

ORIGINAL RESEARCH ARTICLE

Bugbanosides from *Cimicifuga racemosa* target cyclin-dependent kinase 1 degradation to trigger PANoptosis in liver cancerQiang Fu¹, Yanyuan Qiao¹, Rong Lu², Yiwen Ding³, Xuemei Lin⁴, Yufan Bai¹,
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Abstract

Introduction: Liver cancer is a leading cause of cancer-related deaths worldwide, and novel natural-product-based therapeutic strategies are urgently needed. *Cimicifuga racemosa* is a medicinal plant, and its active components exhibit anti-cancer potential. However, the molecular targets and mechanisms of action in liver cancer remain poorly defined.**Objective:** We applied network pharmacology analysis to identify key components of *C. racemosa* that regulate pyroptosis, apoptosis, and necroptosis (PANoptosis) in liver cancer.**Methods:** Transcriptomic data analysis, molecular docking, and experimental approaches were employed to identify cyclin-dependent kinase 1 (CDK1) as a potential target of *C. racemosa* components. The effects of *C. racemosa* extracts and purified compounds on CDK1 activity, cell viability, and PANoptosis induction were evaluated in liver cancer cells.**Results:** Bugbanosides A, C, and F from *C. racemosa* were identified as potential CDK1 inhibitors through molecular docking. *C. racemosa* extracts and these bugbanosides dose-dependently inhibited CDK1 activity, destabilized CDK1 protein, and induced PANoptosis in HepG2 cells, as evidenced by the activation of apoptotic, pyroptotic, and necroptotic markers. Overexpression of CDK1 partially rescued the PANoptosis-inducing effects.**Conclusion:** Our study identifies CDK1 as a target of bioactive components from *C. racemosa* and demonstrates that these components induce PANoptosis in liver cancer cells, suggesting their potential as novel anti-cancer agents for liver cancer treatment.**Keywords:** Liver cancer; *Cimicifuga racemosa*; PANoptosis; Cyclin-dependent kinase 1; Bugbanosides***Corresponding author:**Yong Zhang
(18787038358@163.com)**Citation:** Fu Q, Qiao Y, Lu R, et al. Bugbanosides from *Cimicifuga racemosa* target cyclin-dependent kinase 1 degradation to trigger PANoptosis in liver cancer. *Eurasian J Med Oncol*. doi: 10.36922/EJMO026110127**Received:** March 13, 2026**Revised:** May 7, 2026**Accepted:** May 15, 2026**Published online:** July 13, 2026**Copyright:** © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.**Publisher's Note:** AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Liver cancer is a leading cause of cancer-related deaths worldwide, and hepatocellular carcinoma is the most common form of primary liver cancer.¹ Despite continuous advances in early diagnosis and clinical treatment, the overall prognosis of liver cancer patients remains unsatisfactory, mainly due to high rates of postoperative recurrence, distant metastasis, and resistance to conventional chemotherapy and targeted therapy, emphasizing the need for more effective treatment strategies.^{2,3} Therefore, exploring safe and effective therapeutic agents and elucidating their molecular mechanisms are of great significance for improving the clinical outcomes of liver cancer. Traditional herbal medicines have garnered significant interest as potential sources of anti-cancer agents, owing to their long historical use and unique phytochemical compositions.^{4,5}

Cimicifuga racemosa, also known as black cohosh, is a perennial plant native to North America, widely used in traditional medicine to alleviate menopausal symptoms.^{6,7} In recent years, numerous studies have demonstrated the anti-cancer potential of *C. racemosa* extracts and its active components, including triterpene glycosides (TGs) (e.g., actein, cimicifugoside, and bugbanoside compounds).⁸⁻¹¹ *C. racemosa* extracts have been reported to exhibit cytotoxic effects against various cancer cell lines, including breast, prostate, and liver cancer.⁸⁻¹³ The exploration of the medicinal potential and the molecular targets of *C. racemosa* extracts offers novel perspectives for liver cancer management. However, the specific active components, direct molecular targets, and detailed mechanisms underlying the anti-cancer effects of *C. racemosa* in liver cancer have not been fully elucidated.

Cancer cells can undergo different modes of regulated cell death (RCD), including apoptosis, necroptosis, pyroptosis, and ferroptosis.^{14,15} The simultaneous induction of multiple cell death pathways (pyroptosis, apoptosis, and necroptosis), termed PANoptosis, has emerged as a promising therapeutic strategy to overcome resistance mechanisms and enhance cancer cell elimination. PANoptosis is a unique inflammatory RCD pathway that integrates components of apoptosis, pyroptosis, and necroptosis and is regulated by the PANoptosome complex. Inducing PANoptosis can effectively overcome tumor drug resistance and enhance the killing effect on cancer cells, making it a promising strategy for cancer therapy.^{16,17} Compounds capable of triggering PANoptosis by targeting key regulatory nodes could potentially provide more effective and durable anti-cancer effects.^{18,19}

In our study, we employed a systematic approach integrating transcriptomic data analysis, network pharmacology, molecular docking, and experimental

validation to identify cyclin-dependent kinase 1 (CDK1) as a potential target of bioactive components from *C. racemosa* in the regulation of PANoptosis in liver cancer cells. Our findings suggest that specific bugbanoside compounds from *C. racemosa* can inhibit CDK1 activity and induce PANoptosis, highlighting their potential as novel anti-cancer agents for liver cancer treatment.

2. Methods

2.1. Transcriptome and The Cancer Genome Atlas data analysis

Gene expression data of liver cancer (GSE101685) were obtained from the Gene Expression Omnibus (GEO) database. The Cancer Genome Atlas (TCGA) data for liver cancer were retrieved from the TCGA data portal (<https://portal.gdc.cancer.gov/>). Analysis of transcriptome data was conducted using the limma package in R (v4.1.3, R Foundation for Statistical Computing, Austria), with a threshold of absolute Log2 (fold change) > 1 and adjusted *p*-value < 0.05 to identify differentially expressed genes (DEGs). The protein-protein interaction (PPI) network was generated using protein interaction data from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. The Cytohubba module in Cytoscape (3.9.0, Newman Lab, United States) was used to identify key proteins in the network.

2.2. Retrieval of active component data from *C. racemosa*

The active ingredients in *C. racemosa* were screened using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM), and Traditional Chinese Medicine Integrated Database (TCMID) databases, with a focus on drug-likeness values ≥ 0.18 and oral bioavailability values $\geq 30\%$. Gene targets associated with PANoptosis were obtained from the GeneCards, Online Mendelian Inheritance in Man, and Therapeutic Target Database databases.

2.3. Molecular docking

The two-dimensional structures of the active ingredients from *C. racemosa* were obtained from the PubChem database (<https://PubChem.ncbi.nlm.nih.gov/>) in SDF format. These SDF files were then uploaded to ChemBio3D Ultra (14.0, CambridgeSoft Corporation, USA) to create their three-dimensional (3D) structures in MOL2 format. The 3D structures of the core target proteins were downloaded from the UniProt database (<https://www.UniProt.org/>) in PDB format. Subsequently, water and

small-molecule ligands were removed from the PDB file using PyMOL (3.1.8, Schrödinger, LLC., USA). The active pockets of the protein receptors were plotted, with the active pocket parameters adjusted with AutoDockTools (1.5.6, The Scripps Research Institute, USA). Docking was performed using AutoDock Vina (1.2.6, The Scripps Research Institute, USA), with each object being docked 20 times to determine the optimal result based on the lowest free energy. The optimal model PDB file was then loaded into Discovery Studio (2016, Dassault Systèmes BIOVIA, France) to calculate the interaction forces between residues in the docking region in kcal/mol.

2.4. *C. racemosa* extraction

The *C. racemosa* powder was obtained from Quan Au BioTech (China). The procedures of active component extraction were modified from a previous study.²⁰ The powder was extracted using 75% ethanol/water. The ethanol was then removed under reduced pressure at 45–55 °C. The concentrated extract was partitioned between dichloromethane and water to isolate a 15% TG fraction from the dichloromethane layer. Subsequently, the dichloromethane was removed under reduced pressure at 45–55 °C. The concentrated fraction was further partitioned between n-butanol and water, yielding a 27% TG fraction in the n-butanol phase.

2.5. Cell culture and treatment

The human liver cancer cell line HepG2 was obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA) at 37 °C with 5% carbon dioxide. For treatments, *C. racemosa* extracts were dissolved in dimethyl sulfoxide and diluted in culture medium to the indicated final concentrations for 24-hour treatment. Bugbanosides A, C, and F were purchased from Chao98 Biotechnology Co., Ltd (China).

2.6. Cellular transfection

HepG2 cells were transfected with a CDK1 overexpression plasmid LV_EF1a_CDK1-P2A-Hygro (Addgene plasmid #170221, Addgene, USA) or an empty control vector using FuGENE® HD Transfection Reagent (Promega, USA) following the manufacturer's instructions. Transfected cells were cultured for 48 hours before subsequent experiments.

2.7. Cell-counting kit-8 cell viability assay

Cell viability was measured using the Cell-Counting Kit-8 (CCK-8) kit (CK04, Dojindo Molecular Technologies, USA). Briefly, HepG2 cells (5×10^3 cells/well) were

seeded in 96-well plates and treated with the indicated concentrations of extracts or compounds for 24 hours. After treatment, 10 µL of CCK-8 solution was added, and the mixture was incubated for 2 hours. The absorbance at 570 nm was measured using a microplate reader (MB-580, Mindray Bio-Medical Electronics Co., Ltd, China).

2.8. Lactate dehydrogenase release assay

Lactate dehydrogenase (LDH) release into the culture medium, an indicator of cytotoxicity, was measured using the LDH Cytotoxicity Assay Kit (C0017, Beyotime, China). HepG2 cells (1×10^6 cells/well) in a 6-well plate were treated for 24 hours. Then, 100 µL of culture supernatants from different groups were used to assay LDH activity by reacting with a tetrazolium substrate to generate a colored product. Optical density was quantified at 450 nm using spectrophotometry.

2.9. *In vitro* cyclin-dependent kinase 1 activity assay

The CDK1 kinase activity was determined using the CDK1 Kinase Assay Kit (V2961, Promega, USA). Purified CDK1/cyclin B protein complex was incubated with the ATP/substrate mixture in the presence of vehicle control or indicated concentrations of extracts/compounds for 30 minutes at ambient temperature. After quenching, the ADP formation was quantified using the ADP-Glo reagent on a luminescence plate reader (V9101, Promega Corporation, USA).

2.10. Real-time quantitative polymerase chain reaction

Total RNA was extracted from cells using the RNeasy Mini Kit (74104, Qiagen, Germany) and reverse transcribed to cDNA using the High-Capacity cDNA Reverse Transcription Kit (4368813, Applied Biosystems, USA). Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using PowerUp SYBR Green Master Mix (Applied Biosystems, USA) on an RT-qPCR system (QuantStudio 6 Flex System, Applied Biosystems, USA). Primer sequences for CDK1 were: Forward: 5'-GCCTCCTCTTGGGCATT-3'; Reverse: 5'-CCCTCGGTACCCTGTGAC-3'. *GAPDH* was used as the internal control. Relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method.

2.11. Immunoblotting

Cells were lysed in radioimmunoprecipitation assay buffer with protease/phosphatase inhibitors (Thermo Fisher Scientific, USA). A total of 15 µg denatured protein sample was separated on 12% Mini-PROTEAN TGX precast gels (Bio-Rad, USA) and transferred to polyvinylidene fluoride membranes. After blocking, the membranes were probed

with the following primary antibodies for 24 hours at 1:1,000 dilution: anti-CDK1 (ab133327, Abcam, UK), anti-caspase-3 antibody (#9662, Cell Signaling Technology, USA), anti-cleaved caspase-3 (#9661, Cell Signaling Technology, USA), anti-gasdermin D (GSDMD) (#93709, Cell Signaling Technology, USA), anti-cleaved GSDMD (Asp275) (#36425, Cell Signaling Technology), anti-mixed lineage kinase domain-like protein (MLKL) (ab18471, Abcam) and anti-phospho-MLKL (ab187091, Abcam, UK), and anti- β -actin (#4970, Cell Signaling Technology, USA). Horseradish peroxidase-conjugated secondary antibodies (1:5,000, ab6712 or ab97023, Abcam) were used to detect the primary antibodies, and chemiluminescent substrates (Beyotime, China) were used for signal detection.

2.12. Statistical analyses

Statistical analyses were performed using GraphPad Prism (V8.0, GraphPad Software, USA). Data are presented as mean $\pm$ standard error of the mean from at least three independent experiments. Comparisons between two groups were made using unpaired Student's *t*-tests. For multiple comparisons, one-way analysis of variance with Tukey's post hoc test was used. A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. Identification of *CDK1* as a potential target of *C. racemosa* components in PANoptosis regulation

Initially, we retrieved the microarray profiling data containing 8 normal hepatic tissues and 24 liver cancer specimens for gene expression analysis. We detected 1,424 DEGs, of which 553 were upregulated, and 871 were downregulated (Figure 1A). We identified 21 active compounds from *C. racemosa* in the TCMSP, BATMAN-TCM, and TCMID databases and obtained 316 gene targets through target prediction (Figure 1B, interaction network between the active components and target genes). The Venn diagram showed the intersection among the DEGs, the target genes of *C. racemosa*, and PANoptosis-related genes, and *CDK1* was the only gene shared by the three gene lists (Figure 1C).

We further identified genes associated with liver cancer prognosis using the TCGA database and screened for differential gene sets co-expressed with *CDK1* in GSE101685. The intersection of the two gene sets yielded 386 differentially co-expressed genes (Figure 1D). Enrichment analysis indicated that these genes are associated with cell cycle regulation, cellular senescence, and the p53 signaling pathway (Figure 1E). Furthermore, we extracted the PPI information from the STRING database and identified the top 10 hub genes in the PPI network (Figure 1F). These hub

genes (*CDK1*, *TTK*, *NUF2*, *KIF2C*, *RRM2*, *MELK*, *CCNA2*, *TOP2A*, *ASPM*, and *PBK*) showed significant upregulation (Figure 1G). Moreover, based on liver cancer cohort data from the TCGA database, *CDK1*-interacting hub genes showed higher expression with advancing tumor stage (Figure 2A) and were associated with reduced overall survival in liver cancer patients (Figure 2B). Receiver operating characteristic curve analysis showed that the area under the curve (AUC) was close to 1, suggesting the predictive potential of these genes for liver cancer (Figure 2C). Together, these data indicate that *CDK1* is a potential target of *C. racemosa* components in PANoptosis regulation, and *CDK1* interacts with key genes involved in liver cancer progression.

3.2. Bugbanosides A, C, and F as the active components for targeting cyclin-dependent kinase 1

We next used molecular docking to screen for potential active components in *C. racemosa* targeting *CDK1*. The analysis revealed bugbanosides A, C, and F as the potential components to interact with the *CDK1* protein. The free interaction energy was -9.9 , -8.5 , and -9.9 kcal/mol, respectively, indicating strong molecular interactions (Figure 3).

3.3. Induction of PANoptosis in liver cancer cells by *C. racemosa* extracts

To examine the effect of extracts from *C. racemosa* on liver cancer cells, we conducted a dose-response analysis using different doses. CCK-8 cell viability assay revealed a dose-dependent inhibition of HepG2 cell viability by *C. racemosa* extracts, with a half-maximal inhibitory concentration (IC₅₀) of approximately 20 μ g/mL (Figure 4A). We then applied IC₅₀ and $2 \times$ IC₅₀ concentrations to examine cytotoxicity in HepG2 cells. LDH release analysis showed that the *C. racemosa* extract induced cytotoxicity in HepG2 cells (Figure 4B). To show the effects across different modes of cell death, we analyzed caspase-3 cleavage (an apoptosis marker), GSDMD cleavage (a pyroptosis marker), and MLKL phosphorylation (a necroptosis marker). Immunoblotting results demonstrated cleavage of caspase-3 and GSDMD, and enhanced phosphorylation of MLKL after treatment with *C. racemosa* extracts (Figure 4C). Thus, these data clearly showed that extracts from *C. racemosa* inhibit *CDK1* activity and trigger PANoptosis in liver cancer cells.

3.4. Inhibition and destabilization of cyclin-dependent kinase 1 protein by *C. racemosa* extracts

To examine the effect of *C. racemosa* extracts on *CDK1* activity, we conducted an *in vitro* *CDK1* kinase assay at

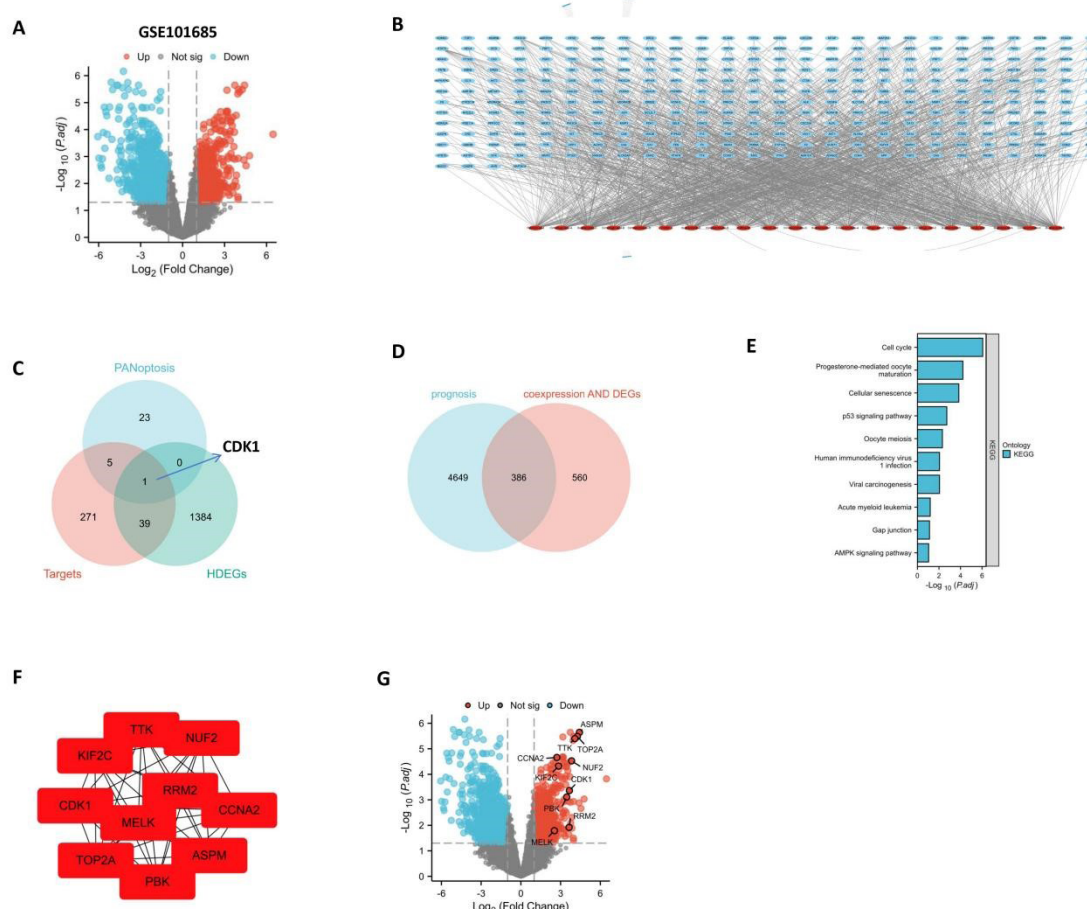


Figure 1. Identification of *CDK1* as a potential target of *C. racemosa* components in PANoptosis regulation. (A) Volcano plot showing differentially expressed genes (DEGs) in liver cancer compared to normal liver tissues (GSE101685). (B) Interaction network between active components from *C. racemosa* and their predicted target genes. (C) Venn diagram depicting the overlap between DEGs in liver cancer, predicted targets of *C. racemosa*, and PANoptosis-related genes, with *CDK1* as the common gene. (D) Venn diagram showing genes co-expressed with *CDK1* in liver cancer samples (GSE101685) in the intersection with genes associated with liver cancer patient prognosis (The Cancer Genome Atlas data). (E) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis for the differentially co-expressed gene set related to *CDK1*. (F) Protein-protein interaction network with top 10 hub genes highlighted. (G) Relative expression levels of the top 10 hub genes between liver cancer tumor and normal samples.

different doses. We detected a dose-dependent inhibition of CDK1 activity by *C. racemosa* extracts, with a half-maximal effective concentration (EC₅₀) of approximately 0.5 µg/mL (Figure 5A). Treatment of HepG2 cells with *C. racemosa* extracts at the IC₅₀ did not significantly alter *CDK1* mRNA levels (Figure 5B). However, the CDK1 protein level was significantly reduced (Figure 5C), suggesting the accelerated protein degradation after the treatment of *C. racemosa* extracts. To investigate this possibility, HepG2 cells with or without *C. racemosa* extracts treatment were subjected to *de novo* protein synthesis inhibition by cycloheximide (CHX). The protein samples were collected at different time points following

CHX addition. Immunoblotting results demonstrated accelerated CDK1 protein loss in HepG2 cells treated with *C. racemosa* extract (Figure 5D). Together, these findings suggest that *C. racemosa* extracts inhibit CDK1 kinase activity and destabilize CDK1 protein.

3.5. Partial rescue of the PANoptosis from cyclin-dependent kinase 1 overexpression induced by *C. racemosa* extracts

We next sought to validate the role of CDK1 inhibition in PANoptosis induction by *C. racemosa* extracts. To this end, the CDK1 expression vector was introduced into HepG2 cells to induce CDK1 overexpression, with the

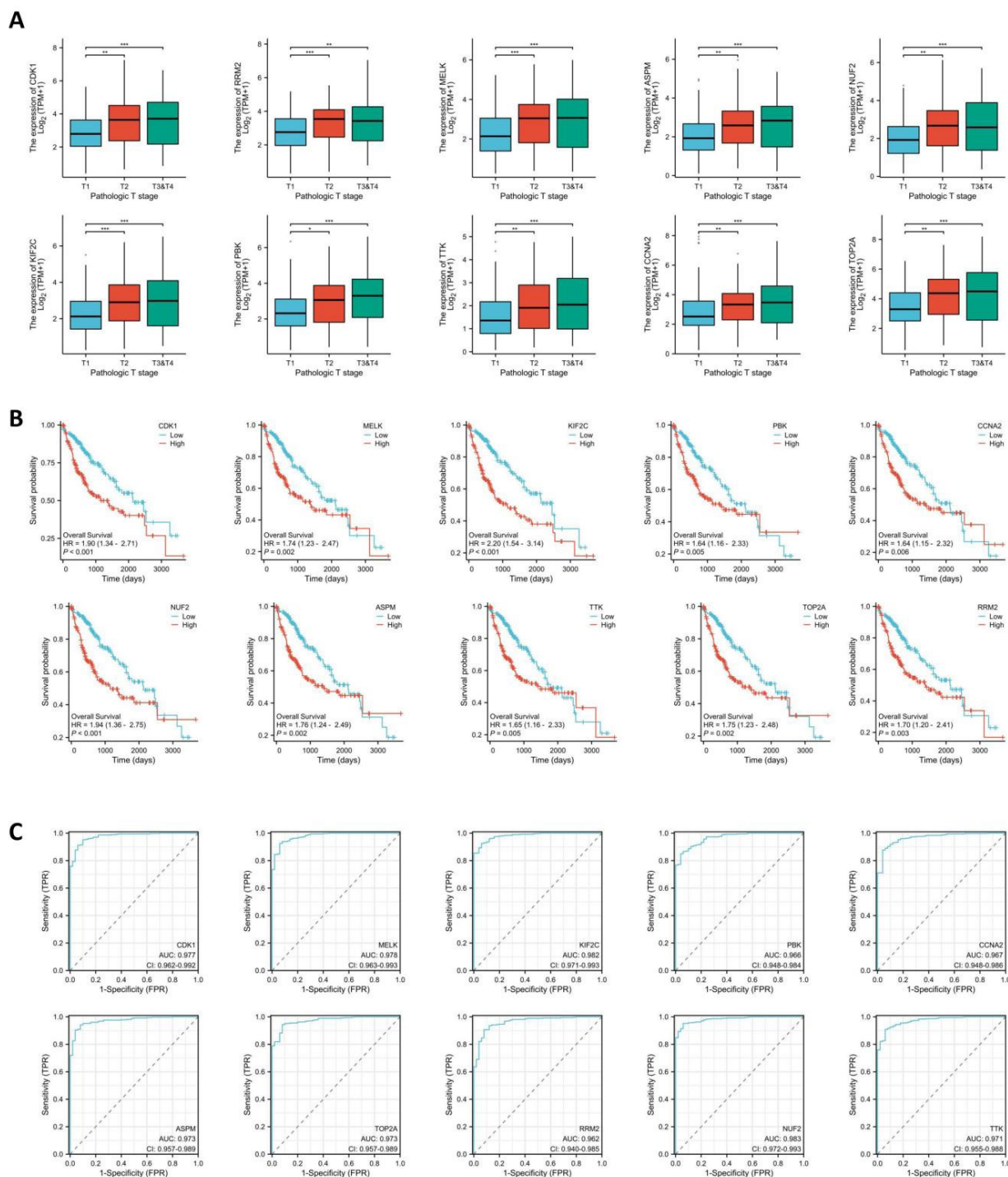


Figure 2. Expression of *CDK1*-interacting genes correlates with liver cancer progression and poor prognosis. (A) Expression levels of *CDK1*-interacting hub genes across different tumor stages in liver cancer patients from The Cancer Genome Atlas database. (B) Kaplan–Meier plots showing reduced overall survival of liver cancer patients with high expression of *CDK1*-interacting hub genes. (C) Receiver operating characteristic curve analysis of *CDK1*-interacting genes as predictive biomarkers for liver cancer.

Abbreviations: AUC: Area under the curve; FPR: False positive rate; HR: Hazard ratio; TPR: True positive rate.

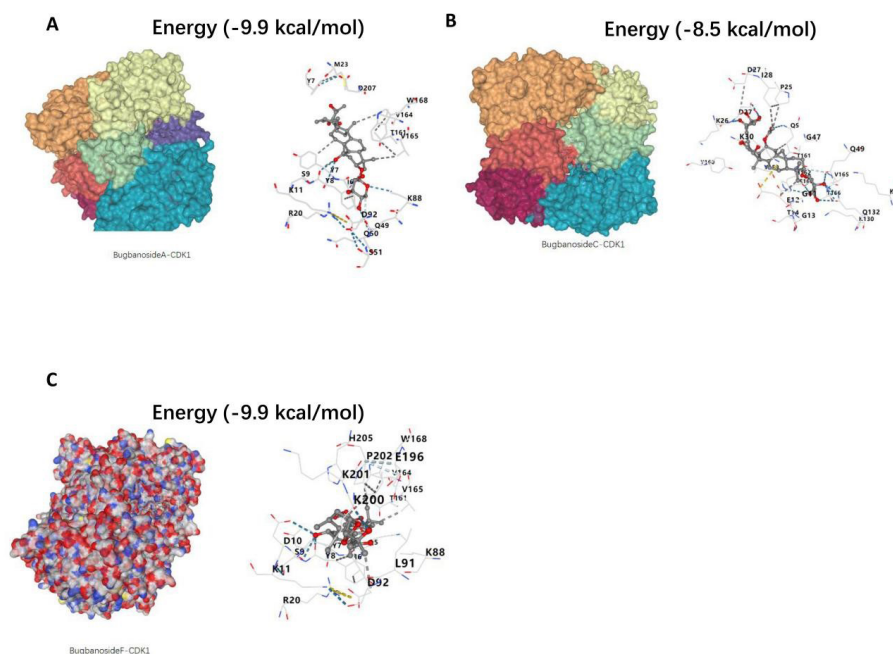


Figure 3. Molecular docking of bugbanosides A, C, and F with cyclin-dependent kinase 1 (CDK1) protein. Predicted binding modes and interactions of (A) bugbanoside A, (B) bugbanoside C, and (C) bugbanoside F with the CDK1 protein structure.

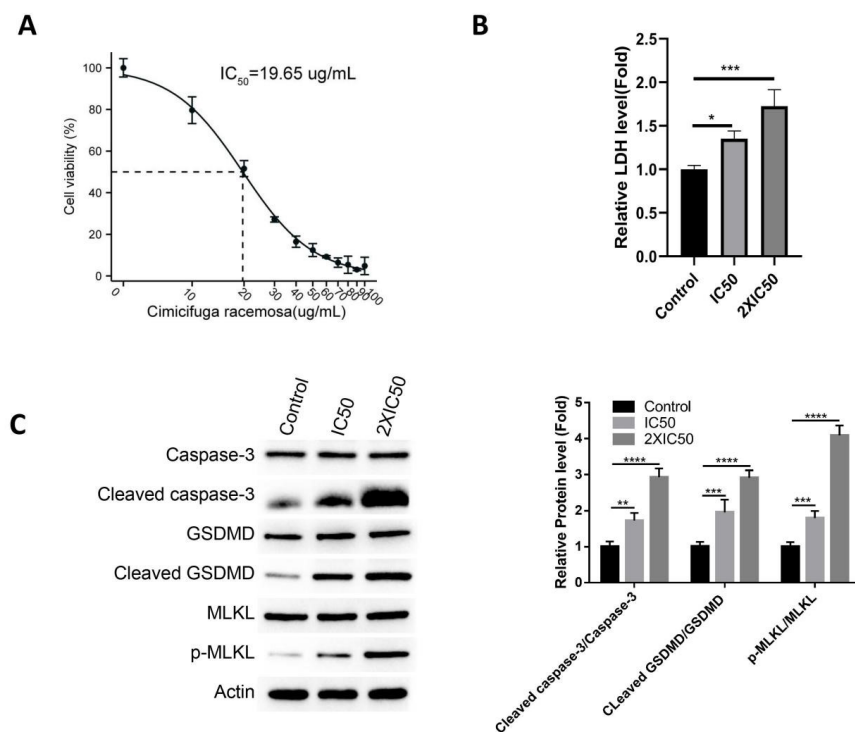


Figure 4. Induction of PANoptosis in liver cancer cells by *C. racemosa* extracts. (A) Dose-response curve of *C. racemosa* extracts on HepG2 cell viability measured using cell-counting kit-8 assay. (B) LDH cytotoxicity assay in HepG2 cells treated with *C. racemosa* extracts at IC₅₀ or 2 × IC₅₀. (C) Immunoblots showing cleavage of caspase-3, GSDMD, and phosphorylation of MLKL in HepG2 cells after treatment with *C. racemosa* extracts.

Notes: Data were summarized from three independent experiments; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Abbreviations: GSDMD: Gasdermin D; IC₅₀: Half-maximal inhibitory concentration; LDH: Lactate dehydrogenase; MLKL: Mixed lineage kinase domain-like protein.

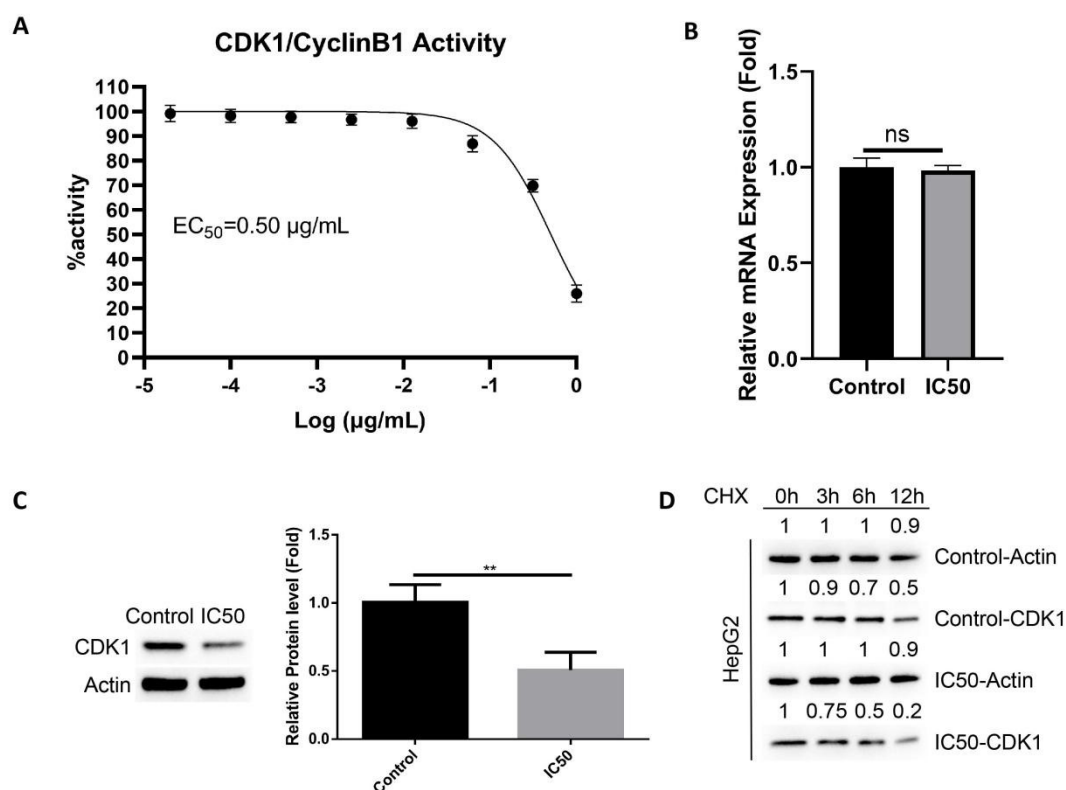


Figure 5. Inhibition and destabilization of CDK1 protein by *C. racemosa* extracts. (A) *In vitro* CDK1 kinase assay with increasing doses of *C. racemosa* extracts. (B) *CDK1* mRNA levels in HepG2 cells treated with *C. racemosa* extracts measured using real-time quantitative polymerase chain reaction. (C) Immunoblot showing reduced CDK1 protein levels in HepG2 cells after *C. racemosa* extract treatment. (D) CHX chase experiment demonstrating accelerated degradation of CDK1 protein upon *C. racemosa* extract treatment in HepG2 cells.

Notes: Data were summarized from three independent experiments; ** $p < 0.01$; ns: not significant.

Abbreviations: CDK1: Cyclin-dependent kinase 1; CHX: Cycloheximide; EC50: Half-maximal effective concentration; IC50: Half-maximal inhibitory concentration.

empty vector as the control. CDK1 overexpression restored CDK1 protein levels upon treatment with *C. racemosa* extract at the IC50 (Figure 6A). CDK1 overexpression partially rescued the reduction in cell viability caused by *C. racemosa* extracts (Figure 6B). Moreover, the cleavage of caspase-3 and GSDMD and the phosphorylation of MLKL induced via *C. racemosa* extract treatment were attenuated by CDK1 overexpression (Figure 6C).

3.6. Inhibition of cyclin-dependent kinase 1 activity and induction of PANoptosis in HepG2 cells by bugbanosides A, C, and F

After demonstrating the PANoptosis-inducing effect of *C. racemosa* extracts, we further examined the activities of bugbanosides A, C, and F. These compounds did not alter *CDK1* mRNA expression in HepG2 cells (Figure 7A). However, these compounds reduced CDK1 protein levels in a dose-dependent manner (Figure 7B). *In vitro* CDK1

kinase assay further demonstrated the inhibitory effect of these compounds on CDK1 activity (Figure 7C, EC50 values: bugbanoside A = 0.1 µM, bugbanoside C = 0.4 µM, bugbanoside F = 0.08 µM). Treatment with bugbanosides A, C, or F reduced HepG2 cell viability (Figure 7D). Moreover, we demonstrated that these compounds were able to induce the cleavage of caspase-3 and GSDMD and enhance the phosphorylation of MLKL (Figure 7E), indicating the PANoptosis-inducing effects in HepG2 cells.

4. Discussion

In this study, we identified CDK1 as a potential target of bioactive components from the medicinal plant *C. racemosa* in the induction of PANoptosis, a form of RCD involving multiple pathways, in liver cancer cells. Our findings suggest that specific bugbanoside compounds from *C. racemosa* can inhibit CDK1 activity, destabilize CDK1 protein, and trigger PANoptosis, highlighting

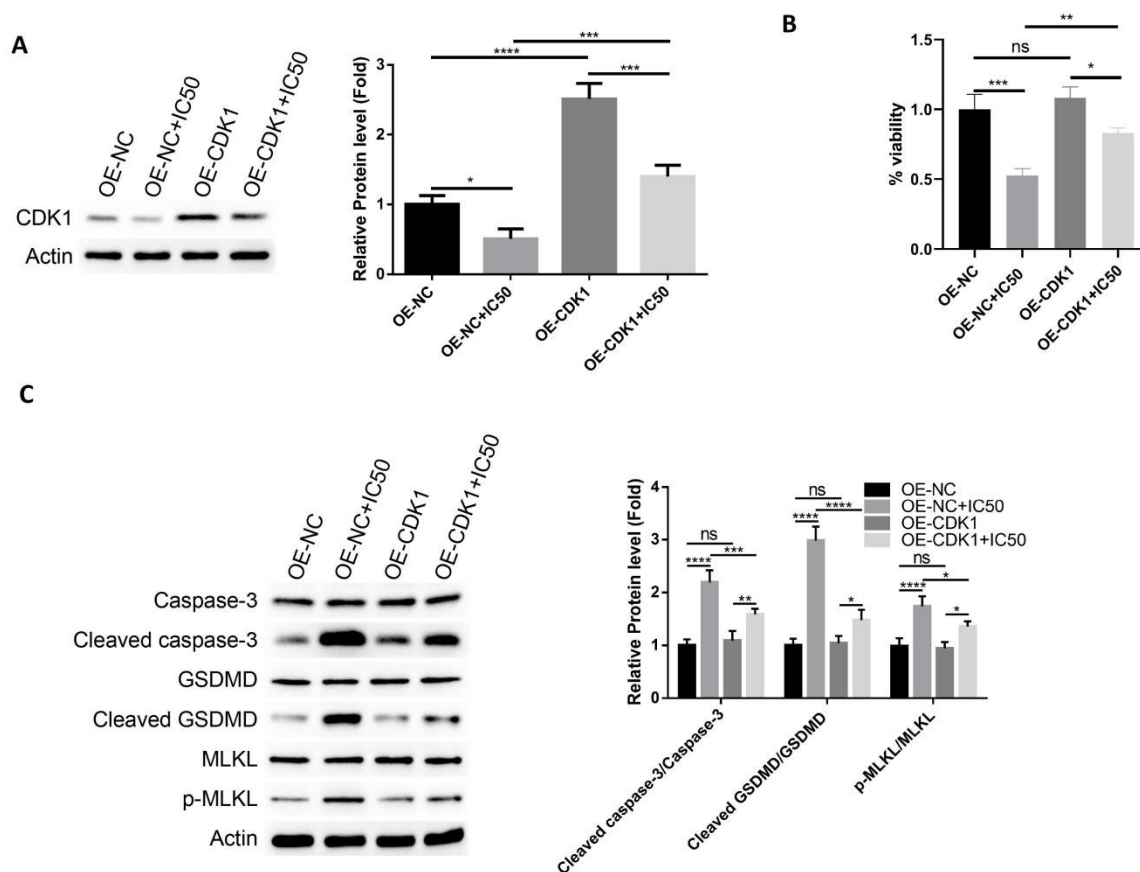


Figure 6. CDK1 OE partially rescues *C. racemosa* extract-induced PANoptosis. (A) Immunoblot confirming CDK1 OE in HepG2 cells transfected with *CDK1* plasmid. (B) Cell counting kit-8 viability assay showing partial rescue of growth inhibition by *C. racemosa* extracts in CDK1-overexpressing HepG2 cells. (C) Immunoblots indicating attenuated cleavage of caspase-3, GSDMD, and phosphorylation of MLKL in CDK1-overexpressing HepG2 cells treated with *C. racemosa* extracts.

Notes: Data were summarized from three independent experiments; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns: not significant.

Abbreviations: CDK1: Cyclin-dependent kinase 1; GSDMD: Gasdermin D; IC50: Half-maximal inhibitory concentration; MLKL: Mixed lineage kinase domain-like protein; NC: Negative control; OE: Overexpression.

their potential as novel anti-cancer agents for liver cancer treatment.

C. racemosa, commonly known as black cohosh, has been extensively studied for its anti-cancer properties across various malignancies. However, there are few studies on its effect and mechanism in liver cancer, and the specific active components and direct targets are not clear. In breast cancer, *C. racemosa* extracts and purified compounds have been shown to selectively disrupt cell cycle progression, induce apoptosis, and inhibit the activity of the human epidermal growth factor receptor 2.^{12,13,21} In addition, TGs and phenolic compounds that trigger cell death in breast cancer cells were also identified from *C. racemosa*.²² Similarly, in prostate cancer, *C.*

racemosa extracts exhibited cytotoxic effects and induced cell cycle arrest and apoptosis.²³ In addition to liver cancer, as demonstrated in our study, *C. racemosa* extracts have been reported to exert anti-proliferative and pro-apoptotic effects in other cancer cell types.^{8,24} This evidence, along with our study, supports the anti-cancer application of *C. racemosa* extracts.

Cyclin-dependent kinase 1, a critical regulator of the cell cycle, has emerged as a promising therapeutic target in cancer due to its pivotal role in cancer progression and resistance to conventional therapies.²⁵⁻²⁷ Cell death can occur through various mechanisms. PANoptosis is a unique form of cell death characterized by the interaction among pyroptosis, apoptosis, and necroptosis. This mode can be

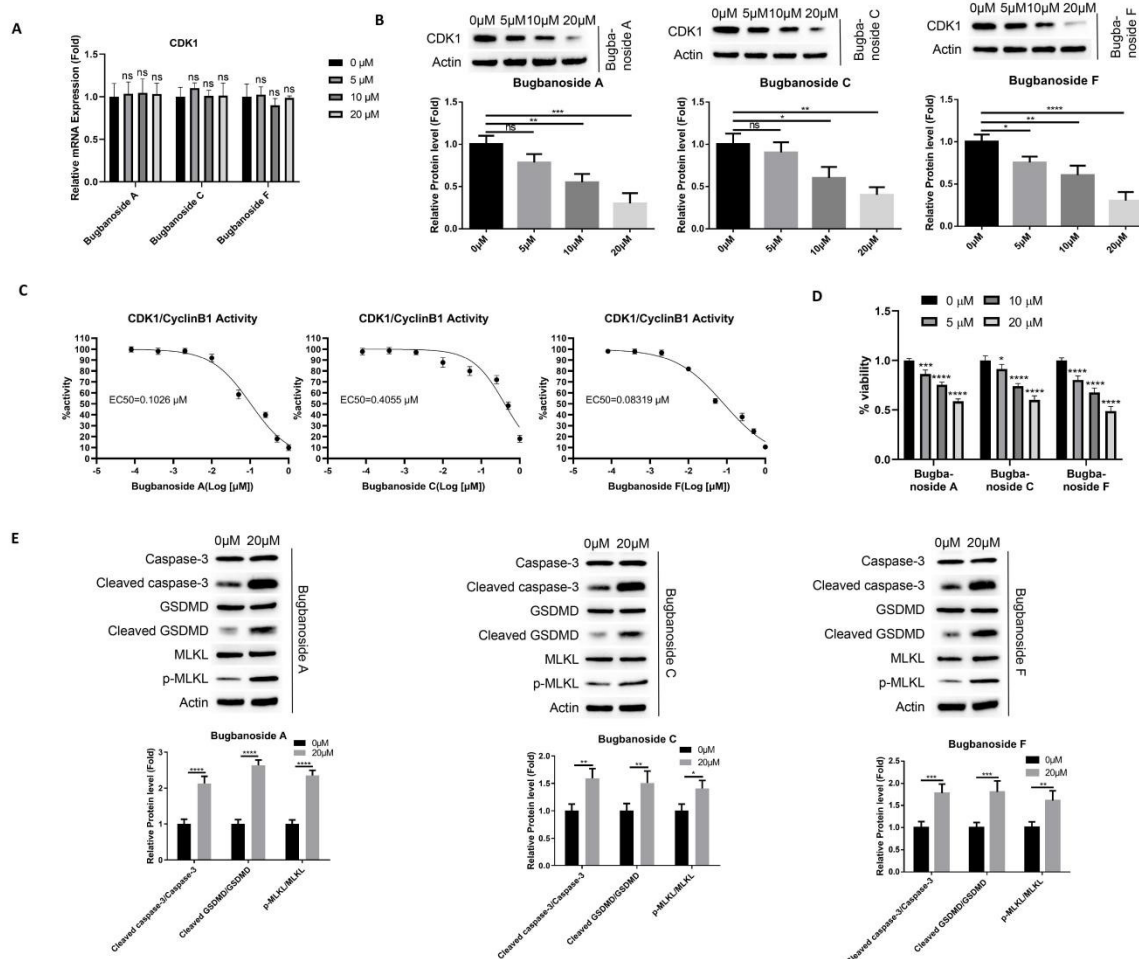


Figure 7. Inhibition of CDK1 activity and induction of PANoptosis in HepG2 cells by bugbanosides A, C, and F. (A) *CDK1* mRNA levels measured using real-time quantitative polymerase chain reaction in HepG2 cells treated with the indicated bugbanosides. (B) Immunoblots showing dose-dependent reduction in CDK1 protein by bugbanoside treatments. (C) *In vitro* CDK1 kinase assay with increasing concentrations of bugbanosides. (D) HepG2 cell viability after treatment with different bugbanosides measured by CCK-8 assay. (E) Immunoblots depicting cleavage of caspase-3, GSDMD, and phosphorylation of MLKL in HepG2 cells induced by bugbanoside treatments.

Notes: Data were summarized from three independent experiments; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns: not significant.

Abbreviations: CDK1: Cyclin-dependent kinase 1; GSDMD: Gasdermin D; IC50: Half-maximal inhibitory concentration; MLKL: Mixed lineage kinase domain-like protein.

mediated by a multifaceted apoptotic complex assembled from components of other forms of cell death.²⁸ Inhibition of CDK1 has been shown to induce PANoptosis in various cancer types, including adrenocortical carcinoma,²⁹ breast cancer,³⁰ and lung cancer.³¹ For instance, Ren *et al.*²⁹ demonstrated that CDK1 inhibition in adrenocortical carcinoma cells led to PANoptosis induction, suppression of epithelial-mesenchymal transition, and G2/M phase arrest. Moreover, CDK1 inhibitors have been proposed as potential PANoptosis inducers in combination with other therapeutic agents, such as RIPK1 inhibitors, to enhance anti-cancer efficacy.^{32,33} Recent studies have also

highlighted the role of CDK1 in regulating PANoptosis through interactions with various signaling pathways, including the p53 and nuclear factor- κ B cascades.^{34,35} Therefore, CDK1 inhibitors may be used in combination with other chemotherapeutic agents to enhance anti-cancer effects through PANoptosis.

Bugbanosides are a class of triterpenoid glycosides that have gained significant attention for their potential medicinal properties. Our study identified bugbanosides A, C, and F as potent inhibitors of CDK1 activity, leading to CDK1 destabilization and PANoptosis induction in liver cancer cells. Bugbanosides are active components isolated

from the *Cimicifuga* species.³⁶ Although there is evidence that bugbanosides A and B exhibit antimalarial activity,³⁷ their other bio-activities remain largely unknown. Our findings highlight the promising anti-cancer potential of bugbanosides and provide a mechanistic basis for their PANoptosis-inducing effects through CDK1 inhibition in liver cancer.

There are certain limitations that warrant further investigation. First, the experiments were conducted primarily in a single liver cancer cell line (HepG2), and it would be essential to validate the findings in additional liver cancer cell lines and patient-derived models to establish the broader applicability of these compounds. Additionally, the molecular mechanisms underlying the CDK1 destabilization and PANoptosis induction by bugbanosides need to be further elucidated, particularly the involvement of specific signaling pathways and regulatory networks. Future studies should also explore the potential synergistic effects of bugbanosides with existing chemotherapeutic agents or targeted therapies, as combination strategies have shown promise in overcoming drug resistance and enhancing therapeutic outcomes in liver cancer.^{38,39} Moreover, *in vivo* studies using appropriate liver cancer xenograft or genetically engineered mouse models are crucial to evaluate the efficacy, pharmacokinetics, and safety profiles of these compounds, paving the way for potential clinical translation.^{40,41} Lastly, the structural optimization and development of more potent and selective CDK1 inhibitors based on the bugbanoside scaffold could further enhance their therapeutic potential for liver cancer and potentially other malignancies.⁴²

5. Conclusion

To conclude, we reported CDK1 as a target of bioactive bugbanoside compounds from *C. racemosa*. *C. racemosa* extracts, as well as bugbanosides A, C, and F, potently inhibit CDK1 activity, destabilize CDK1 protein, and trigger PANoptosis in liver cancer cells. These findings highlight the potential of bugbanosides as novel anti-cancer agents targeting CDK1 for the treatment of liver cancer. These findings reveal a new mechanism of natural products against liver cancer and provide a promising candidate strategy for CDK1-targeted liver cancer therapy. Further studies are needed to verify the *in vivo* efficacy, clarify the detailed mechanism of CDK1 degradation, and explore the combined application of bugbanosides with other therapeutic drugs.

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Conflict of interest

The authors declare there is no conflict of interest.

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Ethics approval and consent to participate

Not applicable.

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Not applicable.

Availability of data

The data generated in this study are available upon request to the corresponding author.

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