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Zineng Zhou

9231810719@stu.njau.edu.cn

Nanjing Agricultural University

Suxiang Huang

Nanjing Agricultural University

Bingjie Guo

Nanjing Agricultural University

Jinhang Zhou

Nanjing Agricultural University

Yifu Hu

Nanjing Agricultural University

Haitao Tian

Nanjing Agricultural University

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Bioinformatics-Based Investigation of the Mechanisms by which *Gastrodia Elata* and Dauricine Enhance Hypoxia Adaptation

Zineng Zhou^{*}, Suxiang Huang, Bingjie Guo, Jinhang Zhou, Yifu Hu, Haitao Tian

¹ College of Food Science and Technology, Nanjing Agricultural University, 210095, Nanjing, China

^{*}corresponding author: 9231810719@stu.njau.edu.cn

Abstract

Gastrodia elata Blume (GE), a renowned traditional Chinese medicine (TCM), has been shown to possess anti-hypoxic properties. However, the primary active components and their specific mechanisms of action remain unclear due to its chemical complexity. To this end, active components of GE were identified from online databases, including dauricine. Potential targets were predicted using SwissTargetPrediction, and hypoxia-related targets were collected from disease databases. The intersection of these targets revealed common candidates. Subsequent protein–protein interaction (PPI) network analysis, gene ontology (GO) enrichment analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis identified AKT1, AKT2, AKT3, PIK3CA, PIK3CB, and PIK3CD as core targets and key signaling pathways. The binding affinity between these core components and targets was further validated through molecular docking and 100-ns molecular dynamics simulations. Notably, 4-(4'-hydroxybenzyloxy)benzyl methyl ether and 4,4'-dihydroxy dibenzyl ether from GE, along with dauricine, enhance hypoxia adaptation primarily via the PI3K/AKT and HIF-1A signaling pathways. These findings provide scientific evidence for the application of TCM in ameliorating hypoxia-related health issues.

Keywords: *Gastrodia elata* Blume, dauricine, hypoxia adaptation, network pharmacology, molecular docking, molecular dynamics simulation

Introduction

Chronic hypoxia, a prevalent pathophysiological condition, is associated with numerous major diseases, significantly impacting patient quality of life and causing cognitive dysfunction, metabolic disorders, and multi-organ damage^[1]. Faced with this global health challenge, developing intervention strategies that can effectively enhance the body's hypoxic adaptation and alleviate

hypoxic injury is of significant clinical and social importance.

Traditional Chinese Medicine (TCM) has attracted widespread attention due to their multi-target and multi-pathway characteristics. *Gastrodia elata* Blume (GE), a well-known medicinal and edible homologous substance, has a long history of use in China and has been shown to protect the nervous system^[2], exhibit antioxidative effects^[3], and demonstrate anti-hypoxic efficacy^[4]. However, its complex chemical composition, including gastrodin, 4-hydroxybenzyl alcohol, parishin compounds, polysaccharides, and various other substances^[5], necessitates a systematic elucidation of the components responsible for its anti-hypoxic effects and the molecular mechanisms. Meanwhile, TCM serves as pure natural compound libraries and offer potential for drug development through minor components, as seen in *Panax notoginseng* where minor saponins often exhibit superior bioavailability and bioactivity to major saponins^[6]. Incorporating these minor components into the research framework is conducive to exploring the common mechanisms of different structural types of natural products against hypoxia and may also provide clues for discovering new active molecules.

In this study, network pharmacology, molecular docking, and molecular dynamics simulation techniques are integrated to systematically investigate the mechanisms by which GE and other potential active components enhance hypoxia adaptation. This integrated computational approach provides a rational, evidence-based framework for prioritizing bioactive compounds worthy of subsequent experimental validation and clinical development as novel anti-hypoxia therapeutics derived from TCM resources.

Results

Identification of bioactive components from GE and intersection targets with hypoxia

Based on pharmacokinetic properties from SwissADME and other literature-reported bioactive compounds, components of GE were screened using the Herb database, resulting in 29 components selected for further analysis (**Table 1**). Potential protein targets were subsequently predicted via SwissTargetPrediction (with a probability threshold > 0), which identified 520 candidates. Separately, a total of 2,836 hypoxia-related genes were retrieved from PharmGKB, OMIM, GeneCards (score ≥ 0.55), and DisGeNET using “hypoxia” as a search keyword. Intersection analysis performed with Venny 2.1.0 revealed 270 common targets shared between the component-related and hypoxia-associated genes (**Fig. 2A, Table 2**).

Core hypoxia-associated targets and pathways identification by PPI network

Based on the 270 common targets, a protein-protein interaction (PPI) network was constructed using the STRING database (Homo sapiens; confidence score ≥ 0.9), resulting in 216 nodes and 692 edges with an average degree of 6.407, which was subsequently visualized in Cytoscape (**Fig. 2B**). Through the application of six CytoHubba algorithms (MCC, MNC, EPC,

Stress, Degree, Radiality), seven core topological targets (AKT1, AKT2, AKT3, PIK3CA, PIK3CB, PIK3CD, and HSP90AA1) were identified (**Fig. 2C, 2D**). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses ($P < 0.01$) of these 270 targets revealed significant involvement in biological processes, cellular components, and molecular functions (**Fig. 3A**). KEGG analysis highlighted 184 significant pathways, with the PI3K-Akt and MAPK signaling pathways being predominant for hypoxia adaptation (**Fig. 3B**). To obtain a more comprehensive functional profile, a GeneMANIA-based functional association (GMFA) network was generated for the seven core genes, expanded to include the top 10 associated genes per input, thereby forming a union set of 319 targets (**Fig. 4A**). Subsequent GO and KEGG analyses of this expanded set reinforced enrichment in similar biological themes and pathways, particularly the PI3K-Akt and HIF-1 signaling pathways (**Fig. 4B, 4C, 4D, 4E–4G**), with a detailed mechanistic schematic provided (**Fig. 5**)^[79]. A component-target-pathway-disease network was then constructed (**Fig. 6**), and topological analysis using CytoNCA pinpointed eight key active components and 11 major functional proteins (**Fig. 7, Table 3**). Ultimately, the intersection of core topological and functional targets yielded six final core targets: AKT1, AKT2, AKT3, PIK3CA, PIK3CB, PIK3CD. All six were successfully validated within the iHypoxia database, thereby confirming their established roles in hypoxia adaptation mechanisms.

Affinity validation between core components of GE and core hypoxia-associated targets

To validate the interactions between the core components and the key targets, molecular docking was performed using the identified core targets (AKT1, AKT2, AKT3, PIK3CA, PIK3CB, PIK3CD). Concurrently, three clinically used pharmaceuticals (acetazolamide, dexamethasone, and nifedipine), known to enhance hypoxia adaptation in humans, were included as positive controls^[10]. Since binding affinity between ligands and receptors is inversely correlated with binding energy, a more negative binding value corresponds to stronger interactions. Based on established criteria, binding energies below -4.25 kcal/mol indicate moderate binding affinity, values below -5.00 kcal/mol reflect good binding affinity, and those below -7.00 kcal/mol represent strong binding affinity^[11]. Results demonstrated that all evaluated small molecules exhibited negative binding energies below zero with their protein targets, indicating spontaneous ligand-receptor binding (**Fig. 8**). Notably, 4-(4'-hydroxybenzyloxy)benzyl methyl ether and 4,4'-dihydroxy dibenzyl ether not only displayed the highest degree values in network analysis but also consistently showed binding energies below -5.00 kcal/mol with nearly all key protein receptors (**Table 4**). These two compounds were then identified as principal contributors to the hypoxia adaptation-enhancing effects of GE. Docking results for gastrodin further reaffirm its excellent capacity to enhance hypoxia adaptation. Furthermore, dauricine exhibits the strongest binding affinity for AKT2, AKT3, and PIK3CD (**Fig. 9**), indicating its considerable research value as a promising candidate for improving hypoxia tolerance.

Confirmation of the stable binding between core components of GE and core hypoxia-associated targets by ADME

profiling and molecular dynamics simulations

The ADME properties of four key active ingredients were evaluated, revealing favorable bioavailability scores and compliance with Lipinski's Rule of Five (**Table 5**). To further validate the binding interactions observed in molecular docking, 100 ns molecular dynamics (MD) simulations were conducted on the top three complexes: AKT2-dauricine, AKT3-dauricine, and PIK3CD-dauricine. Root-mean-square deviation (RMSD) analysis indicated that both the AKT2 and AKT3 complexes reached stable equilibrium within 40 ns, with minimal fluctuations remaining within 0.1–0.2 nm, while the PIK3CD complex exhibited larger fluctuations before stabilizing in the final 20 ns (**Fig. 10A, B**). Root-mean-square fluctuation (RMSF) profiles confirmed overall protein stability, though localized flexible regions, most notably in PIK3CD near residues 200 – 300 and 1000, were observed (**Fig. 10C–E**). Moreover, consistent radius of gyration (Rg) and solvent accessible surface area (SASA) values throughout the simulation suggested that ligand binding maintained compact, native-like protein structures (**Fig. 10F, G**). Hydrogen bond analysis showed dynamic yet persistent interaction patterns, supporting complex stability (**Fig. 10H–J**). Gibbs free energy landscapes constructed from principal components revealed deep, compact global energy minima for all systems, indicating high structural and kinetic stability of the dominant conformations (**Fig. 11A–C**). Finally, MM/PBSA-based binding free energy calculations demonstrated favorable binding affinities for all complexes, with significantly stronger interactions observed for AKT3 and PIK3CD than for AKT2 (**Fig. 12A–C, Table 6**). Energy decomposition analysis revealed van der Waals interactions as the primary binding driving force, with key residues such as ARG-176 (AKT2), PRO-67, PRO-658, VAL-657 (AKT3/PIK3CD) playing major roles. These results collectively provide a mechanistic explanation for the stable binding even during periods of absent hydrogen bonds.

Discussion

Hypoxia is a key factor in a wide range of diseases, with significant advancements in both mechanistic investigations and drug development. The PI3K/AKT signaling pathway is central to cellular hypoxia responses, involved in diverse pathophysiological processes from metabolic adaptation to cell survival. Recent research highlights its critical role in diseases associated with chronic intermittent hypoxia (CIH)^[12], such as pulmonary arterial hypertension (PAH). In a CIH-induced PAH model, hypoxia significantly upregulates the expression of procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2), and PLOD2 knockdown inhibits glycolysis, proliferation, and migration of pulmonary arterial smooth muscle cells (PASMCs) by suppressing the PI3K/AKT pathway, suggesting PLOD2 as a novel therapeutic target for obstructive sleep apnea (OSA)-related PAH^[13]. HIF-1 is the primary transcriptional regulator of cellular oxygen sensing and adaptation^[14]. In mouse embryonic stem cells (mESCs), HIF-1 α displays a dynamic "bind-release-rebind" pattern during normoxia-to-hypoxia transition, modulating pluripotency via the LIFR/JAK-STAT pathway^[15]. Dauricine, a natural bis-benzylisoquinoline alkaloid from TCM GE, shows

promise as a drug candidate with excellent pharmacokinetic properties, inhibiting various cancers by targeting PI3K/AKT, mTOR, HIF-1, etc ^[1617]. However, the role of dauricine in hypoxia-related contexts remains relatively unexplored.

This study delineates the multi-component and multi-target mechanisms mediating the hypoxia adaptation-enhancing effects of GE. Network analysis identified 29 bioactive compounds, among which 4-(4'-hydroxybenzyloxy)benzyl methyl ether and 4,4'-dihydroxy dibenzyl ether, gastrodin, and dauricine emerge as key bioactive components. These compounds synergistically target a core protein network, notably AKT1, AKT2, AKT3, PIK3CA, PIK3CB, and PIK3CD, findings further validated by the iHypoxia database to be hypoxia-associated. Additionally, functional enrichment analyses consistently highlighted the PI3K/AKT and HIF-1 signaling pathways as the central mechanistic routes, aligning with established knowledge where PI3K/AKT activation mitigates apoptosis and inflammation ^[18], and HIF-1 α stabilization orchestrates adaptive responses like erythropoiesis and angiogenesis ^[1920]. Molecular docking assays confirmed robust binding affinities between these core components and targets, particularly for 4-(4'-hydroxybenzyloxy)benzyl methyl ether, 4,4'-dihydroxy dibenzyl ether, and dauricine, which showed binding energies below -5.00 kcal/mol with key targets like AKT2, AKT3, and PIK3CD. Subsequently, 100-ns MD simulations and MM/PBSA calculations for dauricine-target complexes (AKT2, AKT3, PIK3CD) verified stable binding, predominantly driven by van der Waals interactions with critical hydrophobic residues (e.g., PRO-67, VAL-657), a finding that explains the observed high affinity and specificity. Furthermore, favorable ADME profiles of these core compounds further support their drug-likeness. Future experimental validation using hypoxic cell and animal models is essential to confirm the activation of the PI3K/AKT and HIF-1 pathways and to translate these findings into novel anti-hypoxia therapeutics derived from traditional medicinal resources.

Methods

Acquisition of chemical constituents and screening of active components in GE

To ensure the reliability and comprehensiveness of the data, this study collected the primary active components of GE through multiple channels ^[21]. Initially, the active components of GE were retrieved from four TCM-focused databases: HERB (<http://herb.ac.cn>), SymMap (<http://www.symmap.org/>), TCMID (<http://www.megabionet.org/tcmid/>), and TCMSP (https://www.tcmsp-e.com/load_intro.php). Subsequently, SwissADME (<http://www.swissadme.ch>) was utilized to predict additional chemical components based on known drug molecular structures. Chemical components with a GI absorption rating of “high” were deemed to possess high bioavailability. Components receiving at least three affirmative “yes” results across five common pharmacophore rules (Lipinski, Veber, Egan, Ghose, Muegge) were considered to exhibit good pharmacophore properties. These selected compounds, supplemented with those from existing research, were designated as active components

139 for subsequent analysis^[22].

140 **Prediction of targets for active components**

141 Based on the SMILES of known drug molecules, Swiss TargetPrediction (<https://swisstargetprediction.ch/>) was employed to
142 predict the action targets of the screened active components^[23]. Specifically, targets with a non-zero probability were classified
143 as potential drug targets and included in further analyses.

144 **Acquisition of potential hypoxia adaptation-related targets**

145 To identify potential hypoxia adaptation-related targets, “hypoxia” was used as the keyword to retrieve all relevant targets from
146 four biomedical databases: PharmGKB (<https://www.pharmgkb.org/>), OMIM (<https://omim.org>), Genecards
147 (<https://www.genecards.org>), and Disgenet (<https://disgenet.com/>). The disease targets obtained from each database were
148 merged using the online tool jvenn (<https://jvenn.toulouse.inrae.fr/app/example.html>), with a Venn diagram generated to
149 visualize the union of these targets.

150 **PPI network construction and screening of core topological targets**

151 Initially, the intersection between active component targets and potential hypoxia adaptation targets was identified using the
152 online tool Venny 2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/>). Following this, the intersection results were imported into
153 the String database (<https://cn.string-db.org/>) for PPI network analysis, with species restricted to Homo sapiens and a minimum
154 required interaction score of 0.9. Free nodes were hidden to filter the interaction network of key proteins^[24]. Subsequently, the
155 PPI results were visualized using Cytoscape 3.8.0. Each protein's importance was scored using six algorithms from CytoHubba
156 (MCC, MNC, EPC, Stress, Degree, Radiality). The top 20 targets calculated by each algorithm were visualized. Subsequently,
157 their shared targets were selected as core topological targets for subsequent analysis.

158 **GMFA network analysis of core targets**

159 To contextualize core topological targets within a broader functional landscape, GeneMANIA (<https://genemania.org>), a robust
160 tool for exploring gene functional relationships, was employed to construct an association network. For each core gene, the top
161 10 genetically or physically associated partners were incorporated to enrich the network, thereby revealing a more
162 interconnected functional module. Additionally, a union operation was performed between the expanded target set and the
163 original target set, and the results were visualized using Cytoscape 3.8.0.

164 **GO and KEGG enrichment analyses**

165 GO enrichment analysis, a hypergeometric test-based statistical approach, aims to identify statistically significant
166 overrepresentation of a gene set (such as differentially expressed genes) in three functional categories: Biological Process (BP),

Cellular Component (CC), and Molecular Function (MF)^[25]. KEGG is a database integrating genomic, chemical, and systems functional information. It aims to reveal the functions and interactions of genes and molecules within biological networks (e.g., metabolism, signal transduction) through pathway maps^[26].

Specifically, GO functional enrichment analysis was performed using the DAVID database (<https://david.ncifcrf.gov>) to explore their potential biological functions^[27], while KEGG pathway enrichment analysis was conducted using the KOBAS database (<https://bioinfo.org/kobas>) to investigate biological pathways and potential signaling pathways^[28]. A threshold of $P < 0.01$ was set for statistical significance in both analyses. Key results from GO and KEGG analyses were visualized on the MicroBioinformatics online platform (<https://www.bioinformatics.com.cn>)^[29], whereas the association relationships between primary genes and entries were visualized using CNSknowall (<https://www.cnsknowall.com/>).

Construction of component-target-pathway-disease networks and screening of core components/targets

Data on drug-components, component-targets, disease-targets, and pathway-targets were integrated into Cytoscape 3.8.0 for visualization. The CytoNCA module was employed to conduct topological parameter analysis, characterize interaction relationships within the network, and identify core active components and core functional targets.

Core target screening and validation

The intersection of screened core topological targets and core functional targets was identified as core targets for hypoxia adaptation enhancement. To improve prediction accuracy^[30], these core targets were further validated using the iHypoxia database (<https://ihypoxia.omicsbio.info>).

Molecular docking and ADME validation

The affinity between the core components and their core targets was validated through molecular docking. Initially, the crystal structures of the core protein receptors were downloaded from the PDB database (<https://www.rcsb.org/>)^[31]. Based on the SMILES of each component, the 3D structures of small-molecule ligands (core components and three commonly used Western drugs for hypoxia treatment: Acetazolamide, Dexamethasone, Nifedipine) were obtained from NCI/CADD (<https://cactus.nci.nih.gov/>). To verify prediction accuracy, ChemSpider (<https://www.rsc.org/>) was used to re-predict the small-molecule 3D structures, with results compared in PyMOL 2.6. Subsequently, all receptor proteins were processed in PyMOL 2.6 to remove water molecules and small molecules. The 3D structures of the small-molecule ligands were subjected to energy minimization using the MMFF94 force field in Chem3D 22.0.0 software (10,000 iterations; RMSD = 0.001) to obtain stable, low-energy conformations.

Docking simulations were then performed using AutoDock Vina 1.1.2, with 20 iterations set per run and the optimal docking

conformation retained for each ligand-receptor pair^[32]. The three pairs with the best docking affinities were selected, and their interactions were visualized in three dimensions using PyMOL 2.6.

Finally, the ADME properties of the validated core active components were evaluated using SwissADME.

Computational MD simulations and MM/PBSA binding free energy calculations

To further validate the binding affinity between core targets and small molecules, MD simulations were performed on the three optimal complexes identified from molecular docking. The initial structures of the proteins and small molecules were adopted from those processed in Section 3.8.1, with simulations conducted using GROMACS 2025 software^[33]. The AMBER99SB-ILDN force field was employed to describe the protein^{[34],[35]}, while the topology parameters of the ligand were generated using the universal GAFF2 force field^[36]. The topology files of the proteins and ligands were merged to construct the complex system. The complex was then placed in a cubic periodic boundary box, ensuring a minimum distance of 1.2 nm between the complex and box boundaries. The TIP3P water model was used to solvate the systems^[37], and Na⁺/Cl⁻ ions were added to neutralize the net charge and adjust the ionic concentration to 0.15 M, mimicking physiological conditions.

After 1,000 steps of energy minimization via the steepest descent method to eliminate unreasonable interatomic contacts, the systems were equilibrated for 1 ns at 310 K under NVT ensemble conditions using a V-rescale thermostat (coupling constant = 0.1 ps). Subsequently, the systems were equilibrated for 1 ns in the NPT ensemble at 310 K and 1 bar pressure (using a Berendsen pressure bath with a coupling constant of 0.2 ps). Finally, a 100 ns NPT production simulation (310 K, 1 bar) was conducted, with temperature and pressure controlled by a V-rescale thermostat (coupling constant 0.1 ps) and a Parrinello-Rahman pressure bath (coupling constant 0.1 ps), respectively. All simulations used an integration step size of 2 fs, and trajectories were saved every 10 ps for subsequent analysis. Data analysis and visualization of simulation results were performed using Origin software.

Binding free energies were computed using the MM/PBSA method, a widely adopted approach for evaluating protein-ligand interaction affinities^[38], with more negative values indicating more favorable binding. Calculations were based on the 100 ns MD simulation trajectories, following the corresponding formula^[39].

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311 **Author contributions statement**

312 Zineng ZHOU: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Visualization, Writing -
313 Original Draft. Suxiang HUANG: Methodology, Investigation, Data Curation, Writing - Review & Editing. Bingjie GUO:
314 Resources Data Curation, Writing - Review & Editing. Jinhang ZHOU: Investigation, Visualization, Writing - Review &
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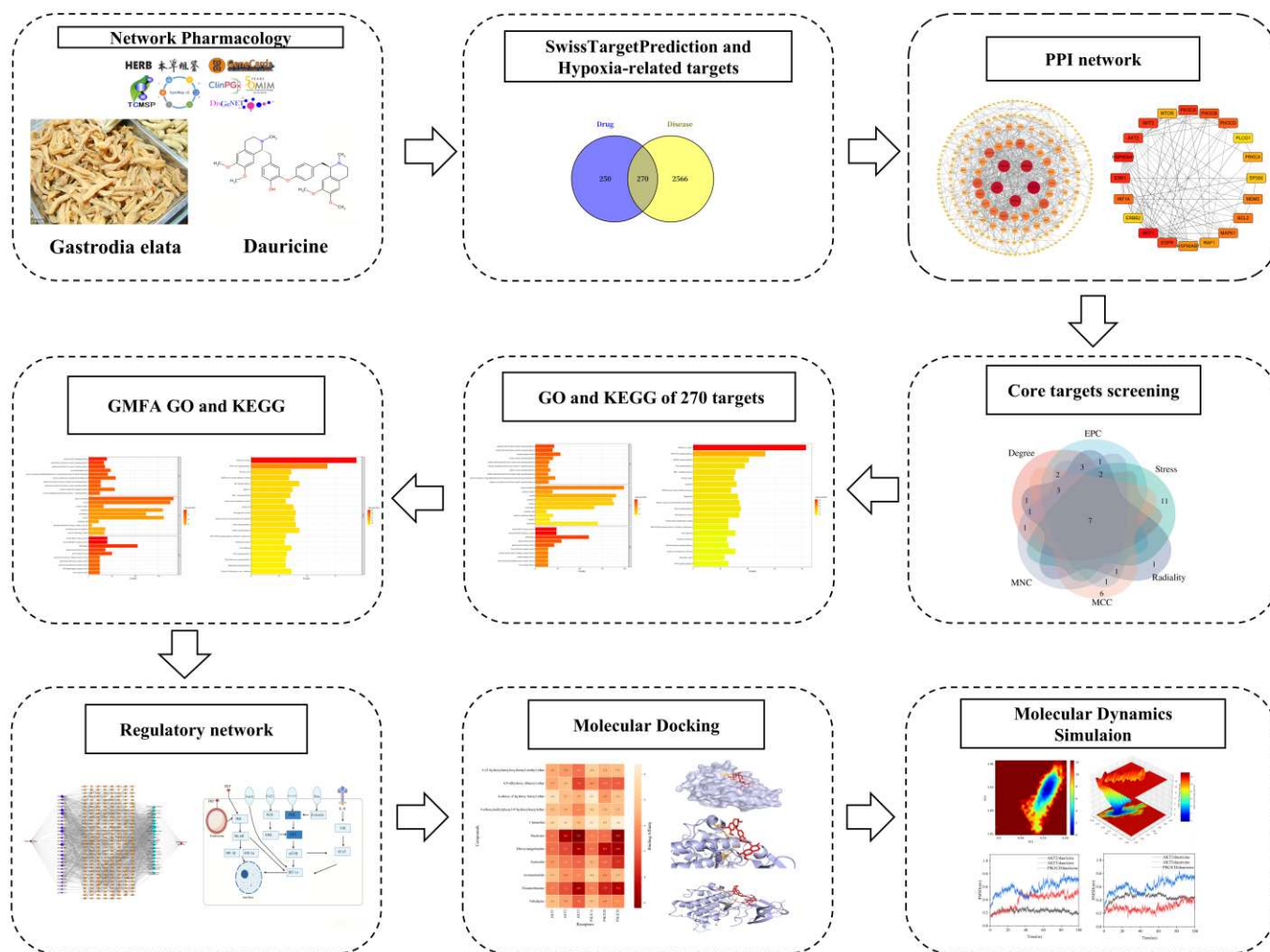
320 The authors declare no competing interests.

321 **Ethics approval and consent to participate**

322 This study did not involve human participants or animals and solely utilized publicly available data; therefore, ethical approval
323 and consent to participate are not applicable.

324 **Data availability statement**

325 The online version contains supplementary material available at <https://data.mendeley.com/datasets/xypv74wsjv/1>.

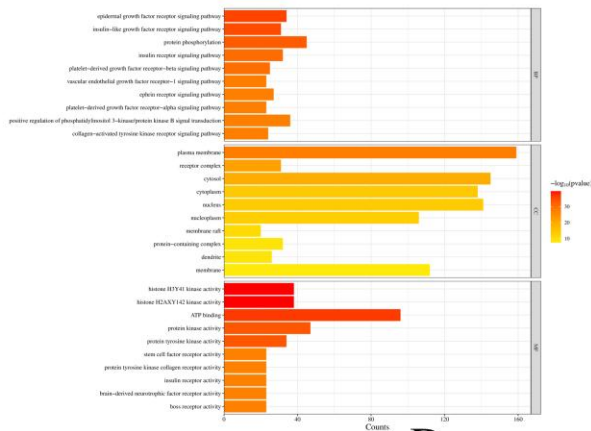


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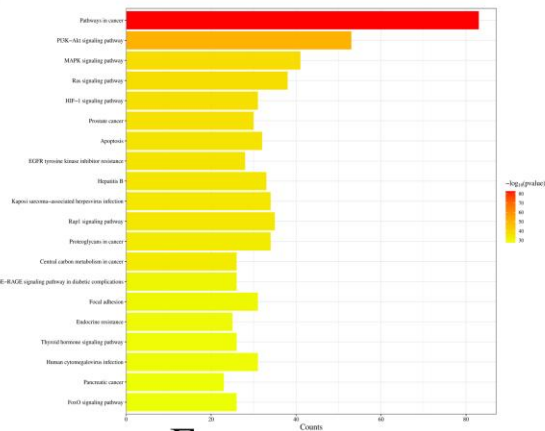
327 **Fig. 1** Research flowchart

329 **Fig. 2** Screening of common targets and core targets. (A) Common targets of components and drugs. (B) PPI network
330 with shared targets. (C) Core target acquisition using six algorithms based on CytoHubba. (D) The top 20 core targets
331 identified through six algorithmic screening processes.

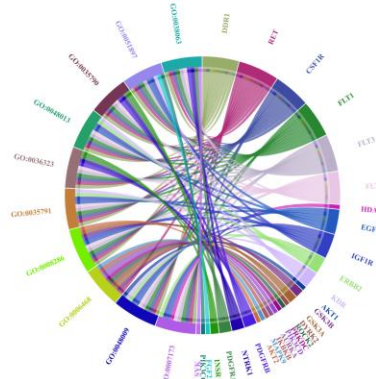
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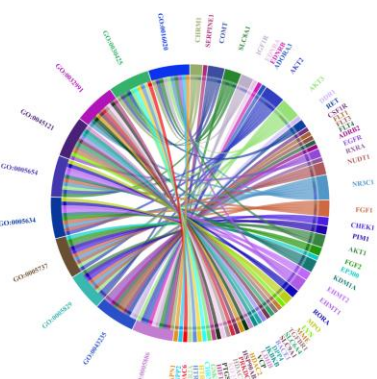
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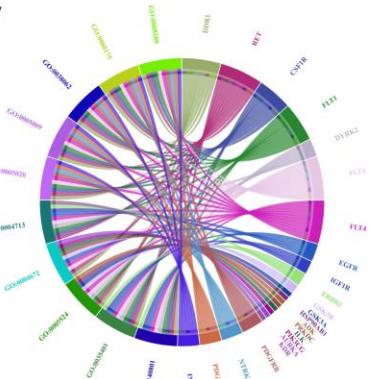
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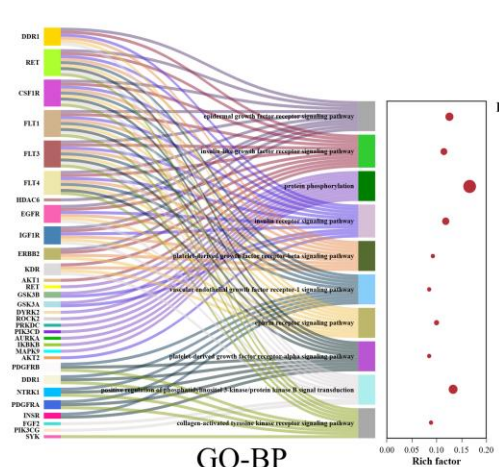
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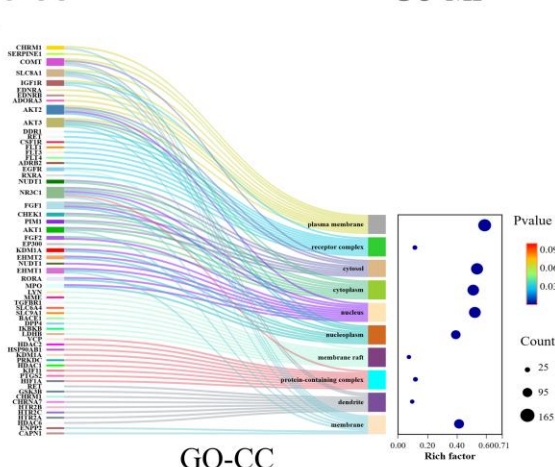
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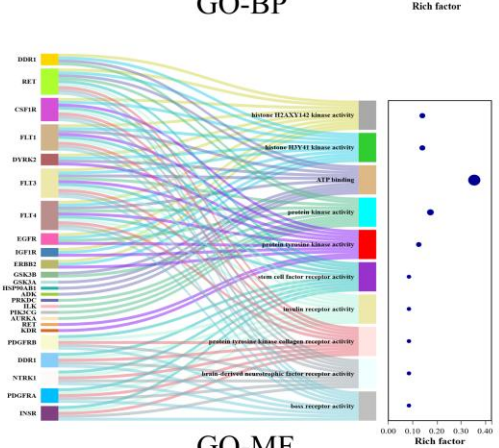
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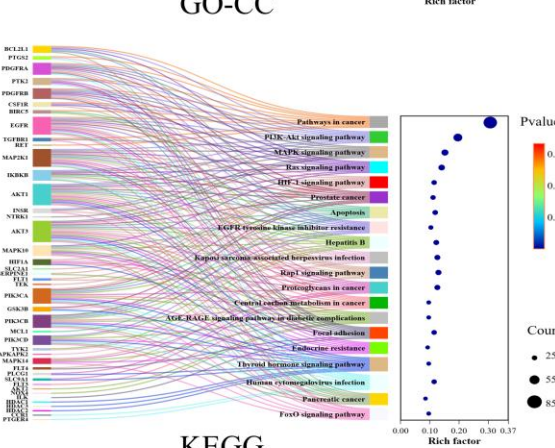
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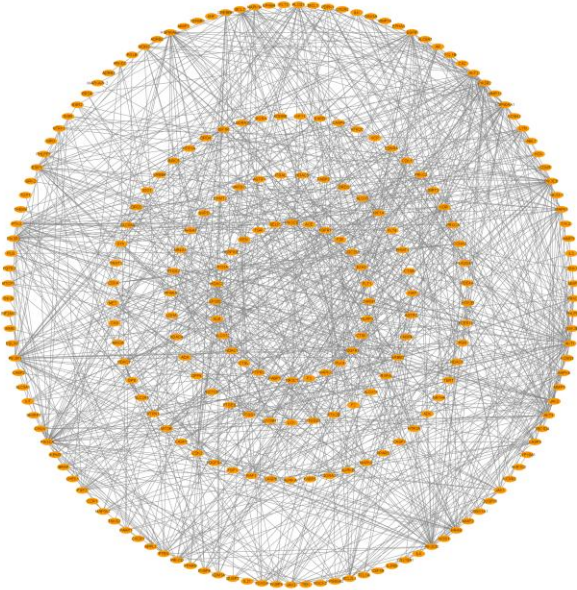


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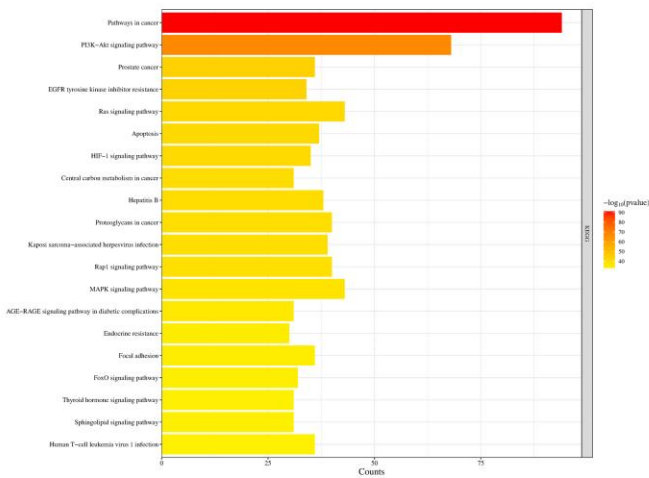


333 **Fig. 3** GO and KEGG enrichment analysis of 270 common target genes. (A) The top 10 entries in BP, CC and MF based
334 on P value. (B) The top 20 entries in KEGG based on P value. (C-E) Key targets associated with the top 10 entries in BP,
335 CC, and MF. (F-H) Key targets associated with the top 10 entries in BP, CC, MF and number of shared genes per entry.
336 (I) Key targets associated with the top 20 entries in KEGG and number of shared genes per entry.

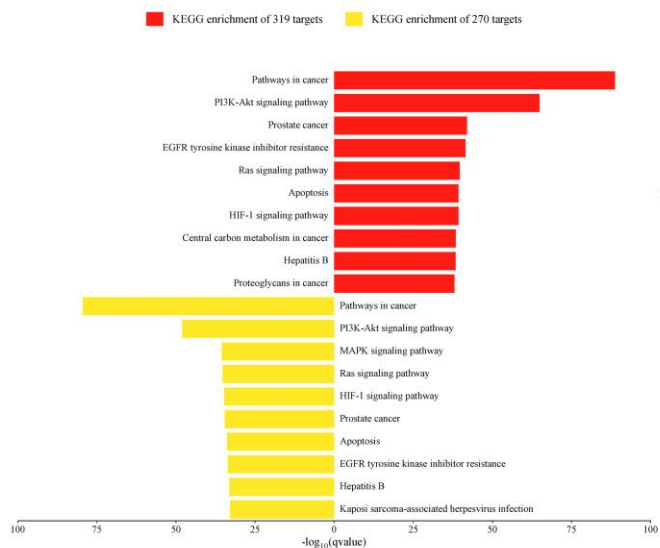
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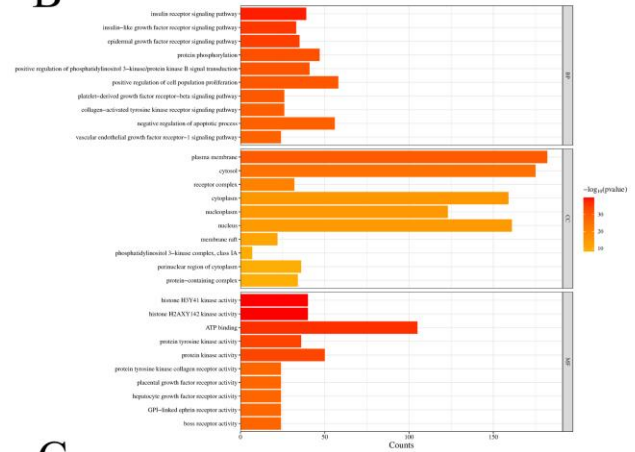
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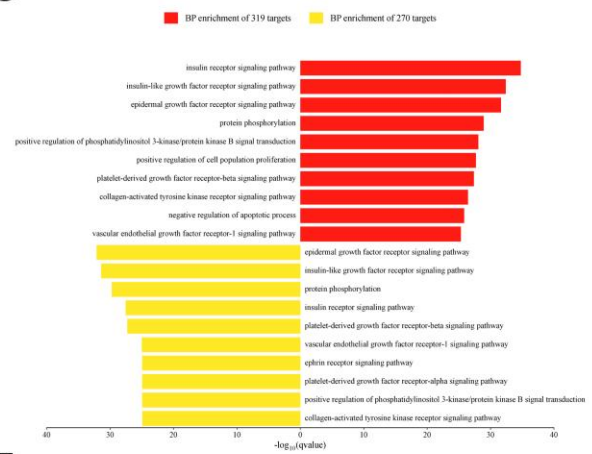
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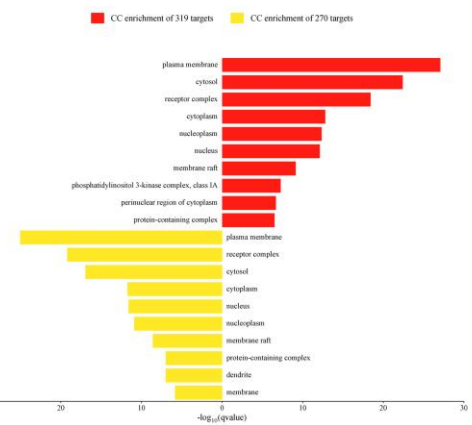
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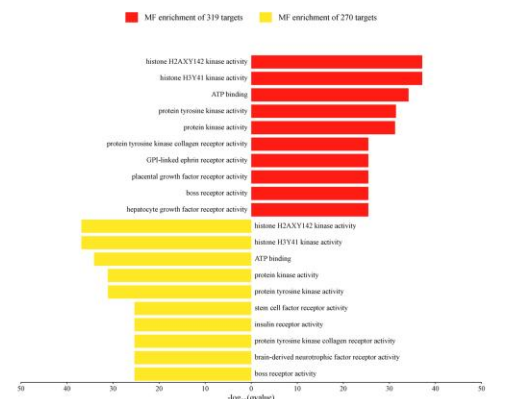
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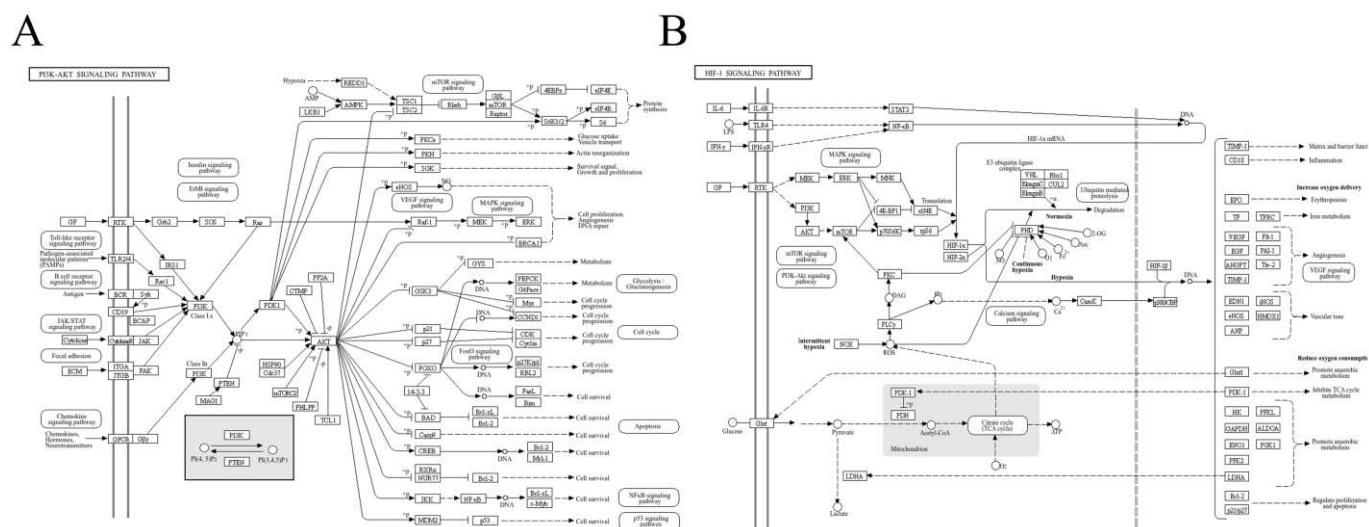


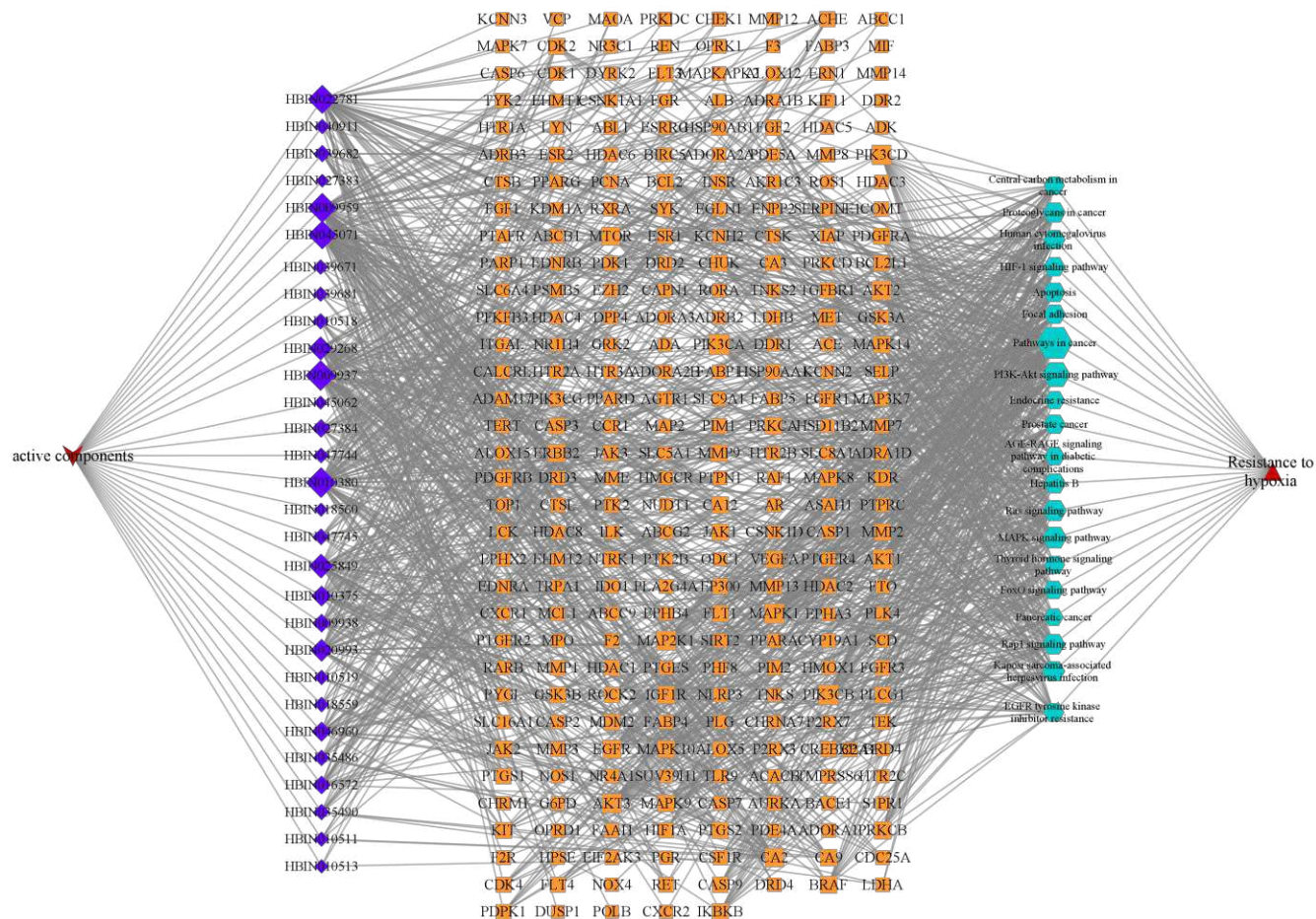
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338 **Fig. 4** GO and KEGG enrichment analysis of 319 common target genes. (A) PPI network with 319 targets. (B) The top 10
339 entries in BP, CC and MF based on P value. (D) The top 20 entries in KEGG based on P value. (C, E, G) Comparison of
340 the top 10 entries for BP, CC, and MF across 319 and 270 target points. (F) Comparison of the top 20 entries for KEGG
341 across 319 and 270 target points.

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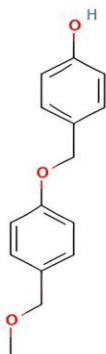




372

373 **Fig. 6** Active component-target-pathway-disease network

A



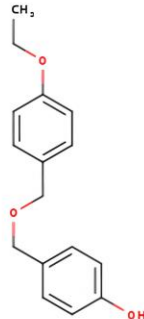
4-(4'-Hydroxybenzyloxy)benzyl methyl ether

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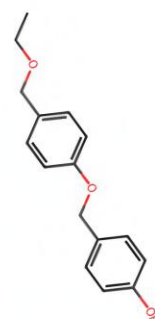
4,4'-dihydroxy dibenzyl ether

C



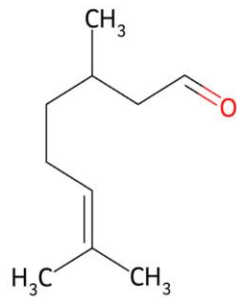
4-ethoxy-4'-hydroxy benzyl ether

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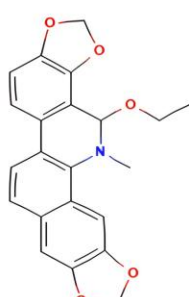
4-ethoxymethylphenyl-4'-hydroxybenzylether

E



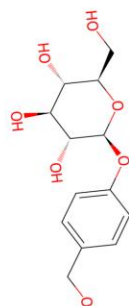
Citronellal

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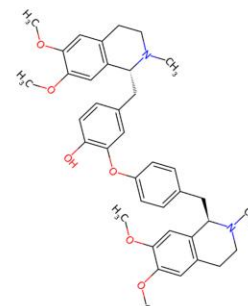
Ethoxysanguinarine

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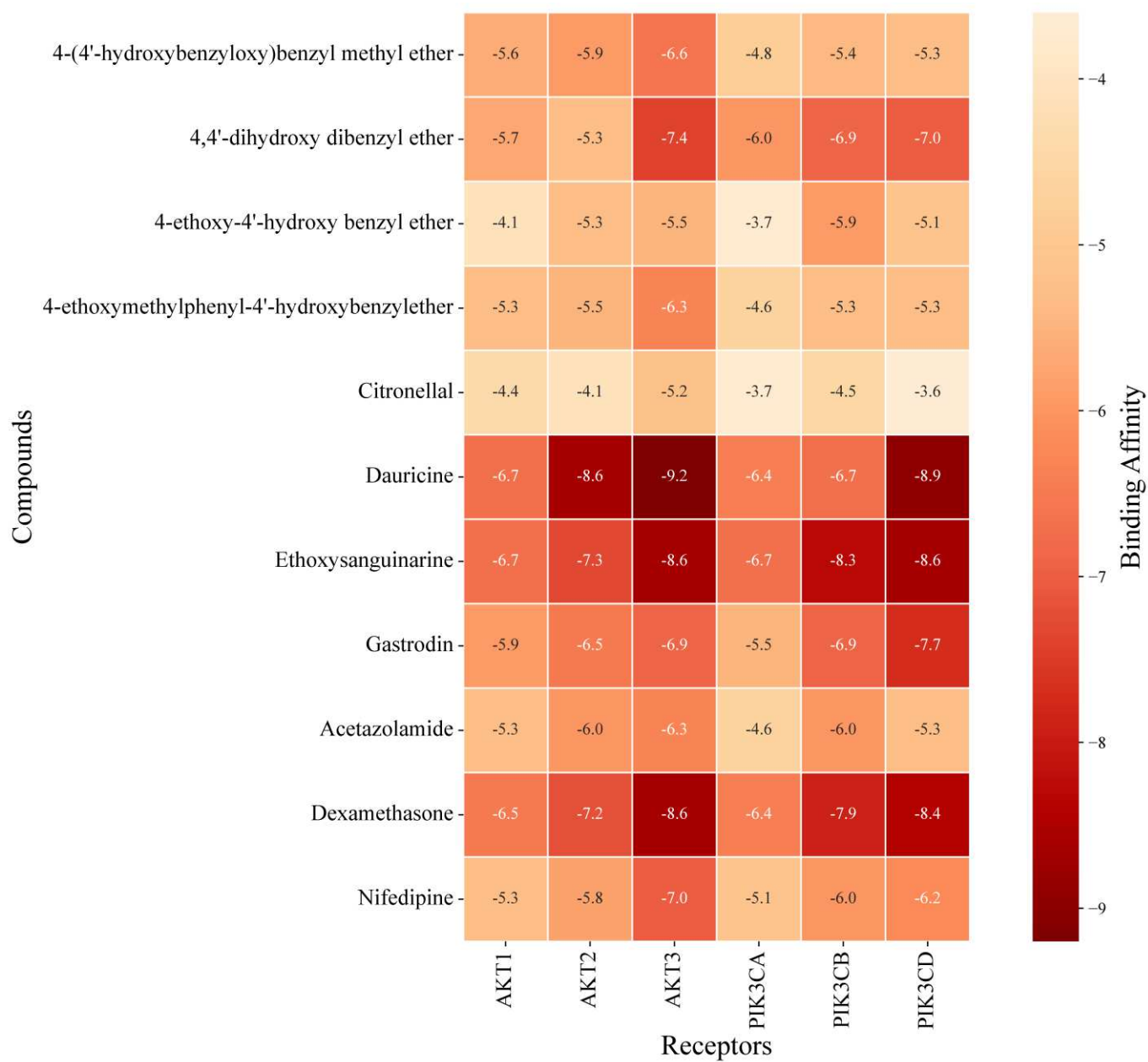
Gastrodin

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Dauricine

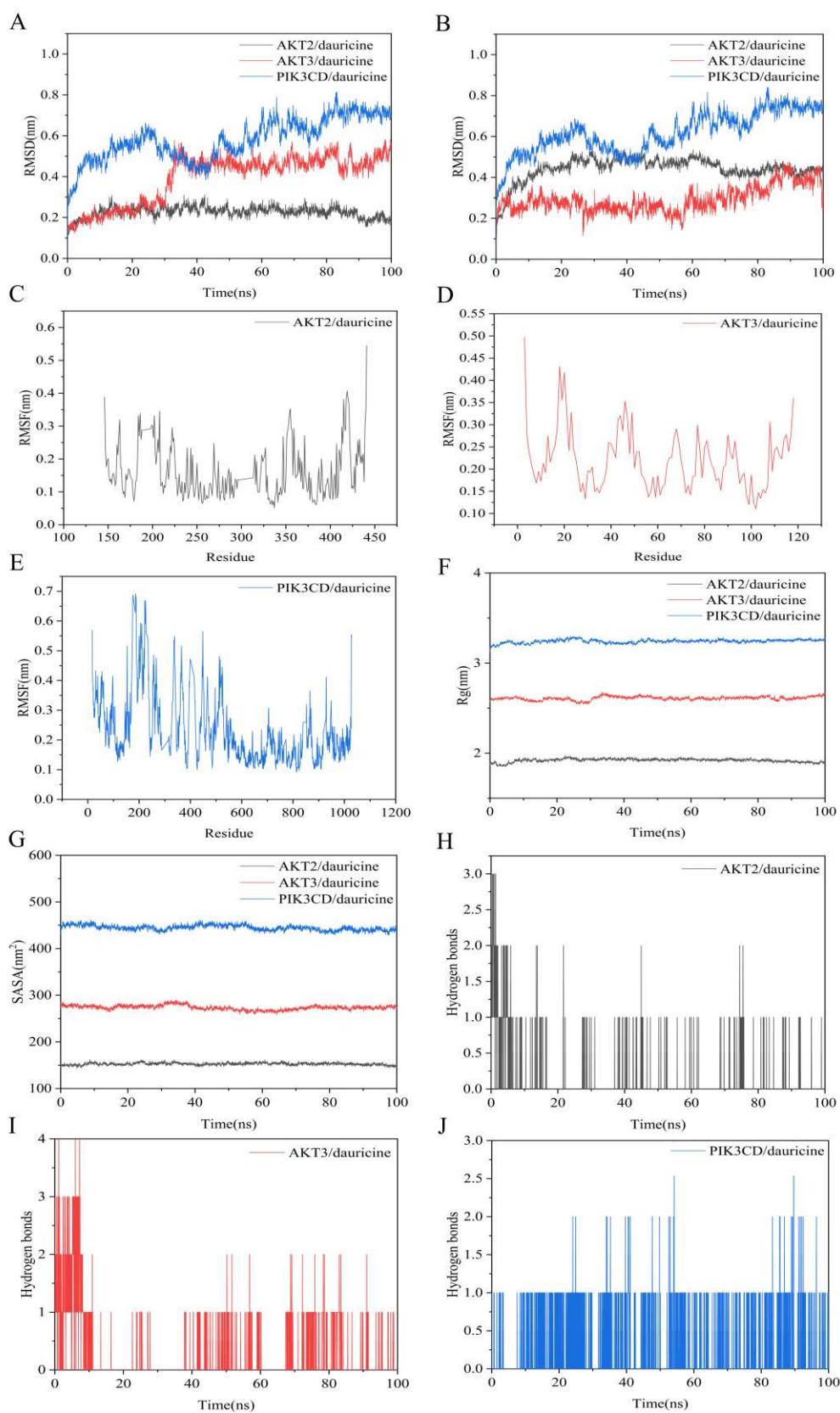
Fig. 7 Planar structure of the primary active ingredient in GE



376

377 **Fig. 8** Molecular docking results between core components and core target molecules.

379 **Fig. 9** Molecular docking visualization results. (A-D) Molecular docking results for AKT2 and four active compounds: 4-
380 (4'-Hydroxybenzyloxy)benzyl methyl ether, 4,4'-dihydroxy dibenzyl ether, dauricine, and gastrodin. (E-H) Molecular
381 docking results for AKT3 and four active compounds: 4-(4'-Hydroxybenzyloxy)benzyl methyl ether, 4,4'-dihydroxy
382 dibenzyl ether, dauricine, and gastrodin. (I-L) Molecular docking results for PIK3CB and four active compounds: 4-(4'-
383 Hydroxybenzyloxy)benzyl methyl ether, 4,4'-dihydroxy dibenzyl ether, dauricine, and gastrodin. (M-P) Molecular docking
384 results for PIK3CD and four active compounds: 4-(4'-Hydroxybenzyloxy)benzyl methyl ether, 4,4'-dihydroxy dibenzyl
385 ether, dauricine, and gastrodin.



387 **Fig. 10** MD simulation results of dauricine-target complex. (A) Root-Mean-Square Deviation (RMSD) of the protein after
388 binding to the ligand. (B) RMSD of dauricine as a ligand after alignment to the protein backbone in different complexes.
389 (C-E) Root-mean-square fluctuation (RMSF) of protein residues in the AKT2/dauricine, AKT3/dauricine and
390 PIK3CD/dauricine complexes, respectively, indicating the flexibility of specific regions. The y-axis represents the RMSF
391 value in nm, reflecting the average fluctuation amplitude of each residue's atomic positions during the simulation process.
392 (F) The radius of gyration (Rg) of a complex. It measures the compactness of a protein, indicates that the lower the value,
393 the more tightly folded the protein is. (G) Solvent Accessible Surface Area (SASA). The y-axis reflects the surface area of
394 the protein exposed to the solvent. (H-J) Number of hydrogen bonds formed between dauricine and AKT2, AKT3 and
395 PIK3CD during the MD simulation, demonstrating the stability of the ligand-protein interactions. The y-axis represents the
396 number of hydrogen bonds formed between the ligand and the protein over the course of the simulation.

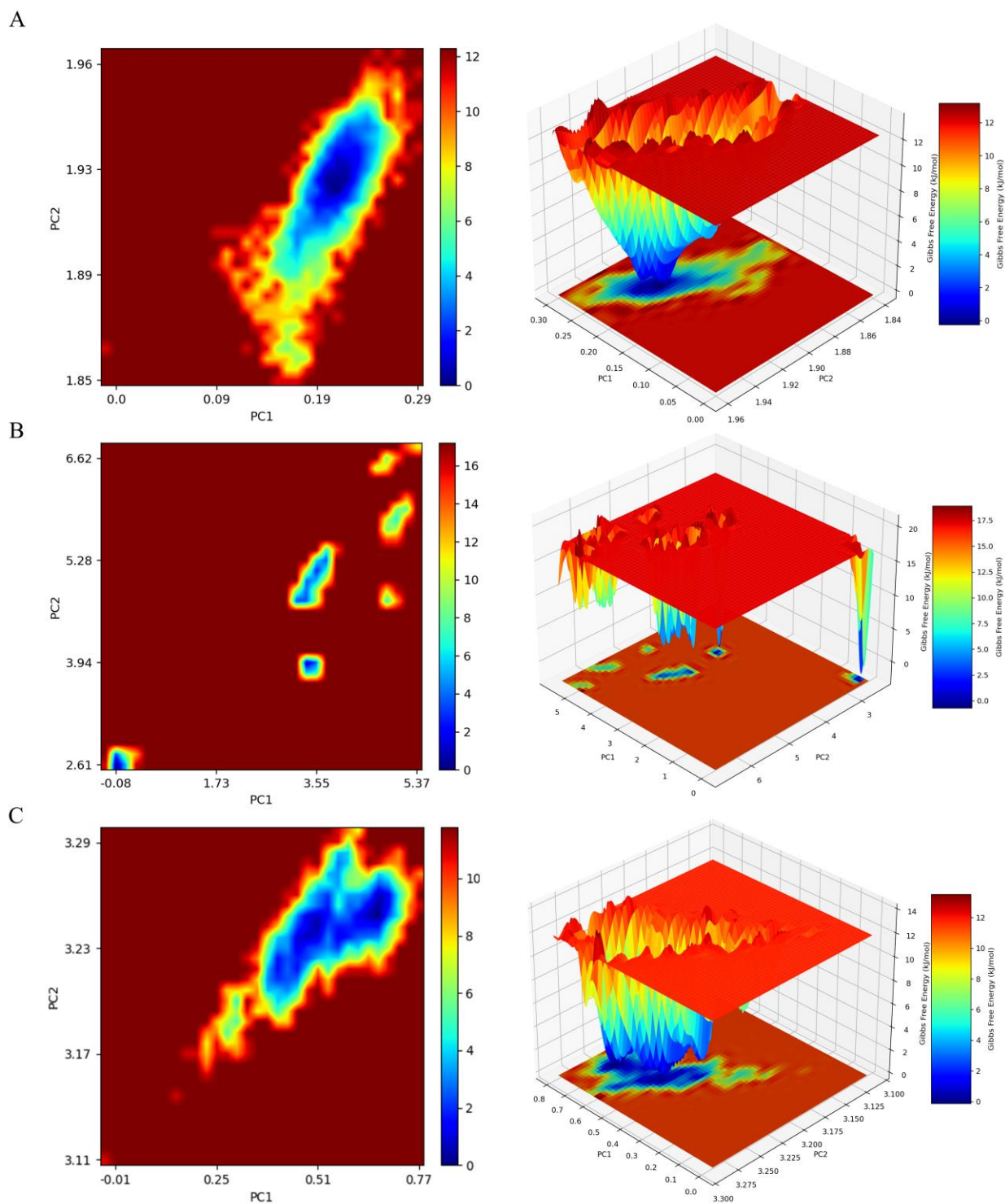


Fig. 11 MD simulation results of dauricine-target complex. (A-C) Gibbs free energy landscapes of AKT2, AKT3 and dauricine in complex with dauricine. Energy troughs (dark blue regions) correspond to high-probability conformational clusters, representing stable states of the system. Energy plateaus or saddle points (yellow/red regions) denote transition states or energy barriers, separating distinct conformational clusters and reflecting the kinetic bottlenecks of conformational transitions.

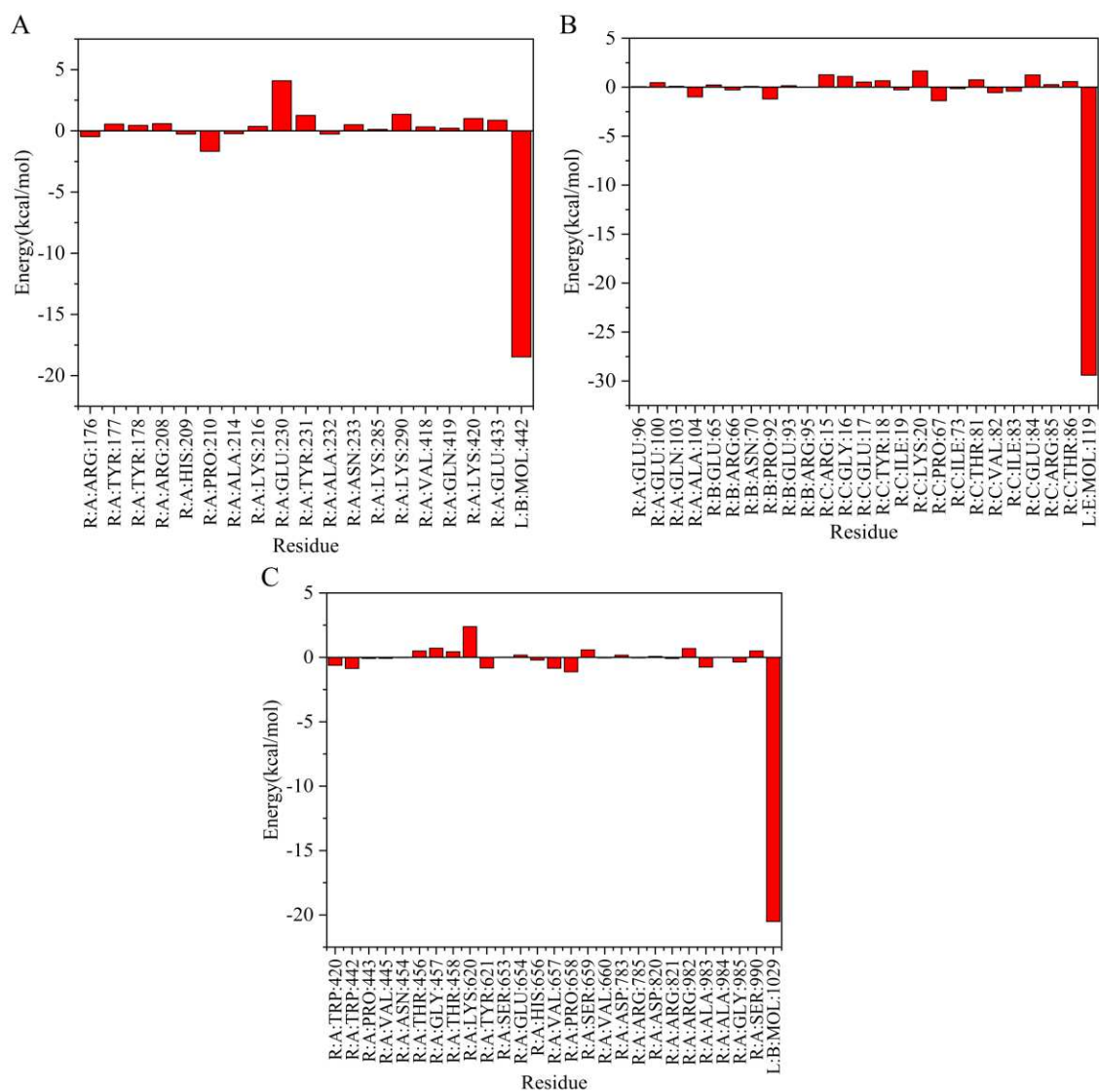


Fig. 12 Molecular dynamics simulation results of dauricine-target complex. (A-C) Residue dissociation energies of dauricine with AKT2, AKT3, and PIK3CD complexes. It reflects the energy contribution of interactions between proteins and ligands. The y-axis represents energy. Negative values indicate that the residue or ligand contributes stabilizing effects to the interaction.

S.No.	Herb ID	Name	GI absorption	Druglikeness
S1	HBIN009937	4,4'-dihydroxy dibenzyl ether	High	Yes
S2	HBIN009938	4,4'-dihydroxy diphenyl methane	High	Yes
S3	HBIN009959	4-(4'-hydroxybenzyloxy)benzyl methyl ether	High	Yes
S4	HBIN010375	4-ethoxy-4'-hydroxy benzyl ether	High	Yes
S5	HBIN010380	4-ethoxymethylphenyl-4'-hydroxybenzylether	High	Yes
S6	HBIN010513	4-hydroxybenzylamine	High	Yes
S7	HBIN010518	4-hydroxybenzyl methyl ether	High	Yes
S8	HBIN010519	4-hydroxybenzyl methyl ether	High	Yes
S9	HBIN018559	bis(4-hydroxybenzyl)ether	High	Yes
S10	HBIN018560	bis(4-hydroxybenzyl)ether mono-beta-d-glucopyranoside	High	Yes
S11	HBIN020993	citronellal	High	Yes
S12	HBIN025849	ethoxysanguinarine	High	Yes
S13	HBIN027383	gastrodamine	High	Yes
S14	HBIN029268	hexadecanoic acid	High	Yes
S15	HBIN035490	m-hydroxybenzoicacid	High	Yes
S16	HBIN039671	p-hydroxybenzaldehyde	High	Yes
S17	HBIN039681	p-hydroxybenzyl alcohol	High	Yes
S18	HBIN039682	p-hydroxy benzyl ethylether	High	Yes
S19	HBIN040911	protocatechuic aldehyde	High	Yes
S20	HBIN045062	succinic acid	High	Yes
S21	HBIN045071	suchilactone	High	Yes
S22	HBIN047744	vanillin	High	Yes
S23	HBIN047745	vanillin acetate	High	Yes
S24	HBIN016572	Arachidonic acid	High	Yes
S25	HBIN046960	Tricin	High	Yes
S26	HBIN035486	3-Hydroxybenzoic acid	High	Yes

S27	HBIN010511	4-Hydroxybenzyl alcohol	High	Yes
S28	HBIN027384	Gastrodin	High	Yes
S29	HBIN022781	dauricine	High	Yes

Table 2 270 common genes identified as potential targets for hypoxia

270 possible target genes for Hypoxia									
REN	HSP90AA1	HSP90AB1	CDK1	PDE5A	JAK2	PIK3CB	PIK3CG	PIK3CA	SYK
INSR	LCK	MAP2K1	SLC2A1	MAPK1	PFKFB3	JAK3	BACE1	PIK3CD	F2R
ACACB	PARP1	MMP9	MMP2	ADAM17	SIRT2	MAPK14	CDK2	ROS1	FLT3
GSK3B	MAPK10	GSK3A	LYN	FGR	EPHA3	DDR2	EPHB4	DDR1	ADORA1
ADORA3	HDAC1	SLC16A1	SUV39H1	EHMT2	CCR1	NTRK1	ABL1	MTOR	TOP1
ERBB2	CXCR2	CXCR1	BIRC5	RET	CASP3	AURKA	AKT3	NOS1	PLCG1
SLC8A1	FLT1	F2	MMP3	ESR1	ESR2	AR	ESRRG	CA2	ALOX5
PTGS1	PTGS2	PRKDC	TMPRSS6	JAK1	TYK2	MMP13	MMP1	MMP14	MMP7
MMP8	BRD4	MME	CREBBP	ROCK2	EIF2AK3	NR3C1	COMT	KIF11	NLRP3
HMGCR	KDR	PRKCB	PIM1	XIAP	PCNA	ABCC9	ACHE	PYGL	AKT1
CHEK1	OPRK1	KIT	CDC25A	VCP	AGTR1	HDAC2	PDGFRB	MDM2	P2RX3
HDAC5	HDAC4	HDAC3	MET	BRAF	OPRD1	KDM1A	EDNRB	ALOX15	ALOX12
CASP1	FAAH	ADORA2B	CAPN1	CASP6	HTR2A	CA12	CA9	MPO	IDO1
NUDT1	MIF	CA3	ADORA2A	SLC5A1	CYP19A1	TRPA1	DRD2	PGR	MAOA
DRD4	CTSK	CTSL	CTSB	PPARG	ABCG2	PPARA	FABP5	PPARD	FABP1
HDAC6	PSMB5	CHRM1	FABP4	FABP3	IKBKB	EPHX2	ACE	ADRA1D	DRD3
HTR1A	F3	ADRA1B	KCNN3	KCNN2	ADRB2	ADRB3	ABCB1	SLC6A4	DPP4
KCNH2	EHMT1	MAP2	TGFBR1	MAPK8	MAPK9	TERT	PRKCD	CSF1R	EZH2
MAPK7	RAF1	FGFR3	TEK	HTR3A	IGF1R	CDK4	PDPK1	SELP	CALCRL
AKT2	FGFR1	HIF1A	EGFR	TLR9	PTAFR	EDNRA	MAP3K7	PTPN1	ILK
FLT4	PDGFRA	HMOX1	POLB	NR1H4	PHF8	G6PD	PTGER2	HSD11B2	PTPRC
RXRA	RARB	PTGER4	ABCC1	CHRNA7	PLA2G4A	PTGES	SCD	PLG	HTR2B
RORA	LDHA	LDHB	AKR1C3	HDAC8	BCL2L1	ALB	ERN1	TNKS2	TNKS
DUSP1	EGLN1	DYRK2	CASP7	PTK2	PLK4	CASP9	GRK2	CASP2	PRKCA
PDE4A	ASAH1	MAPKAPK2	SLC9A1	PIM2	PTK2B	HTR2C	P2RX7	CSNK1A1	CSNK1D
CHUK	EP300	MCL1	BCL2	MMP12	ENPP2	ITGAL	S1PR1	PDK1	NOX4
NR4A1	ODC1	FTO	VEGFA	FGF1	FGF2	HPSE	SERPINE1	ADA	ADK

Table 3 The common core functional targets between active components and hypoxia

Name	Protein name	Degree	Betweenness	Closeness
PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	26	2569.33	0.44
MAPK1	Mitogen-activated protein kinase 1	23	2722.03	0.40
AKT1	RAC-alpha serine/threonine-protein kinase 1	23	1168.58	0.39

PIK3CB	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform	23	1108.62	0.39
PIK3CD	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform	23	1108.62	0.39
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1	22	886.04	0.38
AKT2	RAC-alpha serine/threonine-protein kinase 2	21	767.79	0.37
AKT3	RAC-alpha serine/threonine-protein kinase 3	21	616.11	0.36
CA2	Carbonic anhydrase 2	20	3581.38	0.38
MTOR	Mechanistic target of rapamycin kinase	18	1410.84	0.41
EGFR	Epithelial growth factor receptor	18	642.30	0.35

414

415 **Table 4** Molecular docking results of key components with target sites

Targets	Ligand	Binding affinity
AKT2	4-(4'-Hydroxybenzyloxy)benzyl methyl ether	-5.9
	4,4'-dihydroxy dibenzyl ether	-5.3
	dauricine	-8.6
	Gastrodin	-6.5
	Dexamethasone	-7.2
AKT3	4-(4'-Hydroxybenzyloxy)benzyl methyl ether	-6.6
	4,4'-dihydroxy dibenzyl ether	-7.4
	dauricine	-9.2
	Gastrodin	-6.9
	Dexamethasone	-8.6
PIK3CB	4-(4'-Hydroxybenzyloxy)benzyl methyl ether	-5.4
	4,4'-dihydroxy dibenzyl ether	-6.9
	dauricine	-6.7
	Gastrodin	-6.9

	Dexamethasone	-7.9
	4-(4'-Hydroxybenzyloxy)benzyl methyl ether	-5.3
	4,4'-dihydroxy dibenzyl ether	-7.0
PIK3CD	dauricine	-8.9
	Gastrodin	-7.7
	Dexamethasone	-8.4

416

417 **Table 5** Pharmacological properties assessment of active components. Note: HBA: hydrogen bond acceptor < 10; HBD:
418 hydrogen bond donor ≤ 5; MLog P: Moriguchi octanol-water partition coefficient ≤ 4.15; Lipinski's violations ≤ 1;
419 Bioavailability score > 0.1; TPSA: topological polar surface area. Meeting the above properties indicates that the active
420 ingredient possesses favorable pharmacological properties.

Active ingredient	HBA	HBD	MLog	Lipinski's violations	Bioavailability Score	TPSA
4-(4'-						
Hydroxybenzyloxy)benzyl	3	1	2.32	0	0.55	38.69
methyl ether						
4,4'-dihydroxy dibenzyl ether	4	3	3.32	0	0.55	69.92
dauricine	8	1	3.46	1	0.55	72.86
Gastrodin	7	5	-1.48	0	0.55	119.61

421

422 **Table 6** Binding free energy of the three complex systems

	AKT2-dauricine	AKT3-dauricine	PIK3CD-dauricine
ΔG_{vdw}	-41.12	-62.23	-47.79
ΔG_{eel}	-9.28	-13.15	-0.35
ΔG_{epb}	38.04	49.25	24.69
ΔG_{enp}	-4.43	-6.69	-5.09

ΔG_{Total}

-16.79

-32.82

-28.54

423