

Screening of *Chenopodium hybridum* L. for Anti-ovarian Cancer Compounds Using UPLC-Q-Exactive MS and Partial Least Squares Analysis

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Abstract

Background: Extracts of *Chenopodium hybridum* L. leaves and stems exerted a significant anti-proliferative effect on human A2780 ovarian cancer cells, but *C. hybridum* active components have not been reported.

Materials and Methods: Here, a method is described for screening of *C. hybridum* extracts for potential bioactive components that inhibit A2780 cell proliferation. First, the spectrum–effect relationship between UPLC-Q-Exactive MS chromatograms and *C. hybridum* extract antiproliferative effect against A2780 cells was established to evaluate extract bioactive components using partial least squares (PLS) analysis.

Results: The results indicated that the optimal reflux extraction process for preparing *C. hybridum* extracts with antiproliferative activity involved a suspension of *C. hybridum* material in 8 volumes of 70% ethanol followed by heating and refluxing twice for 60 min/reflux step and then repeating the extraction and pooling of both the extracts. Chromatographic results revealed five compounds with potential anti-ovarian cancer activities based on inhibition of A2780 cell proliferation: isorhamnetin-3-O-β-D-furanosyl(1→2)-O-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside, kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-pyranoside, kaempferol-3-O-[α-L-rhamnopyranosyl(1″→2″)]-β-D-galactopyranoside, quercetin-3,7-di-rhamnose, and isorhamnetin-3-acacia disaccharide. Network pharmacological screening revealed nine core cellular targets that potentially interacted with these compounds.

Conclusion: These results were verified through molecular docking studies that supported the involvement of these compounds in observed *C. hybridum* A2780 cell antiproliferative effects, thus indicating *C. hybridum* active components may have value in ovarian cancer treatments.

Keywords

Chenopodium hybridum L., ovarian cancer, UPLC-Q-Exactive MS, partial least squares, molecular docking

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Introduction

Chenopodium hybridum L. (also known as quinoa, star anise ash dish, etc.) is a commonly used Chinese herb and annual herbaceous plant with high medicinal value that is used to treat menstrual irregularity, metrorrhagia, hemoptysis, hematemesis, and hematuria. Based on its medicinal benefits, *C. hybridum* is distributed worldwide for use in folk medicines (Repo-Carrasco-Valencia et al., 2009; Ye et al., 2015). Notably, phytochemical studies have demonstrated that this herb is rich in numerous bioactive substances, including flavonoids and phenolics that are responsible for its diverse biological effects, which include antioxidant, anti-ovarian cancer, and anti-lung cancer activities.

Ovarian cancer is the most fatal gynecological cancer (Zhang et al., 2020), prompting researchers to intensively work to develop treatments to prevent and cure this terrible disease. Currently, platinum-based drugs are most commonly used to treat ovarian cancer, but cure rates are low despite good initial treatment responses, as most ovarian cancer patients experience

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tumor recurrence and eventually become resistant to salvage treatments (Battista et al., 2016; Ma et al., 2022). Therefore, screening to discover novel highly-effective, less toxic anti-ovarian cancer drugs are important for improving treatment outcomes for these patients. Toward this end, active ingredients of herbal medicines have been shown to possess unique advantages that highlight their potential uses as antitumor agents, due to their chemotherapeutic activities and their abilities to alleviate chemotherapeutic drug toxicity and prevent cancer recurrence (Carter et al., 2017).

Currently, ultra-high performance liquid chromatography-Q-Exactive mass spectrometry (UPLC-Q-Exactive MS) combines powerful chromatographic separation techniques with MS structural identification capabilities to achieve rapid analysis of chemical constituents of complex mixtures (Pavlovic et al., 2018; Wu et al., 2015; Yang et al., 2019), including traditional Chinese medicines (Cohen & Xu, 2015; Song et al., 2017). Moreover, causes of discrepancies in results obtained for different batches of samples have been difficult to diagnose, since current analytical methods cannot effectively determine which molecules are responsible for such discrepancies. To address these issues, multivariate analysis, clustering analysis, neuron network analysis, and other methods have generally been used. Multivariate analysis methods (Peng et al., 2018), including principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and partial least squares discriminant analysis (PLS-DA) (Dai et al., 2020). In practice, for multidimensionally complex samples, PLS-DA and OPLS-DA are used to establish a mathematical model that maximizes the detection of biomarker differences between samples.

To verify results obtained using the abovementioned models, molecular docking-based “lock-and-key” molecular interaction models can be generated to simulate and predict interaction forces and binding modes between ligands and receptors to facilitate the optimization of molecular screening methods (Bolcato et al., 2019; Cuzzolin et al., 2015; Olivero-Acosta et al., 2017). Nevertheless, effective screening generally requires good chemical extraction yields. To meet this goal, here *C. hybridum* extraction methods incorporating different extraction solvents, extraction times, and sample-to-solvent ratios were tested and compared based on flavonoid content as the main indicator of extract quality. Next, the anti-ovarian cancer activities of *C. hybridum* extracts were evaluated using a method combining LC-MS and orthogonal partial least squares analysis to identify flavonoid components with potential anti-ovarian cancer activities. The method developed in this work (Figure 1) was simple, fast, accurate, and sensitive; and thus, it should be suitable for use in identifying flavonoid compounds in plants, foods, and herbal medications (Peng et al., 2019).

Materials and Methods

C. hybridum was purchased from Beijing Tong Ren Tang Co., Ltd. (Changchun, China.). The plant specimen was deposited in the laboratory of the Jilin Ginseng Academy at Changchun University of Chinese Medicine, P.R. China. For use in experiments, 300 g of dried whole *C. hybridum* plants were pulverized and placed in a round-bottom flask followed by two 60-min reflux extractions in eight volumes of 70% ethanol per extraction. Next, both the extractions were pooled, and then, the suspension was mixed and filtered to

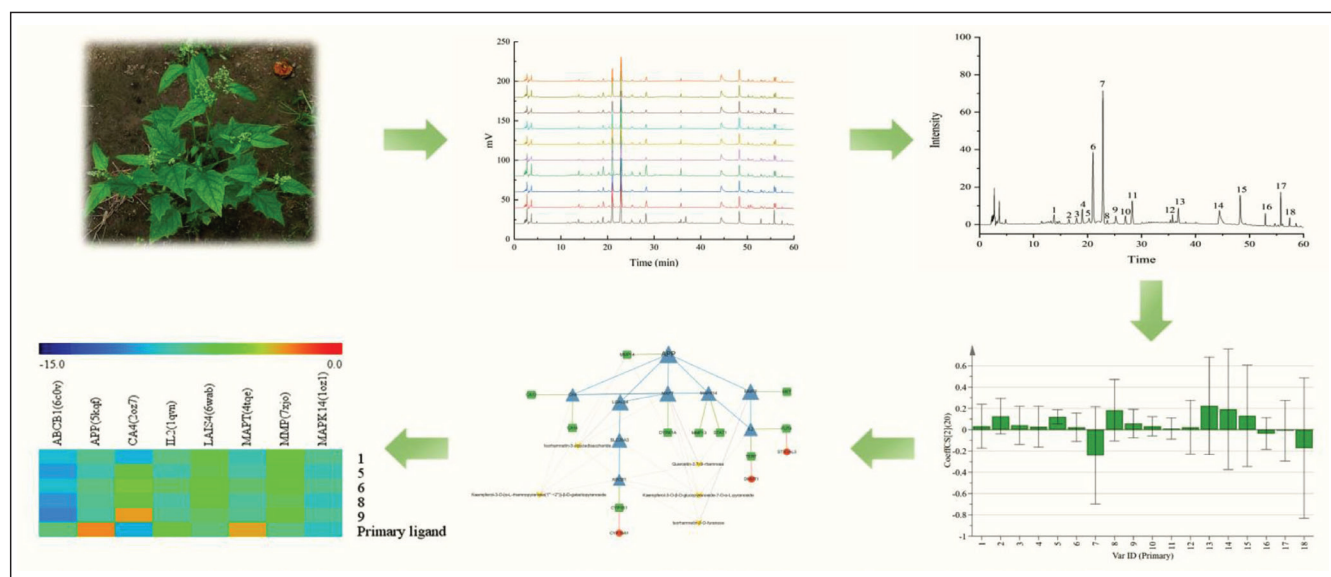


Figure 1. Schematic Showing Screening Strategy Used to Identify Bioactive Compounds Responsible for *C. hybridum* Anti-ovarian Cancer Activity.

remove particulates. Thereafter, the ethanol was removed by evaporation and the remaining solution was concentrated under reduced pressure to obtain a concentrated aqueous solution.

Human ovarian A2780 cells were purchased from the Cell Resource Center of the Institute of Basic Medicine, Chinese Academy of Medical Sciences. Other reagents that were purchased included fetal bovine serum (Gibco; USA), Trypsin (Amresco, USA), phosphate-buffered saline (PBS) and DMEM medium (Hyclone, USA), and dimethyl sulfoxide (Beijing Solebo Technology Co., China). Methanol and acetonitrile purchased from other suppliers were of chromatographic grade and other reagents were of analytical grade.

Equipment used in this study included an UltiMate™ 3000 series HPLC instrument and a Q-Exactive electrostatic field orbitrap high-resolution mass spectrometer (Thermo Fisher, USA), a Sartorius BP211D electronic balance (Beijing Dolis Balance Co., Ltd.), and a KQ3200BE ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.).

Determination of the Total Flavonoid Content

The total flavonoid content (TFC) was determined using a colorimetric method. Total polyphenol content (TPC) values from *C. hybridum* flavonoids extraction were analyzed using a colorimetric method. The various extracts were prepared as a 1 mg/mL 70% ethanol solution. Each extract (1 mL) was mixed with 0.3 mL of a 5% NaNO₂ solution. The mixture was allowed to stay at room temperature for 6 min; 0.3 mL of a 10% AlCl₃ × 9H₂O solution was added for 6 min followed by the addition of a 4 mL 4% NaOH solution. The 60% ethanol solution (0.4 mL) was added to reach a final volume of 10 mL. The solution was mixed and kept at room temperature for 15 min. Absorbance was measured immediately against the prepared blank at 360 nm using a spectrophotometer. Comparisons were made against standards prepared Rutin. All samples were analyzed three times.

Optimization of *C. hybridum* Extraction Parameters

In the present study, the effects of all of the abovementioned factors on flavonoids extraction efficiency were assessed using an orthogonal L9(3⁴) test design (Table 1), and then, the

total evaluation index was determined using statistical analysis (Zhang et al., 2015; Ma'ayan et al., 2014).

UPLC Fingerprint Analysis

Constituent compounds of *C. hybridum* extracts were identified via UPLC-Q-Exactive MS for samples dissolved in methanol to a concentration of 1 mg/mL, with samples applied to the column via automated injection. For chromatography, a Unitary C₁₈ column (4.6 mm × 150 mm, 2.8 μm) was used with a mobile phase consisting of acetonitrile (A) and 1% formic acid (B) aqueous solution. Elution was performed using a binary linear gradient as follows: mobile phase gradient operated using settings of 0–25 min (10–20% A), 25–60 min (20–100% A), a flow rate of 0.3 mL/min, injection volume of 10 μL, column temperature of 30°C, and detection wavelength of 254 nm. For MS, an electrospray ionization source (ESI) was used and samples were scanned in positive and negative ion modes using the following settings: spray voltage of 2.5 kV, capillary temperature of 320°C, lens voltage of 50 eV, sheath gas pressure of 2.76 × 10⁵ Pa, auxiliary gas pressure of 6.89 × 10⁴ Pa, auxiliary gas temperature of 300°C, resolution of 70,000, and mass scanning range of *m/z* 100–1500.

Cell Lines and Cell Culture Conditions

Human ovarian A2780 cells were maintained in RPMI-1640 medium with 10% fetal bovine serum at exponential growth phase in a humidified atmosphere containing 5% CO₂ at 37°C. For A2780 cell inhibition assays, the same medium was used to maintain the cells.

Tests to Determine Cell Viability

Microtiter 96-well plates were seeded with 1 × 10⁴ cells (suspended in 200 μL of medium)/well, and then, the plates were pre-incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO₂. A stock solution of *C. hybridum* extract was prepared and was serially diluted in RPMI-1640 medium. Serial dilutions were next added to wells at time zero. After incubation of plates for 24 h, 10 μL of MTS was added to each well. As assessed based on absorbance readings obtained at 490 nm using a 96-well INFINITE 200 PRO series microplate reader. Finally, the IC₅₀ value of *C. hybridum* was determined for treated and untreated A2780 cells using GraphPad Prism 7 software.

Table 1. Factor-level Table.

Factor Level	Alcohol Concentration (%)	Material-liquid Ratio	Extraction Time (h)	Number of Extractions
1	60	1:8	0.5	1
2	70	1:10	1	2
3	80	1:12	1.5	3

Partial Least Squares Regression Analysis

Partial Least Squares (PLS) is a multivariate data analysis method, was conducted using SIMCA 11 software. For each experiment, data was acquired in triplicate and the results were expressed as the mean $\pm$ standard deviation (SD). To evaluate significant differences, IBM SPSS Statistics 19 was used, with the significance threshold set to $p < 0.05$.

Compound-target Network Construction

A comprehensive list of genes associated with ovarian cancer was compiled from the Therapeutic Target Database (<http://db.idrblab.net/ttd/>) and DrugBank (<http://go.drugbank.com/>). Names of compounds with known positive associations with ovarian cancer were obtained by searching the Swiss Target Prediction Database (<http://www.swisstargetprediction.ch/>). A compound-target interaction network was then generated using compounds and targets as inputs and by applying the layout algorithm (attribute circle layout) during the network construction process (Sun et al., 2017).

Molecular Docking Studies

Molecular structures of extract compounds were downloaded from the PubChem database (<https://www.pubchem.org/>) and

ChemDraw 20.0 was used to draw 2D molecular structures from spatial data files (SDFs) of the five compounds using information obtained from the literature. After energy minimization of each compound structure was conducted using Chem 3D software, structures were converted to PDB format as flexible structures. Next, OpenBabel 2.4.1 was used to confirm molecular sites of rotatable bonds and structures were saved in PDBQT format for future docking (Chen et al., 2019).

Next, crystal structures of targets were downloaded from the RCSB PDB database (<https://www.rcsb.org/>) as PDB files and structures were subjected to water removal, hydrogenation, and other operations using PyMOL. Final target structures were saved as PDBQT files using Autodock. Binding site coordinates of IL-2 (1qvn, 16.435, 17.084, 80.461), MAPT (4tqe, 132.988, -4.307, 6.673), LGALS4 (6wab, -52.203, 24.757, 38.721), CA4, (2oz7, 28.042, 1.761, 3.677), MAPK14 (1oz1, 19.692, 13.039, 32.586), MMP2 (7xjo, 41.070, -61.821, 19.860), APP (5kqf, 17.893, 40.013, -9.034) and ABCB1 (6c0v, 126.375, 126.503, 120.988), and size (26 $\times$ 26 $\times$ 26) were saved in txt format. Thereafter, Autodock Vina was run using the Lamarck genetic algorithm to perform 100 docking operations and the results were visualized and analyzed using PyMOL.

Table 2. Orthogonal Experimental Design Result.

Factor	Alcohol Concentration (%)	Material-liquid Ratio	Extraction Time (h)	Number of Extractions	TFC (%)
I	60	1:10	1.0	1	32.89
2	60	1:12	1.5	2	47.64
3	60	1:14	2.0	3	41.57
4	70	1:10	1.5	3	38.37
5	70	1:12	2.0	1	34.38
6	70	1:14	1.0	2	33.66
7	80	1:10	2.0	2	45.52
8	80	1:12	1.0	3	38.82
9	80	1:14	1.5	1	22.60
I	280.22	367.92	323.88	236.10	
II	340.13	274.65	330.05	371.40	
III	338.85	316.62	305.27	351.69	
R	59.91	93.27	24.79	135.30	

Abbreviation: TFC, total flavonoid content.

Table 3. Variance Analysis on Orthogonal Table.

Source of Variance	Sum of Square of Deviations	Freedom	F Ratio	F Threshold	Significance
A	7028.731	2	7.03	19.000	
B	13091.562	2	13.108	19.000	
C	998.779	2	1.000	19.000	
D	32053.950	2	32.093	19.000	*
Error	998.78	2			

Note: * $p < 0.5$.

Results and Discussion

Effect of solvent to raw material ratio, number of extractions, extraction duration, and temperature on *C. hybridum* extract flavonoids content, (see Table 2).

Based on quantitative analysis of dry extract weights and flavonoids content, the total flavonoids content of the crude extract was determined. While factors influencing extraction included solvent ratio, number of extractions, and duration of extraction. From the intuitive analysis results in Table 3, it can be seen that $D > B > A > C$, the total flavonoids content is the highest under the condition of $A_2B_1C_2D_2$, and the analysis of variance shows that the extraction time has the greatest influence. Combined with the results of the orthogonal test and analysis of variance, the best extraction process of total flavonoids is $A_2B_1C_2D_2$, with optimal values of 70%, 10:1, 2, and 1.5 h, respectively.

Fingerprint Establishment

Extracts of *C. hybridum* obtained using different extraction methods were optimized and then used to optimize HPLC fingerprinting conditions that were used to determine HPLC

fingerprints for 10 batches of samples (Figure 2). An HPLC fingerprint similarity evaluation system was used to determine common peaks and evaluate peak similarity (Chen et al., 2015; Ding et al., 2014; Wu et al., 2016).

According to results of analysis of molecular ion peaks and main fragment peaks of each color spectrum were identified (Figure 3) and compared to results reported in the literature (Agnese et al., 2001; Bi et al., 2022; Cheng et al., 2002; Cui et al., 2003; El-Sayed et al., 1999; Huang et al., 2007; Ji et al., 2001; Lee et al., 2008; Rastrelli et al., 1995; Sun et al., 2020; Wang & Xu, 2015; Wei et al., 2007; Xie et al., 2002; Zhu et al., 2001). Ultimately, 17 flavonoids and one phenolic acid were identified in the crude *C. hybridum* extract (Table 4).

MTS Assay

C. hybridum extract is cytotoxic towards human ovarian A2780 cells. The suppression ratio of the extract on cell proliferation was determined using an MTS assay. The results revealed that the *C. hybridum* extract clearly exerted a significant concentration-dependent antiproliferative effect on A2780 cells (Figure 4).

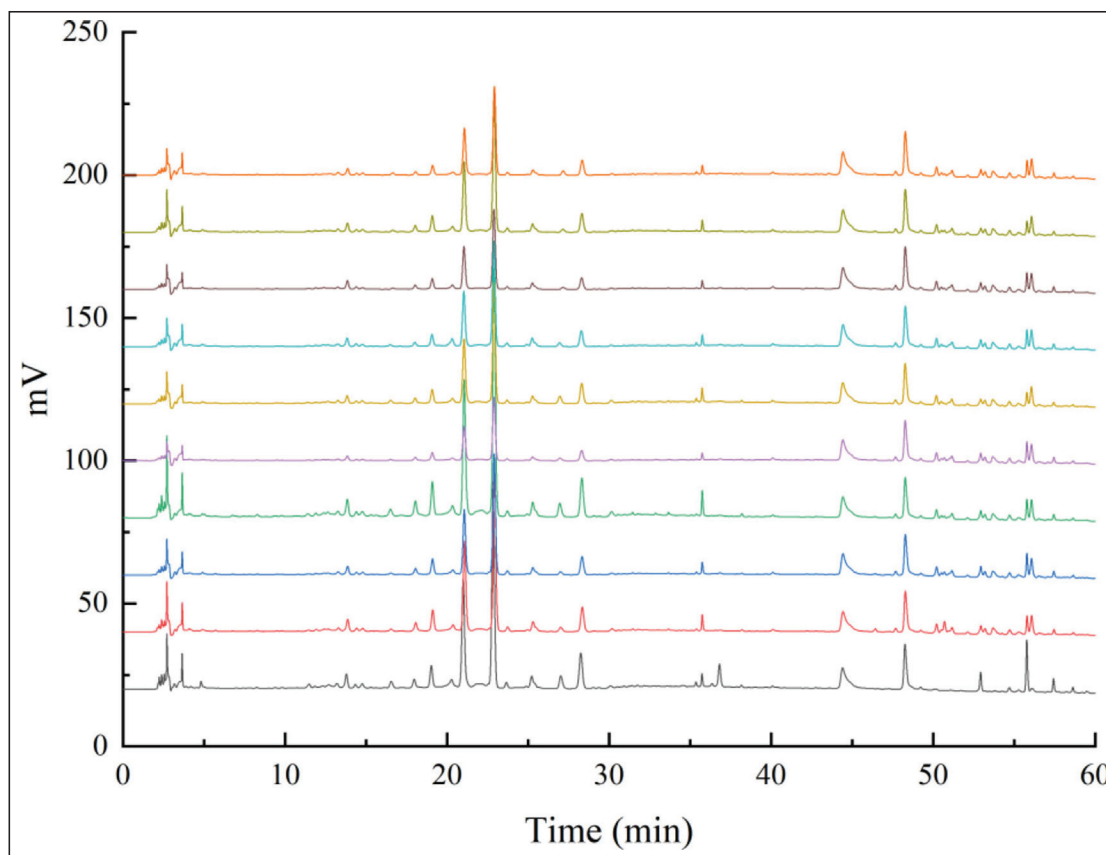


Figure 2. HPLC-ECD Fingerprints for 10 Batches of Samples.

Abbreviation: HPLC-ECD, high performance liquid chromatography-electrochemical detection.

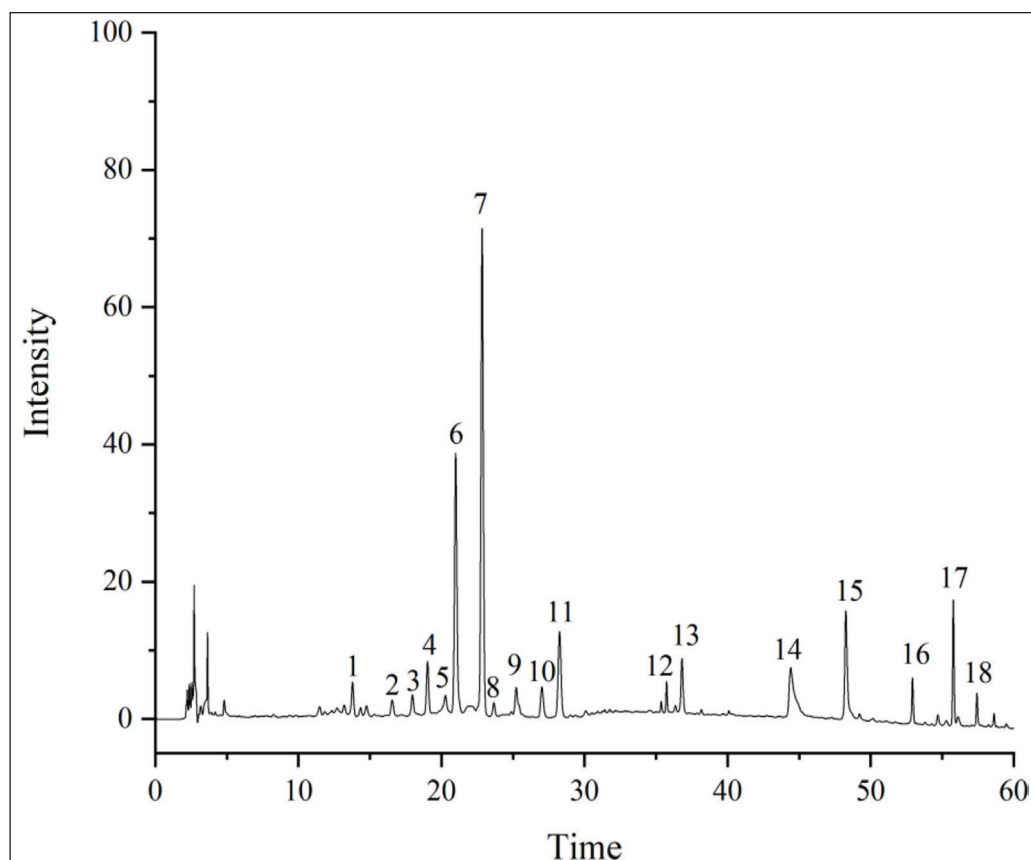


Figure 3. Reference Fingerprint for *C. hybridum*.

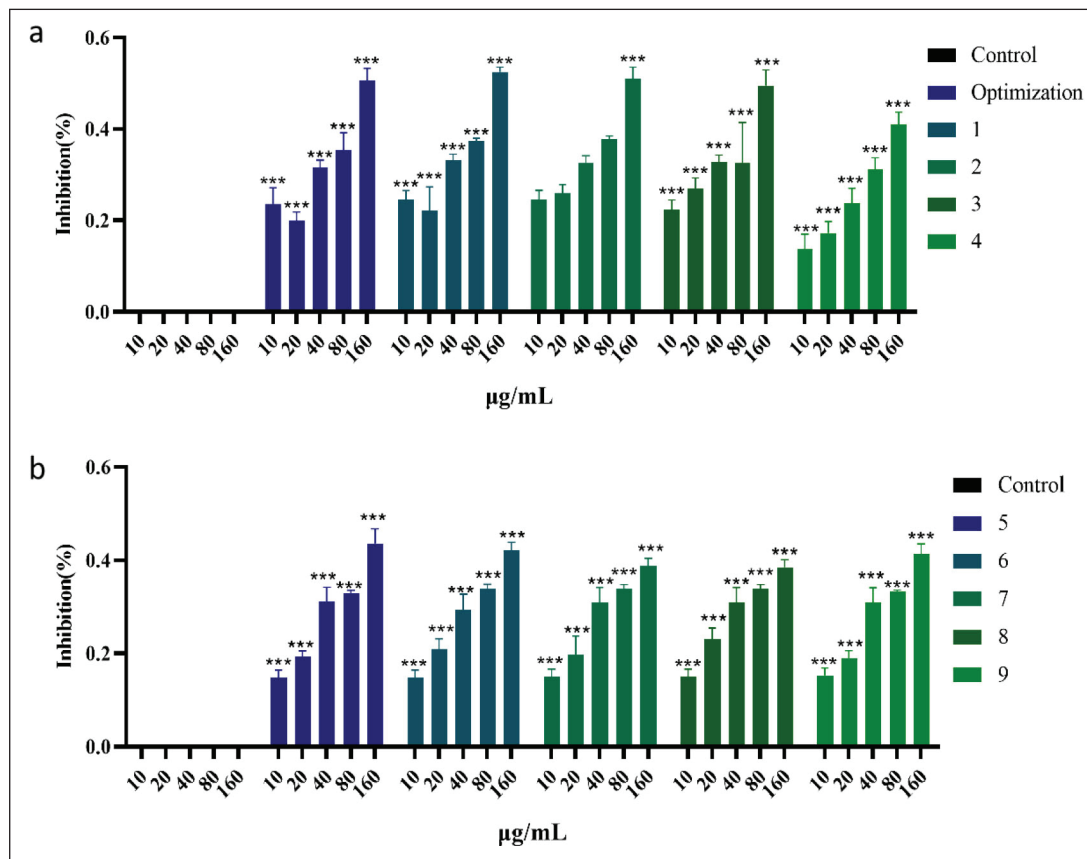
Table 4. UPLC-Q-Exactive MS Data of 18 Peaks.

Sl.No.	Retention Time(min)	Component Name	Contents of Component (%)	Excimer ion Peak (m/z)	Constitutional Formula	Secondary ion (m/z)	References
1	14.3	Isorhamnetin-3-O-β-D-furanosyl(1→2)-O-[α-L-rhamnopyranose(1→6)]-β-D-glucopyranoside	1.8%	756.2113	C ₃₃ H ₄₀ O ₂₁	593.1505, 447.0918, 299.0223	25
2	15.9	Kaempferol-3-O-β-diglucoside	0.9%	610.1534	C ₂₇ H ₃₀ O ₁₆	430.0917, 285.0422	26
3	16.8	Quercetin-3-rhamnocitin-7-glucose	1.2%	610.1534	C ₂₇ H ₃₀ O ₁₆	430.0917, 299.2034	27
4	17.6	Quercetin-3-O-(2,6-di-α-L-rhamnopyran)-β-D-galactopyranoside	3.0%	756.2113	C ₃₃ H ₄₀ O ₂₀	593.1505, 430.0917, 285.0421	28
5	19.2	Kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-pyranoside	1.8%	564.1585	C ₂₇ H ₃₀ O ₁₅	430.0918, 285.0422	29
6	20.7	Kaempferol-3-O-[α-L-rhamnopyranose(1"→2")]-β-D-galactopyranoside	16.7%	594.1585	C ₂₇ H ₃₀ O ₁₅	431.2835, 285.1402	27
7	23.8	Kaempferol-3-acaciadisaccharide	31.0%	594.1585	C ₂₇ H ₃₀ O ₁₅	447.2588, 285.1387	30
8	24.2	Quercetin-3,7-di-rhamnose	0.6%	630.1796	C ₂₇ H ₃₀ O ₁₅	593.1516, 447.0943, 285.0422	31

(Table 4 continued)

(Table 4 continued)

Sl.No.	Retention Time(min)	Component Name	Contents of Component (%)	Excimer ion Peak (m/z)	Constitutional Formula	Secondary ion (m/z)	References
9	25.3	Isorhamnetin-3-acaciadisaccharide	1.2%	624.169	C ₂₈ H ₃₂ O ₁₆	460.1027, 315.0530	32
10	27.8	Kaempferol-3-rhamnocitin-7-xylosid	2.4%	564.1479	C ₂₆ H ₂₈ O ₁₄	563.1384, 430.0918, 285.0423	33
11	28.5	Kaempferol-3-O-β-glucoside	6.0%	448.1006	C ₂₁ H ₂₀ O ₁₁	447.0947, 284.0347	34
12	35.1	Quercetin-7-rhamnocitin	2.4%	448.1006	C ₂₁ H ₂₀ O ₁₁	447.0945, 301.0373	31
13	36.2	Isorhamnetin-3-rutinoside	3.6%	624.169	C ₂₈ H ₃₂ O ₁₆	299.2034	35
14	44.9	Chlorogenic acid	3.6%	354.311	C ₁₆ H ₁₈ O ₉	174.9565, 129.9756	36
15	48.3	Isorhamnetin	4.2%	316.0584	C ₁₆ H ₁₂ O ₇	301.2191, 250.1469	37
16	53.5	Quercetin	3.0%	302.0427	C ₁₅ H ₁₀ O ₇	301.2193, 107.0496	38
17	56.6	Quercetin-3-O-β-D-furanose(1→2)-O-[α-L-rhamnopyranose-(1→6)]-β-D-galactopyranoside	7.7%	742.1956	C ₃₂ H ₃₈ O ₂₀	301.2191	27
18	57.8	Quercetin-3-O-β-D-furanose(1→2)-O-[α-L-rhamnopyranose-(1→6)]-β-D-glucopyranoside	2.4%	742.1956	C ₃₂ H ₃₈ O ₂₀	597.3083, 301.2191	25

**Figure 4.** Different Concentrations of 10 Extract Samples on the Inhibition Rate of A2780 Cells.**Note:** *** $p < 0.001$.

Partial Least Squares Screening of Active Compounds

To predict potential *C. hybridum* active components, PLS was applied to selected initial data using parameter settings of the confidence level of 95%, $R^2 = (0.0, 0.954)$, $Q^2 = (0.0, -0.425)$ in order to establish an effective PLS model. The PLS model was then used to analyze weights of common peak areas exported from rapid resolution liquid chromatography (RRLC) spectra of 18 compounds in 10 extract samples (x-axis) that were plotted against cancer cell inhibition rate (y-axis) with a concentration of 80 µg/mL in order to screen for major compounds with anticancer cell bioactivity. The results revealed some Cr values that were greater than 0, which indicated positive correlations existed between some extract samples and cancer cell inhibition rate (Figure 5), with positive Cr values obtained for five potential *C. hybridum* compounds of isorhamnetin-3-O-β-D-furanosyl(1→2)-O-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside (1), kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-pyranoside (5), kaempferol-3-O-[α-L-rhamnopyranosyl(1"→2")]-β-D-galactopyranoside (6), quercetin-3,7-di-rhamnose (8), and isorhamnetin-3-acacia disaccharide (9). Based on these results, additional studies were conducted to evaluate these compounds as potential anti-ovarian cancer bioactive compounds.

Network Pharmacological Analysis

In this study, a total of five compounds with potential anti-ovarian cancer activity were identified via PLS analysis. According to the Therapeutic Target Database and DrugBank,

9491 directly-related ovarian cancer targets were screened as potential *C. hybridum* targets using Swiss Target Prediction, yielding 51 potential targets of the 5 *C. hybridum* compounds with anti-ovarian cancer activity. A Venn analysis of these targets and the 5 *C. hybridum* compounds with anti-ovarian cancer activity revealed 38 potential targets related to potential anti-ovarian cancer activities of the five *C. hybridum* compounds (Figure 6).

After evaluating the topological parameters of the basic network, a compound-target network was constructed that contained five compounds and 20 targets (Figure 7). Through comprehensive evaluation of topological parameters and network stabilization, a final compound-target network containing putative major targets of *C. hybridum* was constructed. Analysis of the network revealed that isorhamnetin-3-O-β-D-furanosyl(1→2)-O-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside (1), kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-pyranoside (5), kaempferol-3-O-[α-L-rhamnopyranosyl(1"→2")]-β-D-galactopyranoside (6), quercetin-3,7-di-rhamnose (8), and isorhamnetin-3-acacia disaccharide (9) were key bioactive compounds that were potentially responsible for *C. hybridum* anti-ovarian cancer activity.

Based on component-target predictions obtained using the SWISS platform and intersecting ovarian cancer targets, here we identified 38 putative targets of the five selected *C. hybridum* compounds. Subsequent screening of putative targets using protein-protein interaction (PPI) analysis led to the identification of nine key potential targets, namely ABCB1, APP, CA4, IL-2, LGALS4, MAPT, MMP2,

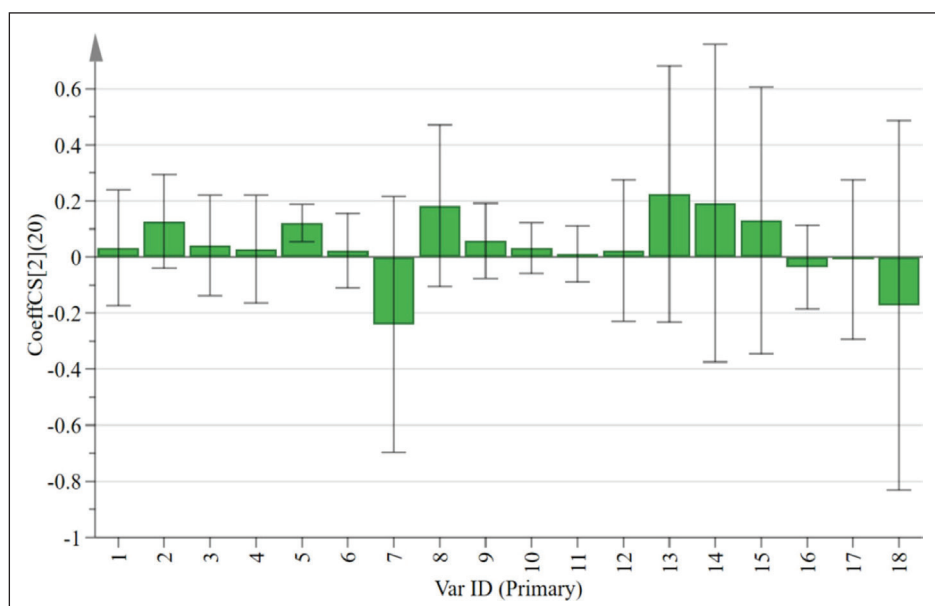


Figure 5. PLS-DA Score Plot for 18 Components.

Abbreviation: PLS-DA, partial least squares-discriminant analysis.

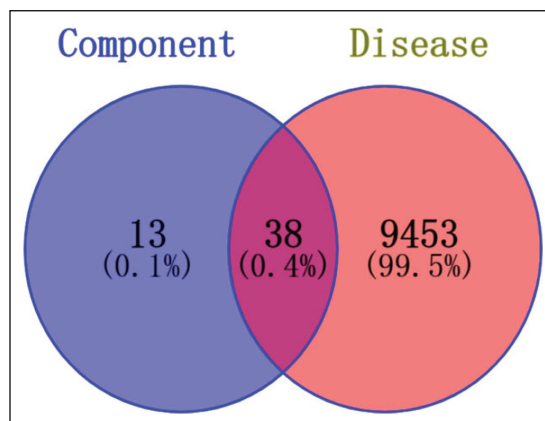


Figure 6. Venn Diagram of Components-targets.

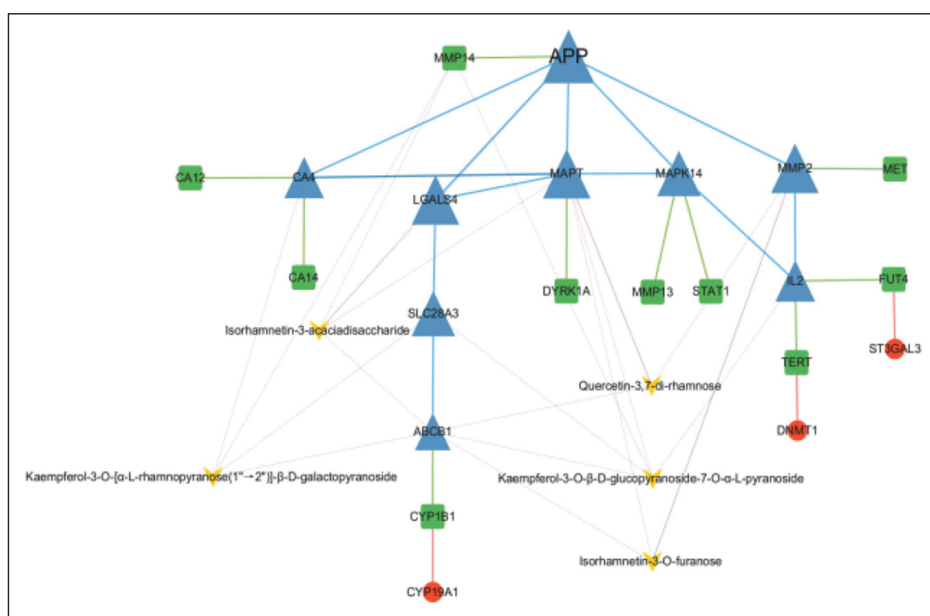


Figure 7. Target-compound Network. Yellow Represents the Ingredient, Blue Represents the Primary Target, Green Represents the Secondary Target, and Red Represents the Tertiary Target.

SLC28A3, and MAPK14. These nine targets were then imported into Cytoscape in order to generate a component tertiary target contact diagram (see Figure 7).

Molecular Docking Analysis

Table 5 shows molecular docking results between IL-2, ABCB1, APP, and MAPT targets and the five selected *C. hybridum* compounds. Notably, interactions between the five *C. hybridum* compounds and IL-2, ABCB1, APP, and MAPT targets were more substantial than docking interactions between these targets and their corresponding Primary ligands, warranting further study.

From Figure 8, it can be seen that the five *C. hybridum* compounds are each connected by at least one hydrogen bond to amino acid residues within the active centers of their respective targets, thus suggesting that these compounds mainly interact with their target proteins via hydrogen bonds and hydrophobic interactions, as consistent with results shown in Table 5 and Figure 8 and those reported in the literature. For example, results of a 2014 study by Gao B found that an ABCB1 blocker inhibited paclitaxel efflux from all ovarian cancer cell lines regardless of ABCB1 expression level, thus indicating that an ABCB1 transporter plays a key role in paclitaxel resistance of ovarian cancer cells. In another study (Gao et al., 2014), Duan et al. (2014) found that APP negatively regulated APP precursor protein hydrolysate. A activity during an *in vitro* fertilization study of plasma

Table 5. Binding of Compounds to Targets.

	ABCB1 (6c0v)	APP (5kqf)	CA4(2oz7)	IL2(1qvn)	LGALS4 (6wab)	MAPT (4tqe)	MMP2 (7xjo)	MAPK14 (1oz1)
1	-9.5	-7.9	9.7	-7.7	-6.5	-8.7	-5.6	-7.9
5	-10.2	-8.6	-6.2	-8.5	-6.6	-8.4	-6.3	-8.7
6	-9.4	-7.7	-5	-7.1	-5.7	-7.2	-5.9	-7.9
8	-10.4	-8	-5.8	-8.3	-6	-8.2	-6.8	-8.9
9	-10.8	-8.2	-3	-7.9	-7	-7.9	-5.8	-8.1
Primary ligand	-7.4	-2.6	-9.8	-5.6	-7.4	-3.1	-7.5	-9

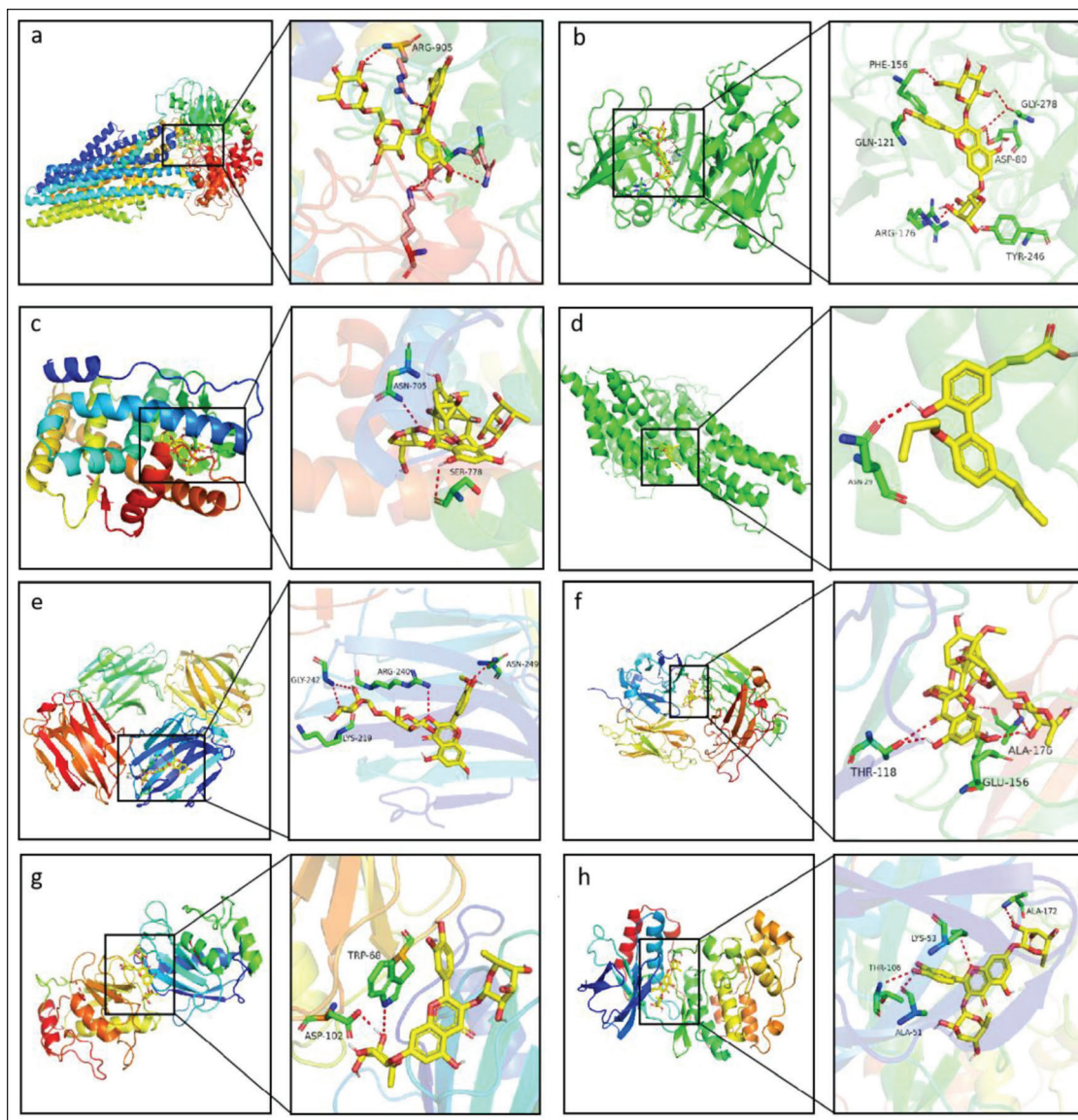


Figure 8. Molecular Docking of the Five *C. hybridum* Active Compounds and Primary Ligands to Nine Potential Targets Based on the Lowest Binding Energy Configurations. The Figure was Prepared Using PyMOL, with a Panoramic View Shown at Left and Ligand Detail Map Shown at Right (a) ABCB1, 6c0v (isorhamnetin-3-acacia disaccharide), (b) APP, 5kqf (kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-pyranoside), (c) CA4, 2oz7 (isorhamnetin-3-O-β-D-furanosyl(1→2)-O-[α-L-rhamnopyranoside(1→6)]-β-D-glucopyranoside), (d) IL-2, 1qvn (kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-pyranoside), (e) LGALS4, 6wab (isorhamnetin-3-acacia disaccharide), (f) MAPT, 4tqe (isorhamnetin-3-O-β-D-furanosyl(1→2)-O-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside), (g) MMP2, 7xjo (quercetin-3,7-di-rhamnose), and (h) MAPK14, 1oz1 (quercetin-3,7-di-rhamnose).

amyloid- β 40 regulation of ovarian cancer development (Duan et al., 2014). More recently, in 2020, Chulpanova et al. confirmed that amniotic fluid-derived mesenchymal stem cells (MSCs) expressing IL-2 could induce ovarian cancer cell apoptosis *in vivo* (Chulpanova et al., 2020), while the results of another study revealed that high expression of MAPT was linked to poor ovarian cancer prognosis, thus indicating that inhibition of MAPT expression could improve ovarian cancer prognosis (Schroeder et al., 2019).

Conclusion

In this study, the best extraction process of the total flavonoids content from *C. hybridum* was screened by orthogonal experiment; several *C. hybridum* compounds with potential anti-ovarian cancer activity were identified using UPLC-Q-Exactive MS combined with multivariate analysis (PLS analysis). Correlation analysis was studied to explore internal relationships between *C. hybridum* chemical constituents and pharmacological effects to discover active compounds responsible for known anti-ovarian cancer therapeutic effects of *C. hybridum*. It is a method for feasible, rapid, and useful screening of potential components. Molecular docking results revealed five potential bioactive *C. hybridum* compounds with anti-ovarian cancer activity that were predicted to competitively bind to IL-2, MAPT, LGALS4, and MMP2 cancer cell targets. And from the results of molecular docking, it can be clearly found that these components are supported by relevant literature, which proves the accuracy and feasibility of screening active components by this method. Furthermore, screening methods based on such models offer advantages of simple execution, rapid and accurate results, high-throughput capacity, and low cost that make it possible to quickly screen TCMs for potentially active compounds from a large number of chemical components. This provides a reference for the application and further development of *C. hybridum* in anti-ovarian cancer.

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Abbreviations

UPLC: Ultra performance liquid chromatography; MS: Mass spectrometry; m/z : mass-to-charge ratio; HPLC: High performance liquid chromatography; ECD: Electrochemical detection; DMEM: Dulbecco's modified eagle medium; PBS: Phosphate-buffered saline; TFC: Total flavonoid content; TPC: Total polyphenol content.

Authors Contribution

Conceptualization, R.-X.L. and S.-L.Y.; Methodology, X.-Q.F.; validation, D.-D.C.; Formal analysis, X.-Q.F. and D.-D.C.; Investigation, X.-Q.F.; Resources, R.-X.L.; Data curation, S.-L.Y.

and X.-Q.F.; Writing—original draft preparation, D.-D.C.; Writing, review and editing, R.-X.L.; Visualization, D.-D.C.; Supervision, R.-X.L.; Project administration, S.-L.Y.; Funding acquisition, R.-X.L. and S.-L.Y. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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