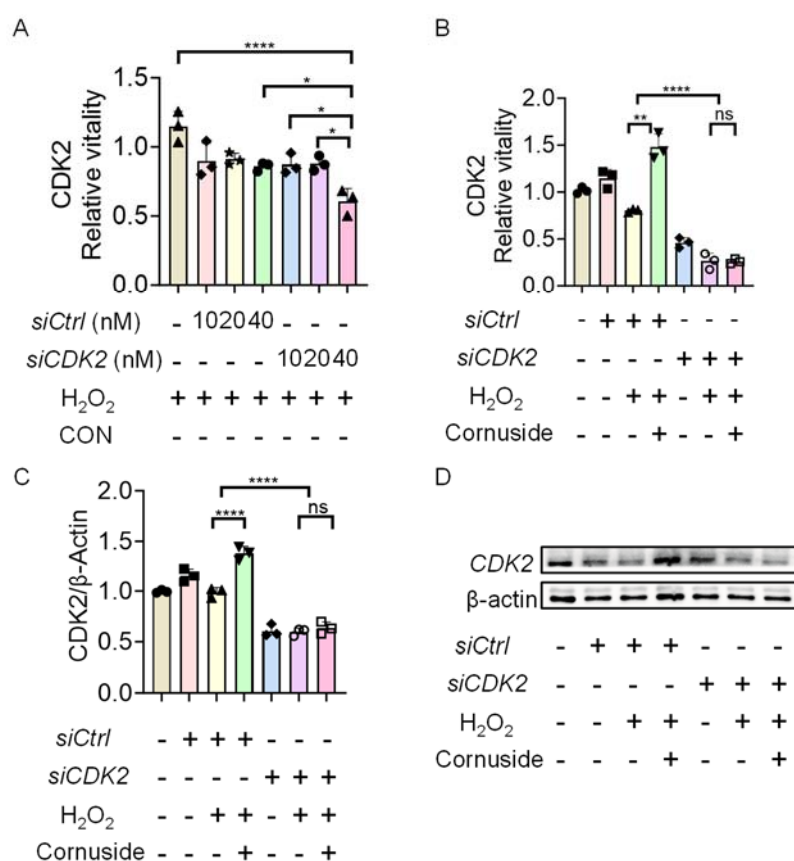
Figure 7. Cornuside feedback regulates the NRF2-NQO1 signaling pathway through direct binding to CDK2. The relative mRNA expression levels of *NRF2* and *NQO1* in (A) HepG2 cells and (B) the zebrafish model were analyzed by RT-qPCR. Protein expression levels of (E) *NRF2* and (F) *NQO1* were determined by Western blot. (G) The docking diagrams for CDK2 protein with cornuside. (H) Cellular thermal shift assay (CETSA) assays confirmed the binding of cornuside to CDK2 in HepG2 cells, with β-Actin serving as the internal control. Data represent mean ± SEM. #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.005, ####*P* < 0.001 vs CON; **P* < 0.05, ***P* < 0.01, ****P* < 0.005, *****P* < 0.001 vs H₂O₂.

Firstly, a preliminary screening was conducted based on the centrality of the protein-protein interaction (PPI) network. Further focus was placed on its characteristic as a common target of multiple components, and with reference to its known association with the NRF2 pathway, CDK2 was finally selected for experimental validation. Molecular docking using AutoDock revealed a binding energy of -9.3 kcal/mol

between cornuside and CDK2, indicating strong binding affinity and supporting CDK2 as a potential target of cornuside. The binding mode was visualized using PyMOL 2.5. Analysis revealed that cornuside forms van der Waals contacts with Thr14, hydrogen bonds with Lys33 and Lys129, and hydrophobic interactions with Ile10, Val18, Ala31, Val64, Phe80, Leu134, and Ala144 of CDK2 (Fig. 7G). These interactions demonstrate strong binding affinity between cornuside and CDK2. CETSA detects ligand-target engagement by measuring protein thermal stabilization upon binding. To experimentally validate direct binding, we performed CETSA. CETSA results (Fig. 7H) showed significantly higher CDK2 stability in cornuside-treated samples compared to DMSO controls at 50–59°C, confirming direct target engagement. Collectively, these results demonstrate that cornuside alleviates oxidative stress in HepG2 cells through direct binding to CDK2 and subsequent activation of the NRF2-NQO1 signaling pathway.

3.6 CDK2 is Required for the Antioxidant Activity of Cornuside.

To further examine if the antioxidant effects of cornuside was mediated by CDK2, we knockdown CDK2 in HepG2 cells. HepG2 cells were transfected with CDK2-specific siRNA. After 24 h, CDK2 knockdown efficiency was verified by RT-qPCR and Western blot analysis. As shown in Figures 8A–D, CDK2-specific siRNA (40 nM) effectively knocked down CDK2 expression, as evidenced by significantly reduced mRNA and protein levels compared to the negative control (NC) siRNA group ($P < 0.01$). Cornuside treatment failed to restore CDK2 expression in CDK2-knockdown cells. Accordingly, CDK2 knockdown also suppressed the expression of oxidative stress-related factors NRF2, NQO1, and CAT, and cornuside failed to rescue their expression (Fig. 8E–G). These results demonstrate that the antioxidant effects of cornuside are dependent on CDK2, through which it reduces oxidative stress in HepG2 cells.



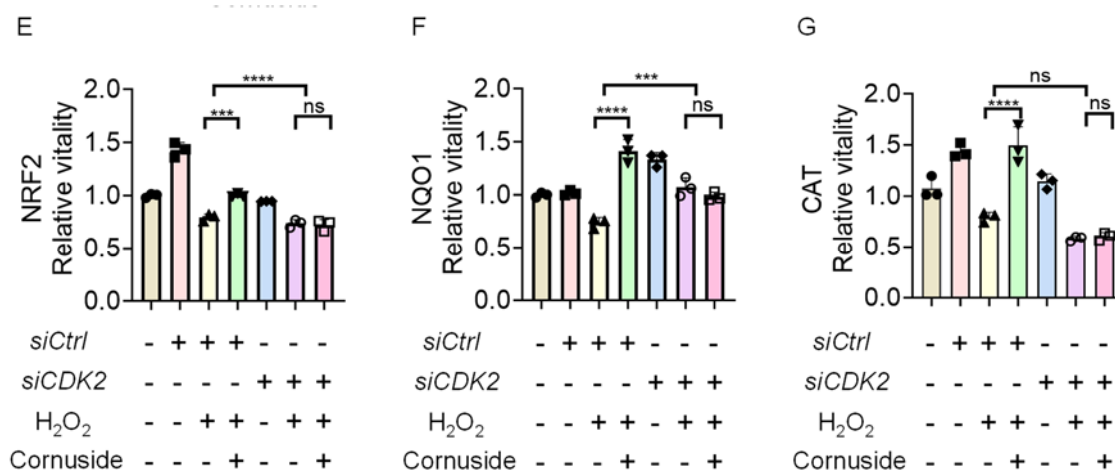


Figure 8. The antioxidant effect of cornuside depends on CDK2. (A) Screening of CDK2 siRNA transfection concentrations in HepG2 cells. (B) After CDK2 knockdown, the restorative effects of cornuside on the mRNA expression of CDK2 was abolished. (C-D) Western blot analysis assay and quantitative results of CDK2-knockdown cells. CDK2 knockdown also prevented cornuside from upregulating the mRNA expression of downstream antioxidant factors (E) NRF2, (F) NQO1, and (G) CAT.

4. Discussion

While the pathogenesis of oxidative stress is clear, its role as a key driver in the processes of ageing and disease is widely recognized by accumulating evidence[22]. *Cornus officinalis* is a medicinal and edible plant possessing diverse bioactive compounds. It is rich in iridoid glycosides, triterpenoids, and polysaccharides, which exhibit beneficial effects such as hypoglycemic activity, immune enhancement, anti-tumor, anti-inflammatory, antibacterial, and antioxidant activities. In this study, we elucidated the mechanism by which cornuside alleviates oxidative stress through an integrated strategy combining spectrum-effect relationship analysis, metabolomics, and network pharmacology.

The spectrum-effect relationship approach integrates mathematical modeling with pharmacological experimentation. This methodology analyzes chemical fingerprints of traditional Chinese medicine in conjunction with pharmacological data to identify bioactive components, establishing correlations between chemical composition and bioactivity through experimental validation[23]. In this study, we investigated the spectrum-effect relationship of *Cornus officinalis* extracts by combining HPLC fingerprinting with ABTS radical scavenging activity assessment. HPLC analysis revealed similar chromatographic profiles across 13 batches from different regions, with 12 consistent common peaks. Cluster analysis and PCA provided a comprehensive evaluation of the chemical composition of *Cornus officinalis*. Similarly, Liu et al. integrated PCA with cluster analysis to identify ferulic acid glycosides and coumaric acid as key tyrosinase-active components in crab apple[24]. Integrated analysis of clustering, PCA, and ABTS scavenging activity indicated that samples from Liuzhong Village in the Funiu Mountains exhibited superior quality. This region provides optimal growing conditions for *Cornus officinalis*, including suitable shade, temperature, and well-drained sandy loam soil. Seven peaks (F2, F3, F4, F7, F9, F10, F12) were predicted to positively contribute to radical scavenging activity. In recent years, most studies on the chemical components of TCM have focused on reducing component complexity and investigating single components for the development

of new drugs. However, given the diverse chemical components in medicinal plants, their pharmacodynamic effects are exerted through multi-component and multi-target interactions; therefore, the interactions between components also play an important role[25]. Shi et al. used component knockout technology to verify the spectrum-effect relationship results of active compounds on tyrosinase from the fruits of *Psoralea corylifolia* L., confirming that daidzein and neobavaisoflavone could activate tyrosinase[26]. Our component-knockout validation confirmed that kingiside, cornin, and cornuside significantly enhanced antioxidant activity compared to negative controls, corroborating the spectrum-effect predictions and underscoring the importance of component interplay.

Untargeted metabolomics facilitates large-scale, semi-quantitative analysis of measurable analytes in biological samples[27], employing high-throughput technologies to generate comprehensive metabolic fingerprints with maximal coverage[28]. Luo et al. conducted metabolomic screening of *Ophiopogon japonicus* and *Liriope spicata*, identifying isoflavones and steroidal saponins as key discriminatory markers between these species[29]. Meanwhile, metabolomic data provide a basis for studying the functional effects of active components in traditional Chinese medicine (TCM)[30]. Wirginia et al. performed metabolomic analysis of turmeric curcuminoids, demonstrating that three curcuminoid compounds act synergistically to inhibit cancer cell proliferation[31]. Deyani et al. analyzed metabolomic profiles of kiwifruit leaf extracts, identifying ursolic acid and stearic acid in methanol fractions as participants in apoptotic processes[32]. Its application to the systematic antioxidant evaluation of *C. officinalis* has remained limited. In this study, LC-MS/MS-based untargeted metabolomics identified a total of 82 compounds from *C. officinalis* extracts, providing a comprehensive chemical inventory for subsequent network pharmacology analysis.

Network pharmacology leverages large-scale interaction networks (disease-gene-protein-compound) to enable efficient target prediction and mechanism exploration[33-35]. This approach has been successfully applied to predict targets for *Ilex pubescens* against thromboangiitis obliterans[36] and to elucidate cantharidin-induced nephrotoxicity pathways[37]. Given the multi-component, multi-target nature of TCM, network pharmacology is particularly well-suited to identify bioactive compounds and disease-related targets from large-scale datasets[38]. By integrating untargeted metabolomics with network pharmacology, we constructed component-target, oxidative stress-target, and PPI networks, identifying 55 antioxidant compounds and 13 key targets (PGR, TNF, MAPK1, CDK2, ESR1, GSK3B, ESR2, HSPA8, HSP90AA1, CASP3, PPARG, and EGFR).

Elevated ROS levels cause oxidative damage to biomacromolecules, disrupt membrane integrity, impair enzymatic function, and induce DNA damage. The biological antioxidant enzyme system in the human body, including SOD, CAT, MDA, and GSH, plays a crucial role in reducing ROS levels and mitigating oxidative damage to cells and tissues[39]. Our mechanistic investigation focused on the NRF2-KEAP1 axis, a central pathway in oxidative stress defense.

Under normal redox conditions, KEAP1 binds to NRF2, leading to the inactivation of NRF2. Under oxidative stress, NRF2 dissociates from KEAP1 and translocates to the nucleus to activate antioxidant gene

expression[40]. Multiple iridoid components have been reported to activate the NRF2 pathway: geniposide alleviates oxidative damage in rat retinas[41]; aucubin attenuates neurological damage in cerebral ischemia-reperfusion injury[42]; and catalpol promotes NRF2 expression to mitigate ferroptosis-induced neurotoxicity[43, 44]. These findings suggest that NRF2 may be a high-efficiency target for iridoid components. Previous studies have also demonstrated that *C. officinalis* constituents, including ursolic acid, betulinic acid, and monotropein, promote NRF2 expression[45]. In this study, we confirmed that cornuside, a characteristic iridoid unique to *C. officinalis*, alleviates oxidative stress via the NRF2-NQO1 pathway, as verified by RT-qPCR and Western blot analysis, thereby establishing a mechanistic basis for its antioxidant activity that extends beyond previous structural characterizations.

CDK2 is a cyclin-dependent kinase that plays a vital regulatory role in the cell proliferation cycle[46]. CDK2 promotes the transition of cells from the G1 phase to the S phase during cell division and is degraded at the end of the S phase to facilitate the next stage of the cell cycle. Under oxidative stress, CDK2 expression is suppressed concomitant with NRF2 downregulation. After promoting NRF2 expression via drug administration, CDK2 expression is restored, and oxidative stress symptoms are alleviated[47]. Knockdown experiments on NRF2 and NQO1 have confirmed that NQO1 can inhibit tumor cells and alleviate oxidative stress by regulating CDK2[48]. Shi et al. reported that geniposide from *Gardenia jasminoides* promotes CDK2 expression to exert antithrombotic effects in zebrafish[49]. Given the structural similarity between cornuside and geniposide, we investigated CDK2 as a potential direct target. Our results demonstrate that cornuside directly binds CDK2 in HepG2 cells and modulates the expression of NRF2 and NQO1, revealing a novel target for cornuside activity and providing a molecular link between iridoid structure and antioxidant function.

5. Conclusion

In conclusion, this study systematically identifies cornuside as the principal antioxidant and anti-apoptotic constituent of *Cornus officinalis*. Moving beyond the traditional evaluation of crude extracts, we established a rigorous pharmacological evidence chain. Our findings unequivocally demonstrate that cornuside mitigates oxidative damage both in vitro (HepG2 cells) and in vivo (zebrafish model) by directly engaging the CDK2 protein. This direct physical binding, validated by CETSA, serves as a molecular switch that simultaneously activates the NRF2/NQO1 antioxidant defense axis and suppresses CASP3-mediated apoptosis (Fig. 9). Crucially, the complete abolishment of these protective effects following CDK2 knockdown confirms that cornuside acts in a strictly CDK2-dependent manner. Collectively, this work not only elucidates the definitive material basis and precise molecular targets of *Cornus officinalis* against oxidative stress but also highlights cornuside as a promising naturally derived candidate for the management of oxidative stress-related pathologies.

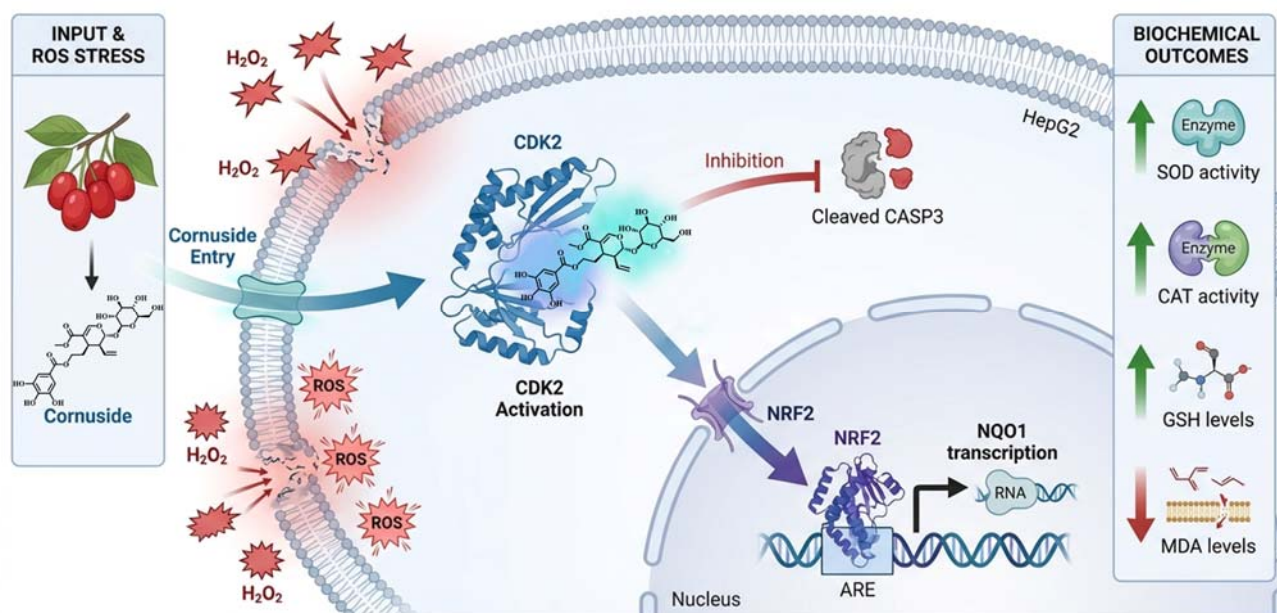


Figure 9. The molecular mechanism of cornuside from *Cornus officinalis* in alleviating oxidative stress and apoptosis.

Declaration of competing interest

We declare that we have no financial or personal relationships with individuals or organizations that could inappropriately influence our work. There are no professional or personal interests in any product, service, or company that could be perceived as influencing the position presented in the manuscript or the review thereof.

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Data availability

The data will be made available upon request.

Appendix A. Supplementary data

Supplemental information can be found online.

References

- [1] C. Vidjeyamannane, A. Joy, K. Prakash, et al., A comprehensive review on the role of plant-derived bioactive metabolites driving ROS-mediated apoptosis in cancer, *Medical Oncology* 42 (2025) 420.<http://doi.org/10.1007/s12032-025-02985-x>.
- [2] D. Przybylska, A.Z. Kucharska and T. Sozański, A Review on Bioactive Iridoids in Edible Fruits – from Garden to Food and Pharmaceutical Products, *Food Reviews International* 39 (2023) 6447-6477.<http://doi.org/10.1080/87559129.2022.2117375>.
- [3] X. Gao, Y. Liu, Z. An, et al., Active Components and Pharmacological Effects of *Cornus officinalis*: Literature Review, *Front Pharmacol* 12 (2021) 633447.<http://doi.org/10.3389/fphar.2021.633447>.
- [4] X. Zhang, H. Zhang, P. Jiao, et al., Preparation and Evaluation of Antioxidant Activities of Bioactive Peptides Obtained from *Cornus officinalis*, *Molecules* 27 (2022) 1232

- [5] H. Yang, H. Liu, Y. Zheng, et al., Cornus Officinalis Total Glycosides Alleviate Granulomatous Lobular Mastitis via the B7-CD28/CTLA-4 Costimulatory Pathway, *Chemistry & Biodiversity* 22 (2025) e202401539.<http://doi.org/https://doi.org/10.1002/cbdv.202401539>.
- [6] L.L. Ji, X. Wang, J.J. Li, et al., New Iridoid Derivatives from the Fruits of Cornus officinalis and Their Neuroprotective Activities, *Molecules* 24 (2019).<http://doi.org/10.3390/molecules24030625>.
- [7] N. Yamabe, K.S. Kang, E. Goto, et al., Beneficial effect of Corni Fructus, a constituent of Hachimi-jio-gan, on advanced glycation end-product-mediated renal injury in Streptozotocin-treated diabetic rats, *Biol Pharm Bull* 30 (2007) 520-526.<http://doi.org/10.1248/bpb.30.520>.
- [8] C.H. Park, J.S. Noh, T. Tanaka, et al., The effects of corni fructus extract and its fractions against α -glucosidase inhibitory activities in vitro and sucrose tolerance in normal rats, *Am J Chin Med* 39 (2011) 367-380.<http://doi.org/10.1142/s0192415x11008889>.
- [9] Y.-P. Hao, L. Wang, Q.-W. Li, et al., Unraveling the functional components in medicinal Aloe species based on UPLC-MS/MS metabolomics, *Food Med. Homol.*, DOI: 10.26599/FMH.2026.9420098 (2025).<http://doi.org/10.26599/FMH.2026.9420098>.
- [10] X. Liu, X. Meng, D. Liu, et al., A rapid analysis method to discover antioxidant active components and mechanisms in soybean: untargeted metabolomics combined with network pharmacology and spectrum-effect relationship-component knockout-identification techniques, *Food Sci. Hum. Wellness* 14 (2025) 9250620.<http://doi.org/10.26599/FSHW.2025.9250620>.
- [11] D. Jiang, J. Xu, S. Liu, et al., Rosmanol induces breast cancer cells apoptosis by regulating PI3K/AKT and STAT3/JAK2 signaling pathways, *Oncol. Lett.* 22 (2021) 631.<http://doi.org/10.3892/ol.2021.12892>.
- [12] Q. Wang, S. Wang, L. Cui, et al., Flammulina velutipes polysaccharide exerts immunomodulatory function involving RSAD2 to regulate the NF- κ B/MAPK signaling pathway in RAW264.7 macrophage cells and in mouse spleen cells, *Int. J. Biol. Macromol.* 309 (2025) 142985.<http://doi.org/10.1016/j.ijbiomac.2025.142985>.
- [13] Z. Liang, Q. Tang, H. Liang, et al., Glucomannogalactan inhibits senescence by promoting nuclear translocation of NRF2, *Int J Biol Macromol* 305 (2025) 141059.<http://doi.org/10.1016/j.ijbiomac.2025.141059>.
- [14] Q. Tang, Z.-H. Liang, J.-J. Yuan, et al., FER1L6 ameliorates insulin resistance by regulating GLUT4 expression, *Food Med. Homol.* 2 (2025) 9420070.<http://doi.org/10.26599/FMH.2025.9420070>.
- [15] Z.-H. Liu, B.-B. Yu, H.-H. Zhou, et al., Characteristic components from Rehmannia radix and their effects on insulin resistance through PI-3K/AKT signaling pathway in HepG2 Cells, *Food Med. Homol.* 2 (2025) 9420073.<http://doi.org/10.26599/FMH.2025.9420073>.
- [16] Y. Zheng, Y. Yu, J. Feng, et al., Unveiling the Potential of Nelumbo nucifera-Derived Liensinine to Target The Myostatin Protein and to Counteract Muscle Atrophy, *J. Agric. Food Chem.* 72 (2024) 2240-2249.<http://doi.org/10.1021/acs.jafc.3c09002>.
- [17] L.-L. Gao, X.-T. Fang, S.-H. Zhao, et al., Naphthoquinone Derivatives from the Endophytic Fungus Fusarium solani Induce Pancreatic Cancer Cells Apoptosis via Targeting EGFR-Mediated PI3K/Akt Pathway, *J. Agric. Food Chem.* 72 (2024) 26209-26223.<http://doi.org/10.1021/acs.jafc.4c08652>.
- [18] Y. Dong, Z.L. Feng, H.B. Chen, et al., Corni Fructus: a review of chemical constituents and pharmacological activities, *Chin Med* 13 (2018) 34.<http://doi.org/10.1186/s13020-018-0191-z>.
- [19] H. Cai, G. Cao and B. Cai, Rapid simultaneous identification and determination of the multiple compounds in crude Fructus Corni and its processed products by HPLC-MS/MS with multiple reaction monitoring mode, *Pharm Biol* 51 (2013) 273-278.<http://doi.org/10.3109/13880209.2012.720689>.
- [20] Y.-J. Qian, W.-X. Pi, G.-F. Zhu, et al., Quality evaluation of raw and processed Corni Fructus by UHPLC-QTOF-MS and HPLC coupled with color determination, *Journal of Pharmaceutical and Biomedical Analysis* 218 (2022) 114842.<http://doi.org/https://doi.org/10.1016/j.jpba.2022.114842>.
- [21] M.V. Deligiorgi, C. Liapi and D.T. Trafalis, How Far Are We from Prescribing Fasting as Anticancer Medicine?, *Int J Mol Sci* 21 (2020).<http://doi.org/10.3390/ijms21239175>.
- [22] M.-Y. Li, G.-F. Wu, F. Lu, et al., Food and Medicine Homology Focus in 2026, *Food Med. Homol.* 3 (2026) 9420133.<http://doi.org/10.26599/FMH.2026.9420133>.

- [23] C. Li, Y. Cui, J. Lu, et al., Spectrum-effect relationship of immunologic activity of *Ganoderma lucidum* by UPLC-MS/MS and component knock-out method, Food Science and Human Wellness 10 (2021) 278-288.<http://doi.org/https://doi.org/10.1016/j.fshw.2021.02.019>.
- [24] W. Li, Y. Zhang, S. Shi, et al., Spectrum-effect relationship of antioxidant and tyrosinase activity with *Malus pumila* flowers by UPLC-MS/MS and component knock-out method, Food and Chemical Toxicology 133 (2019) 110754.<http://doi.org/https://doi.org/10.1016/j.fct.2019.110754>.
- [25] C. Liu, C. Ma, J. Lu, et al., A rapid method and mechanism to identify the active compounds in *Malus micromalus* Makino fruit with spectrum-effect relationship, components knock-out and molecular docking technology, Food and Chemical Toxicology 150 (2021) 112086.<http://doi.org/https://doi.org/10.1016/j.fct.2021.112086>.
- [26] M. Shi, Y. Zhang, M. Song, et al., Screening the Marker Components in *Psoralea corylifolia* L. with the Aids of Spectrum-Effect Relationship and Component Knock-Out by UPLC-MS2, International Journal of Molecular Sciences 19 (2018) 3439
- [27] J. Wang, L. Guo, X. Hao, et al., Nontargeted metabolomics combining with intestinal microbiota and hypolipidemia targets elucidates anti-hyperlipidemia effect of Sangju yinzha tea in hyperlipidemia rats, Food Biosci. 65 (2025) 106012.<http://doi.org/10.1016/j.fbio.2025.106012>.
- [28] L. Mattoli, M. Gianni and M. Burico, Mass spectrometry-based metabolomic analysis as a tool for quality control of natural complex products, Mass Spectrom Rev 42 (2023) 1358-1396.<http://doi.org/10.1002/mas.21773>.
- [29] H. Luo, L.S. Ming, T.T. Tong, et al., Chemical Comparison of *Ophiopogonis radix* and *Liriope radix* Based on Quantitative Analysis of Multiple Components by HPLC Coupled with Electrospray Ionization Tandem Triple Quadrupole Mass Spectrometry, J AOAC Int 103 (2020) 1148-1159.<http://doi.org/10.1093/jaoacint/qsaa010>.
- [30] O.R. Oyenih, A.B. Oyenih, J.O. Erhabor, et al., Unravelling the Anticancer Mechanisms of Traditional Herbal Medicines with Metabolomics, Molecules 26 (2021).<http://doi.org/10.3390/molecules26216541>.
- [31] W. Kukula-Koch, A. Grabarska, J. Łuszczki, et al., Superior anticancer activity is demonstrated by total extract of *Curcuma longa* L. as opposed to individual curcuminoids separated by centrifugal partition chromatography, Phytother Res 32 (2018) 933-942.<http://doi.org/10.1002/ptr.6035>.
- [32] D. Nocedo-Mena, M.Y. Ríos, M.Á. Ramírez-Cisneros, et al., Metabolomic Profile and Cytotoxic Activity of *Cissus incisa* Leaves Extracts, Plants 10 (2021) 1389
- [33] L. Xu, Y. Zhang, P. Zhang, et al., Integrated Metabolomics and Network Pharmacology Strategy-Driven Active Traditional Chinese Medicine Ingredients Discovery for the Alleviation of Cisplatin Nephrotoxicity, Chemical Research in Toxicology 32 (2019) 2411-2421.<http://doi.org/10.1021/acs.chemrestox.9b00180>.
- [34] R.-l. Zhao and Y.-m. He, Network pharmacology analysis of the anti-cancer pharmacological mechanisms of *Ganoderma lucidum* extract with experimental support using Hepa1-6-bearing C57 BL/6 mice, Journal of Ethnopharmacology 210 (2018) 287-295.<http://doi.org/https://doi.org/10.1016/j.jep.2017.08.041>.
- [35] L. Chen, Y. Cao, H. Zhang, et al., Network pharmacology-based strategy for predicting active ingredients and potential targets of Yangxinshi tablet for treating heart failure, J Ethnopharmacol 219 (2018) 359-368.<http://doi.org/10.1016/j.jep.2017.12.011>.
- [36] J. Chen, Y. Wang, C. Chen, et al., Integrated network pharmacology and metabolomics reveal vascular protective effects of *Ilex pubescens* on thromboangiitis obliterans, Phytomedicine 130 (2024) 155720.<http://doi.org/10.1016/j.phymed.2024.155720>.
- [37] T. He, L. Xiong, Y. Zhang, et al., Mice kidney biometabolic process analysis after cantharidin exposure using widely-targeted metabolomics combined with network pharmacology, Food and Chemical Toxicology 171 (2023) 113541.<http://doi.org/https://doi.org/10.1016/j.fct.2022.113541>.
- [38] W.U. Jiashuo, Z. Fangqing, L.I. Zhuangzhuang, et al., Integration strategy of network pharmacology in Traditional Chinese Medicine: a narrative review, J Tradit Chin Med 42 (2022) 479-486.<http://doi.org/10.19852/j.cnki.jtcm.20220408.003>.
- [39] D. Zhang, Y. Wang, M. Xu, et al., Antioxidant Synergetic Effect Between the Peptides Derived from the Egg White Pentapeptide Trp-Asn-Trp-Ala-Asp, International Journal of Peptide Research and Therapeutics 23 (2017) 509-518.<http://doi.org/10.1007/s10989-017-9585-5>.

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- [40] J.W. Shin, K.S. Chun, D.H. Kim, et al., Curcumin induces stabilization of Nrf2 protein through Keap1 cysteine modification, *Biochem Pharmacol* 173 (2020) 113820.<http://doi.org/10.1016/j.bcp.2020.113820>.
- [41] Y. Koriyama, K. Chiba, M. Yamazaki, et al., Long-acting genipin derivative protects retinal ganglion cells from oxidative stress models in vitro and in vivo through the Nrf2/antioxidant response element signaling pathway, *J Neurochem* 115 (2010) 79-91.<http://doi.org/10.1111/j.1471-4159.2010.06903.x>.
- [42] F. Yang, Q. Lian, X. Zhang, et al., Aucubin provides protection against cerebral ischaemia-reperfusion injury by suppressing neuronal apoptosis, oxidative stress, and inflammation through the modulation of the AKT-GSK-3 β -Nrf2 signal cascade, *Toxicol Appl Pharmacol* 483 (2024) 116829.<http://doi.org/10.1016/j.taap.2024.116829>.
- [43] J. Lin, L. Deng, A. Qi, et al., Catalpol alleviates hypoxia ischemia-induced brain damage by inhibiting ferroptosis through the PI3K/NRF2/system Xc-/GPX4 axis in neonatal rats, *European Journal of Pharmacology* 968 (2024) 176406.<http://doi.org/https://doi.org/10.1016/j.ejphar.2024.176406>.
- [44] Z.-H. Liu, W.-J. Guo, X.-J. Meng, et al., Exploration strategies for food and medicine homologous resources: future direction in food development, *Food Med. Homol.*, DOI: 10.26599/FMH.2026.9420134 (2026).<http://doi.org/10.26599/FMH.2026.9420134>.
- [45] K.A. Hwang, Y.J. Hwang and J. Song, Antioxidant activities and oxidative stress inhibitory effects of ethanol extracts from *Cornus officinalis* on raw 264.7 cells, *BMC Complement Altern Med* 16 (2016) 196.<http://doi.org/10.1186/s12906-016-1172-3>.
- [46] R. Fagundes and L.K. Teixeira, Cyclin E/CDK2: DNA Replication, Replication Stress and Genomic Instability, *Front Cell Dev Biol* 9 (2021) 774845.<http://doi.org/10.3389/fcell.2021.774845>.
- [47] J.J. Ren, X.W. Yuan, Z.L. Meng, et al., Diosmetin Promotes Early Embryonic Development in Pigs by Alleviating Oxidative Stress, *Mol Reprod Dev* 91 (2024) e23775.<http://doi.org/10.1002/mrd.23775>.
- [48] Q. Kong, H. Deng, C. Li, et al., Sustained high expression of NRF2 and its target genes induces dysregulation of cellular proliferation and apoptosis is associated with arsenite-induced malignant transformation of human bronchial epithelial cells, *Sci Total Environ* 756 (2021) 143840.<http://doi.org/10.1016/j.scitotenv.2020.143840>.
- [49] Y.-p. Shi, Y.-g. Zhang, H.-n. Li, et al., Discovery and identification of antithrombotic chemical markers in *Gardenia Fructus* by herbal metabolomics and zebrafish model, *Journal of Ethnopharmacology* 253 (2020) 112679.<http://doi.org/https://doi.org/10.1016/j.jep.2020.112679>.