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Network pharmacology and molecular docking-based prediction of the multi-target mechanisms by which icariin intervenes in perimenopausal depressive-like symptoms

Xiaoyu Chen*
Obstetrics and Gynecology Department, Xianju County Traditional Chinese Medicine Hospital, Taizhou City, Zhejiang Province 317300, China

Abstract: **Background**: Perimenopausal depressive-like symptoms are closely related to estrogen fluctuations, neuroinflammation and impaired neuroplasticity. Icariin has phytoestrogen-like and neuroprotective activities, but its multi-target mechanism in intervening in this pathological state remains unclear. **Objectives**: To predict the key targets and action pathways of icariin in intervening in perimenopausal depressive-like symptoms based on network pharmacology and molecular docking. **Methods**: Potential targets of icariin and targets related to the perimenopausal module and the depression module were obtained through public databases and drug-disease common targets were screened; a protein-protein interaction (PPI) network was constructed and core targets were screened; GO and KEGG enrichment analyses and integrated network analysis of "Icariin-common targets-pathways-disease" were performed; finally, molecular docking validation was carried out for the core targets. **Results**: A total of 219 potential targets of icariin were obtained and 74 common targets were identified after intersection with the disease modules. The PPI network contained 68 connected nodes and 387 interaction edges and AKT1, ESR1, TNF, IL6 and TP53 ranked in the top 5 by MCC. The common targets were significantly enriched in GO terms and the top 20 KEGG pathways (all adjusted $P < 0.05$), mainly involving regulation of protein phosphorylation, apoptotic process, steroid hormone response and the PI3K-Akt, MAPK, estrogen, TNF and neurotrophin signaling pathways. The integrated network included 96 nodes and 330 edges. The redocking RMSD values of the 9 core targets were all <2.00 A; the optimal docking binding energies of icariin with ESR1, PTGS2 and MAPK1 were -10.12, -9.28 and -8.76 kcal/mol, respectively. **Conclusion**: Icariin may take ESR1, AKT1, MAPK1 and PTGS2 as key nodes and synergistically intervene in perimenopausal depressive-like symptoms through estrogen regulation, inflammatory response regulation and pathways related to cell survival and apoptosis.

Keywords: Icariin; Molecular docking; Network pharmacology; Perimenopausal depressive-like symptoms

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INTRODUCTION

Perimenopause is a critical window during which women transition from the reproductive period to the non-reproductive period and susceptibility to depression increases significantly (Herson and Kulkarni, 2022). Longitudinal studies suggest that women in the menopausal transition have a higher risk of developing depressive symptoms or depressive disorders than those in the premenopausal stage and community samples also show that about one quarter of women have depressive symptoms of varying degrees (Santoro *et al*., 2021). Current understanding holds that such emotional problems are not caused simply by aging, but are jointly related to estradiol fluctuations, imbalance of the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal axis, monoamine neurotransmitter abnormalities, neuroinflammation and oxidative stress (Han *et al*., 2023). Clinical interventions still mainly include antidepressants, psychotherapy and some hormone-related therapies, but symptom heterogeneity is substantial and the development of treatments targeting the perimenopause-specific pathological processes is still relatively slow (Wang L *et al*., 2025; Liang *et al*., 2024). Icariin is the main active component of *Epimedium* and has pharmacological activities such as phytoestrogen-like, anti-inflammatory, antioxidant, anti-apoptotic and neuroprotective effects (Sanchez-Gutierrez *et al*., 2024). Previous experimental studies have suggested that icariin can improve the behavioral performance of perimenopausal depressive-like animals, regulate sex hormones and monoamine neurotransmitters in the brain and affect PI3K/Akt, Bcl-2/Bax and other signaling pathways (Dong *et al*., 2025). Related reviews have also shown that it is associated with HPA axis regulation, neuroinflammation inhibition and neuroplasticity protection in models of depression and other neuropsychiatric diseases (Wang S *et al*., 2021). The problem is that most of this evidence is scattered across single pathways or single models and is still insufficient to explain the complex pathology of perimenopausal depressive-like symptoms, which simultaneously feature hormone fluctuations, inflammatory responses and neural

**Corresponding author:* e-mail: fivestar1992220@163.com

network damage (Zhang Y *et al*., 2024). There is still a lack of systematic clarification regarding the multi-target basis of action, key hub molecules and pathway coupling relationships of icariin in this specific disease context. Compared with previous network pharmacology studies of icariin in general depression or other neuropsychiatric disorders, the novelty of the present study lies in focusing on the specific pathological context of perimenopausal depressive-like symptoms and integrating the shared molecular features of the perimenopausal and depression modules into a disease-context-oriented mechanistic framework. Therefore, using icariin as the sole study compound, this study uses network pharmacology and molecular docking to predict its multi-target mechanisms in perimenopausal depressive-like symptoms from multiple levels, including potential drug targets, the shared molecular features of the perimenopausal and depression modules, PPI core nodes, GO and KEGG enrichment and protein-ligand spatial matching. The aim is to integrate scattered pharmacological clues into a disease-context-oriented mechanistic framework, provide candidate targets for subsequent validation around estrogen signaling, PI3K/Akt and MAPK cascades and inflammation and apoptosis related pathways and support the further development of icariin in perimenopausal mood disorders.

MATERIALS AND METHODS

Study design

This study was a computational biology study based on public databases and molecular simulation, with icariin as the only research object and no compound formula decomposition or re-screening of active ingredients was performed. Icariin was selected as the sole study compound because it is a clearly defined monomeric component with prior pharmacological evidence relevant to perimenopausal depressive-like symptoms and the aim of this study was focused mechanistic prediction rather than comparative analysis among multiple compounds. The research process was as follows: standardized chemical information of icariin was obtained and its potential targets were predicted; perimenopause-related targets and depression-related targets were retrieved separately; the intersection of the two disease target sets was defined as the shared targets between the two modules, representing shared molecular features related to perimenopausal depressive-like symptoms; common targets between icariin and this shared target set were further screened; a protein-protein interaction (PPI) network was constructed and core targets were screened; GO and KEGG enrichment analyses were performed; an integrated network of "Icariin-common targets-pathways-shared molecular features" was constructed; finally, molecular docking validation was carried out using the core targets as receptors. All data were derived from public databases and did not involve human subjects, animal experiments, or identifiable personal information and as such this study did not require ethical approval or informed consent because it used only publicly available data.

Data sources and software platforms

In this study, all database retrieval, data collation and computational analyses were completed from March 12, 2026 to March 26, 2026 and March 26, 2026 was used as the database lock date. Compound information was obtained from PubChem; the drug target prediction platforms were SwissTargetPrediction and PharmMapper; the databases for retrieval of disease-related targets were GeneCards, OMIM, DisGeNET and CTD; the protein structure database was RCSB PDB; and the PPI platform was STRING.

Network construction and visualization were performed using Cytoscape 3.10.4 and its cytoHubba plugin; functional enrichment analyses were performed using R 4.5.3, Bioconductor 3.22, clusterProfiler 4.18.4 and org.Hs.eg.db; molecular docking was performed using AutoDock Vina 1.2.0, MGLTools/AutoDockTools 1.5.7, Open Babel 3.1.1 and PyMOL 3.1. Unless otherwise specified, all retrieved and analyzed species were limited to Homo sapiens.

Prediction of potential targets of icariin

Icariin was retrieved from PubChem to obtain its canonical SMILES, two-dimensional structure and three-dimensional SDF file. Since the research object was a clearly defined monomeric component, no oral bioavailability or drug-likeness screening step was set and target prediction was performed directly. SwissTargetPrediction, as a ligand-similarity-based target prediction platform, was set to the *Homo sapiens* species and the canonical SMILES was entered and predicted targets with probability > 0 were retained. In PharmMapper, the SDF file was uploaded, the target set was selected as Human Targets only, the platform default conformation generation parameters were used and candidate targets with z′-score > 0 were retained (Yin *et al*., 2023). Considering that icariin is a structurally complex glycosylated flavonoid, the two platforms were used in a complementary manner and the predicted targets were treated only as candidate targets for subsequent analysis. The results from the two platforms were merged and then entered in the subsequent standardization process.

Retrieval of targets related to perimenopausal depressive-like symptoms

Because "perimenopausal depressive-like symptoms" lacks a standard disease ontology term that can be directly matched, a disease module decomposition strategy was adopted in this study. Because perimenopause is a dynamic endocrine transition rather than a static

hormone-deficiency state, the perimenopausal module in this study was used only to capture related molecular features at the database level and not to model the temporal dynamics of hormonal fluctuations. The perimenopause-related search terms were set as "perimenopause", "menopausal transition" and "climacteric"; the depression-related search terms were set as "depression", "depressive disorder" and "major depressive disorder". The above keywords were searched separately in GeneCards, OMIM, DisGeNET and CTD and the original results were exported according to the "perimenopausal module" and the "depression module", respectively. To reduce text retrieval noise in GeneCards, the results were limited to the Disorders section and inferred associations from MalaCards were excluded; in OMIM, DisGeNET and CTD, human gene entries directly corresponding to the search terms were retained.

Target standardization, integration and screening of drug common targets

Drug targets and disease targets were uniformly imported into UniProt for name standardization, with the species limited to *Homo sapiens* and only reviewed (Swiss-Prot) entries were retained and unified as official gene symbols. After deleting non-human entries, entries without reviewed protein annotation, duplicate symbols and unmappable entries, the intersection of the perimenopausal module and the depression module was first obtained and defined as the shared targets between the two modules, representing shared molecular features related to perimenopausal depressive-like symptoms; this set was then intersected with the potential targets of icariin to obtain drug-shared common targets. Set operations were completed in R. All common targets were included in the enrichment analysis; among them, targets with STRING interaction information were included in the screening of PPI core targets.

Construction of the PPI network of common targets and screening of core targets

The common targets were uploaded to STRING v12.0 to construct a protein-protein interaction (PPI) network, with the species limited to *Homo sapiens*, the minimum required interaction score set to 0.700 and no external shell interactors added (Szklarczyk *et al*., 2023). The network results were exported in TSV format and then imported into Cytoscape. Isolated nodes without interaction edges were not included in topological ranking and only the connected network was retained for core target analysis. The Maximal Clique Centrality (MCC) algorithm in the cytoHubba plugin was used to rank the nodes and to balance topological priority with interpretability and to keep a manageable set for subsequent analysis, the top 10 targets were defined as candidate core targets for subsequent key pathway association analysis and molecular docking.

Functional enrichment analysis of common targets

After converting the common targets into Entrez IDs, GO and KEGG enrichment analyses were performed based on R/clusterProfiler, with the background set defined as the full set of human genes annotated in org.Hs.eg.db. The GO analysis covered the three categories of biological process, cellular component and molecular function; the species code for the KEGG analysis was set as “hsa”. The statistical test was based on the hypergeometric distribution model, the P values were corrected by the Benjamini-Hochberg method and adjusted $P < 0.05$ was considered significant enrichment. Ranked in ascending order of adjusted P values, the top 10 terms in each GO category and the top 20 KEGG pathways were displayed, respectively.

Construction of the drug common targets-pathways-network

Based on the common targets and significant KEGG pathways, the "Icariin-common targets-pathways-shared molecular features" integrated network was constructed in Cytoscape. The network included 1 compound node, 1 shared-feature node, common target nodes and the nodes of the top 20 significant KEGG pathways. The edges between the compound and the targets represented predicted targeting relationships, the edges between the targets and the pathways represented KEGG annotation relationships and the edges between the pathways and the shared-feature node represented the potential molecular associations of these pathways with perimenopausal depressive-like symptoms. Node size was mapped according to the degree value and was used to describe the connection strength of nodes in the integrated network.

Molecular docking analysis of core targets and icariin

Receptor proteins were screened from the candidate core targets. Any target for which a human experimentally resolved structure, resolution ≤3.0 Å, a complete binding pocket and a co-crystallized ligand were available in the RCSB PDB was included in molecular docking; when multiple structures were available for the same target, the X-ray crystal structure with the highest resolution and the most complete binding domain was preferentially selected (Yang *et al*., 2022). Protein preprocessing was completed in PyMOL and AutoDockTools, retaining the protein chain directly related to ligand recognition and deleting free water molecules and unnecessary heterologous ligands; metal ions or cofactors located in the active pocket and involved in ligand recognition were retained; polar hydrogens were then added, Gasteiger charges were assigned and the structures were converted into PDBQT format. For the icariin ligand, the PubChem three-dimensional structure file was used, hydrogen atoms were added using Open Babel, geometric optimization was performed with the MMFF94 force field and the ligand was then converted into PDBQT format.

Before formal docking, the original co-crystallized ligand was first extracted from the receptor structure and subjected to redocking. If the RMSD between the redocked conformation and the crystal conformation was < 2.0 A, the receptor and parameter combination was considered suitable for formal docking (Hosseini *et al*., 2021); structures that did not meet this criterion were not included in the final analysis. Formal docking was performed using AutoDock Vina 1.2.0, with the mode set as rigid receptor-flexible ligand, the center of the search box set as the geometric center of the original co-crystallized ligand, the box range covering the original ligand and the pocket residues within 5 A around it, exhaustiveness set to 32 and num_modes set to 20 (Eberhardt *et al*., 2021). For each receptor, the conformation located within the validated active pocket and with the lowest binding energy was retained as the final result and hydrogen bonds, hydrophobic interactions and π interactions were analyzed in PyMOL. Binding energy was used only as an indicator of relative affinity within the same study and not as direct evidence of confirmed binding.

Statistical analysis

All set operations, ID conversion, counting statistics, enrichment matrix collation and figure plotting were completed in the R environment. Statistical inference was used only for enrichment analysis: the hypergeometric distribution test was adopted, followed by multiple comparison correction using the Benjamini-Hochberg method, with adjusted $P < 0.05$ as the threshold for significance. PPI topological parameters and molecular docking binding energies were both used as descriptive indicators and no parametric test or rank-sum test was performed; this study did not involve sample size estimation, missing value imputation, or randomized grouping.

RESULTS

Results of screening of potential targets of icariin and targets related to perimenopausal depressive-like symptoms

After prediction by SwissTargetPrediction and PharmMapper, UniProt standardization and deduplication, a total of 219 potential targets of icariin were obtained; the perimenopausal module and the depression module yielded 807 and 1935 related targets, respectively (Table 1). The perimenopausal module and the depression module had 337 intersecting targets, representing the shared molecular features between the two modules; after further intersection of this 337-target set with the potential targets of icariin, 74 common targets shared by all three sets were obtained (Fig. 1).

Results of construction of the PPI network of common targets and screening of core targets

After construction of the PPI network based on 74 common targets, the PPI network contained 68 connected nodes and 387 interaction edges and 6 isolated nodes that lacked interaction edges under the preset STRING threshold, were not included in figure 2 or in the subsequent topological ranking. Core targets such as AKT1, ESR1, TNF and IL6 were located in the central region of the network, suggesting that they had relatively high topological importance in the common target network (Fig. 2). According to MCC topological analysis, AKT1, ESR1, TNF, IL6 and TP53 ranked in the top 5 by MCC, with MCC values of 1736, 1612, 1497, 1384 and 1269, respectively; MAPK1, EGFR, SRC, CASP3 and PTGS2 also ranked in the top 10 and were identified as candidate core targets in the PPI network (Table 2).

Results of functional enrichment and integrated network analysis of common targets

GO enrichment analysis showed that, in the BP category, the common targets were mainly involved in positive regulation of protein phosphorylation, regulation of apoptotic process, steroid hormone response and cellular response to oxidative stress; in the CC category, they were mainly enriched in membrane rafts, membrane microdomains and receptor complexes; in the MF category, they were mainly involved in transcription factor binding and protein kinase binding. All of the above terms reached significant enrichment (adjusted $P < 0.05$) (Fig. 3A). KEGG enrichment analysis showed that the common targets were mainly enriched in the PI3K-Akt signaling pathway, MAPK signaling pathway, estrogen signaling pathway, TNF signaling pathway, apoptosis and neurotrophin signaling pathway. The top 20 pathways were all significantly enriched (all adjusted $P < 0.05$) (Fig. 3B). The integrated network included 96 nodes and 330 edges, covering 1 icariin node, 74 common target nodes, 20 KEGG pathway nodes and 1 perimenopausal depressive-like symptoms node. Targets such as AKT1, MAPK1, EGFR and IL6 had higher degree values and were connected to multiple pathway nodes, suggesting that icariin may intervene in perimenopausal depressive-like symptoms through the synergistic action of multiple targets and multiple pathways (Fig. 3C).

Molecular docking results of core targets and icariin

According to the preset criteria, 9 core targets were finally included in molecular docking and the redocking RMSD values were all <2.00 A, suggesting that the docking parameters were reliable. Icariin showed lower predicted binding energies with ESR1, PTGS2 and MAPK1, with optimal binding energy values of -10.12, -9.28 and -8.76 kcal/mol, respectively (Table 3). The spatial binding patterns showed that icariin could be embedded in the validated active pockets of ESR1, AKT1, MAPK1 and PTGS2 and form hydrogen bonds with key residues such as Glu353, Ser205, Asp106 and Arg513, together with hydrophobic or π interactions, suggesting good spatial matching with the above targets (Fig. 4).

Table 1: Results of potential targets of icariin and targets related to perimenopause and depression.

Module/Object	Data source/platform	Entries included in analysis, n	Successfully standardized by UniProt, n	Final unique targets, n
Potential targets of icariin	SwissTargetPrediction	104	98	94
	PharmMapper	247	185	172
	Merged results	351	283	219
Targets related to the perimenopausal module	GeneCards	589	573	546
	OMIM	42	33	31
	DisGeNET	176	171	165
	CTD	327	309	296
	Merged results	1134	1086	807
Targets related to the depression module	GeneCards	1693	1638	1557
	OMIM	69	56	52
	DisGeNET	709	686	649
	CTD	1041	987	941
	Merged results	3512	3367	1935

Note: Entries included in analysis refer to the number of entries entering the standardization process; successfully standardized by UniProt refers to the number of targets that could be mapped to Homo sapiens reviewed entries; final unique targets refer to the number of unique official gene symbols after merging and deduplication within each module. The perimenopausal module was integrated from the retrieval results of perimenopause, menopausal transition, and climacteric; the depression module was integrated from the retrieval results of depression, depressive disorder, and major depressive disorder. The merged results row represents the integrated results across databases.

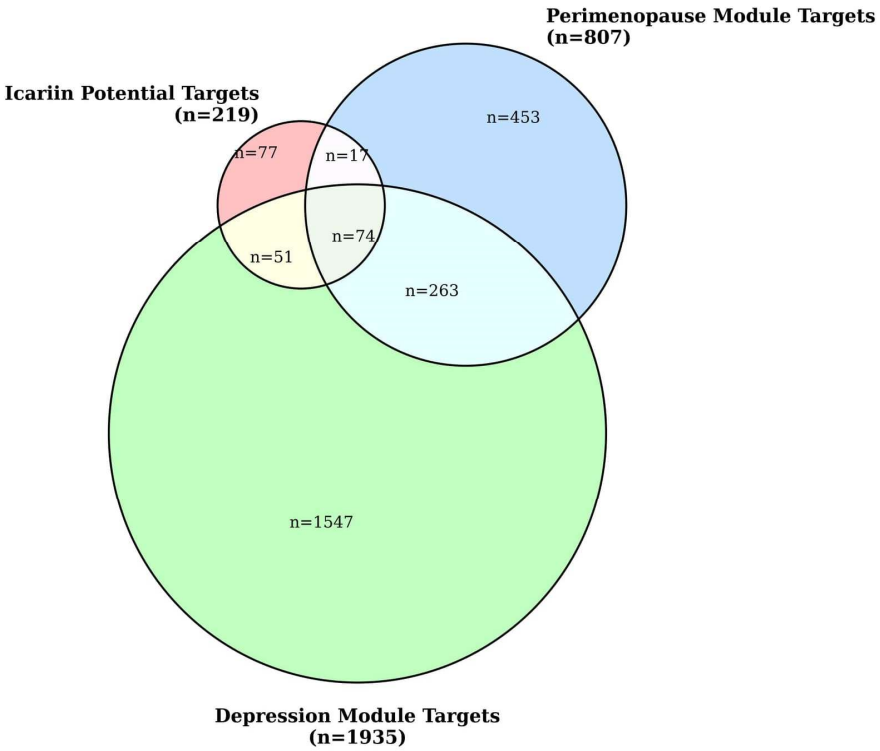

Fig. 1: Intersection diagram of the potential targets of icariin and targets related to perimenopause and depression. The central intersection represents the number of common targets among the potential targets of icariin, the targets related to the perimenopausal module, and the targets related to the depression module. The intersection of the perimenopausal module and the depression module was defined as targets related to perimenopausal depressive-like symptoms.

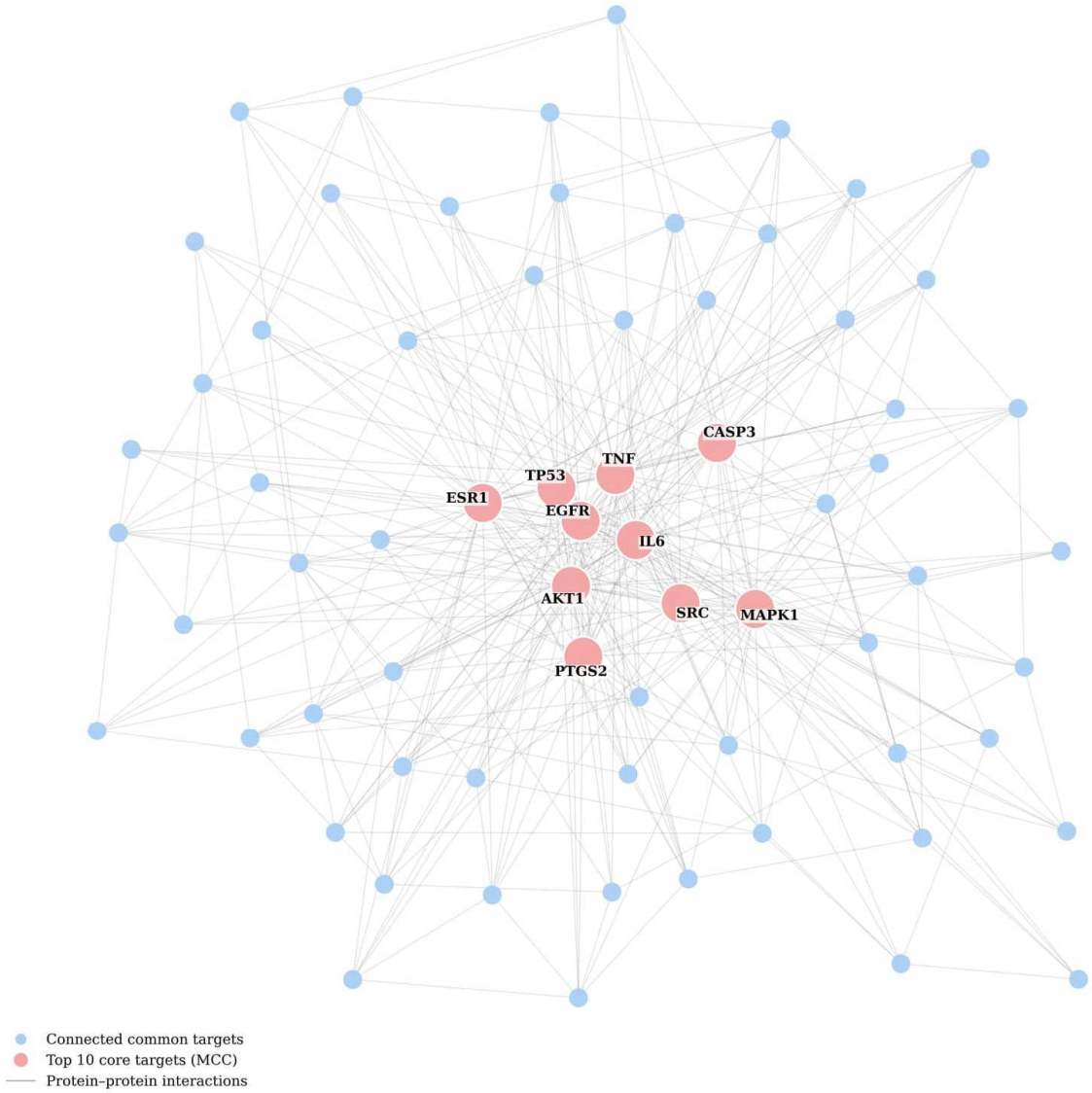

Fig. 2: PPI network of common targets. Nodes represent common targets, and edges represent protein-protein interaction relationships; only connected nodes under the preset STRING high-confidence threshold are shown, and isolated nodes were not included in the topological ranking. The labeled nodes are the top 10 core targets ranked by MCC.

Table 2: Top 10 core targets ranked by MCC in the PPI network of common targets.

Rank	Gene symbol	Protein name	MCC
1	AKT1	RAC-alpha serine/threonine-protein kinase	1736
2	ESR1	Estrogen receptor alpha	1612
3	TNF	Tumor necrosis factor	1497
4	IL6	Interleukin-6	1384
5	TP53	Tumor protein p53	1269
6	MAPK1	Mitogen-activated protein kinase 1	1183
7	EGFR	Epidermal growth factor receptor	1072
8	SRC	Proto-oncogene tyrosine-protein kinase Src	994
9	CASP3	Caspase-3	913
10	PTGS2	Prostaglandin-endoperoxide synthase 2	846

Note: MCC stands for Maximal Clique Centrality. A higher MCC value indicates higher topological importance of the node in the PPI network.

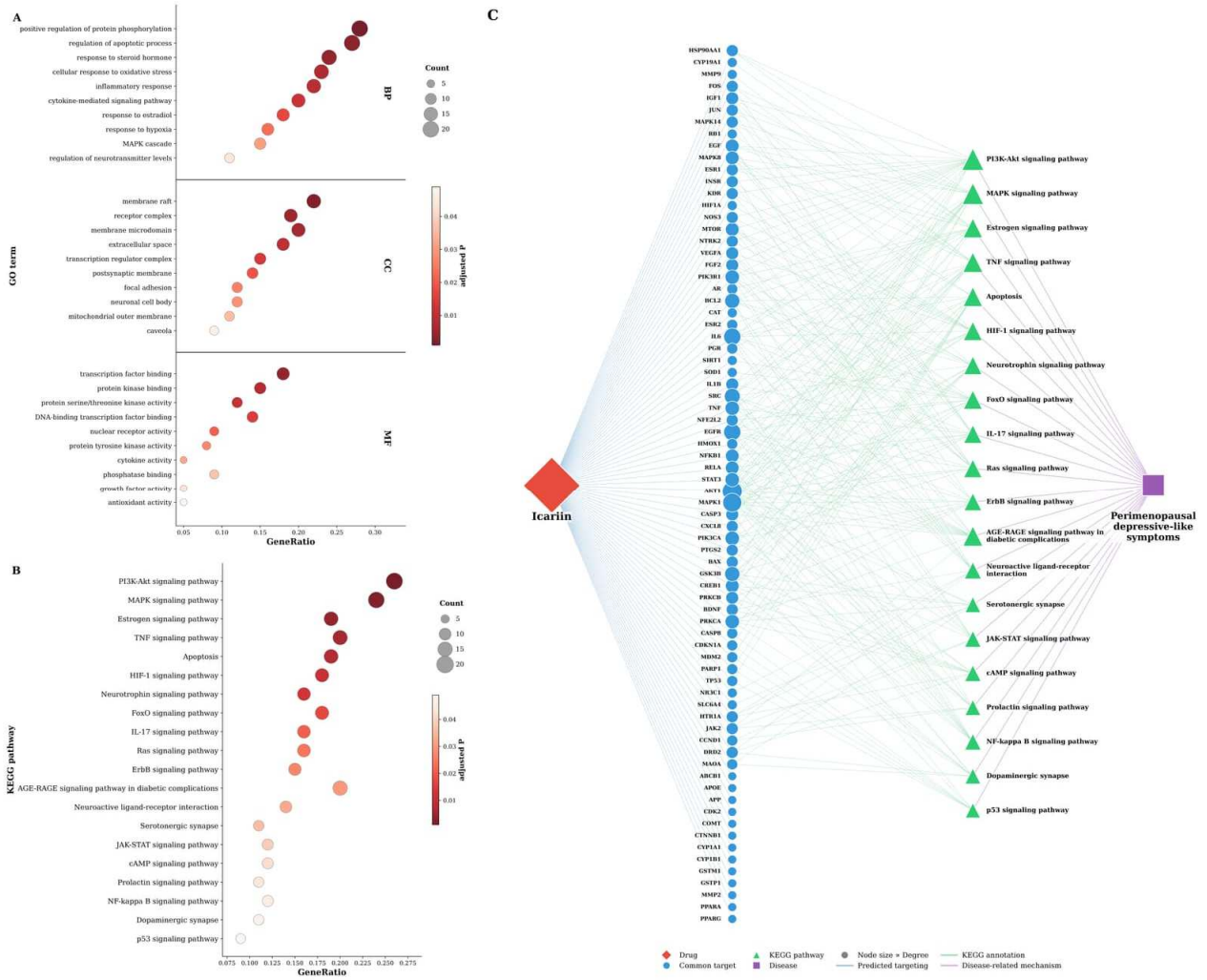

Fig. 3: GO and KEGG enrichment and integrated network analysis of common targets. (A) GO enrichment analysis of common targets. GeneRatio = number of common targets enriched in a certain GO term/total number of common targets included in the GO enrichment analysis; Count = number of common targets enriched in the GO term; adjusted P = P value corrected by the Benjamini-Hochberg method. Only the top 10 terms with the smallest adjusted P values in the BP, CC, and MF categories are shown; (B) KEGG pathway enrichment analysis of common targets. GeneRatio = number of common targets enriched in a certain pathway/total number of common targets included in the KEGG enrichment analysis; Count = number of common targets enriched in the pathway; adjusted P = P value corrected by the Benjamini-Hochberg method. Only the top 20 KEGG pathways with the smallest adjusted P values are shown; (C) Icariin-common targets-pathways-shared molecular features integrated network. Node size is proportional to degree, and degree represents the number of edges connected to a node. Pathway nodes only include the top 20 KEGG pathways with adjusted $P < 0.05$; different types of edges represent predicted targeting relationships, KEGG annotation relationships, and mechanistic associations with the shared molecular features, respectively. The network is unweighted and does not encode cross-database evidence levels for disease-target associations.

DISCUSSION

A clear overlap was observed between the perimenopausal module and the depression module and this overlap further intersected with the potential targets of icariin, suggesting that perimenopausal depressive-like symptoms may have an identifiable drug-disease network basis and may not simply represent a simple juxtaposition of endocrine changes in perimenopause and general depressive states. The core pathology during perimenopause is the imbalance of estrogen signaling caused by fluctuations in ovarian function and this imbalance affects the regulation of the hypothalamic-pituitary-ovarian axis and the hypothalamic-pituitary-adrenal axis, influences monoamine neurotransmitter homeostasis, inflammatory mediator release, oxidative stress and neuronal survival status and ultimately manifests as depressive-like changes in emotion, cognition and behavior (Lang *et al*., 2025).

Table 3: Structural information of core targets and molecular docking results.

Gene symbol	PDB ID	Resolution (A)	Co-crystallized ligand	Redocking RMSD (A)	Optimal binding affinity (kcal/mol)
AKT1	3O96	2.7	Inhibitor VIII	1.07	-8.31
ESR1	3ERT	1.9	4-Hydroxytamoxifen	0.74	-10.12
TNF	2AZ5	2.1	SPD304	1.43	-8.24
TP53	8XCB	1.83	B20	1.36	-7.58
MAPK1	4QTA	1.45	SCH772984	0.68	-8.76
EGFR	1XKK	2.4	GW572016 (Lapatinib)	0.83	-8.47
SRC	4MXO	2.1	Bosutinib	0.79	-8.19
CASP3	2H5I	1.69	Ac-DEVD-CHO	1.57	-7.36
PTGS2	5KIR	2.7	Rofecoxib	0.84	-9.28

Note: The rows are arranged according to the MCC ranking order in table 2. RMSD < 2.00 A was regarded as passing redocking validation. The optimal binding affinity value was predicted by AutoDock Vina and a lower value indicates a stronger predicted binding tendency.

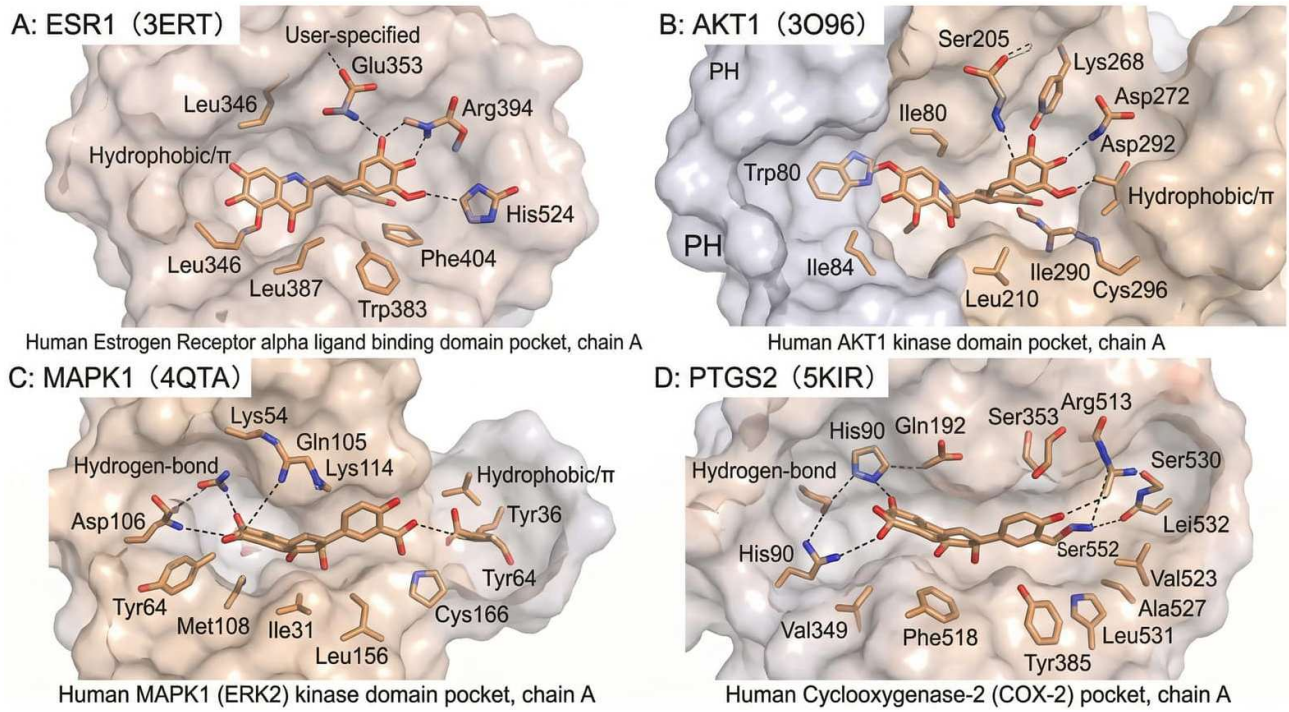

Fig. 4: Three-dimensional binding conformations and interaction patterns of icariin with representative core targets. Each panel shows the binding pose of icariin in the active pockets of ESR1, AKT1, MAPK1 and PTGS2 and the key interacting residues; hydrogen bonds are shown as dashed lines. The interactions shown are docking-predicted structural matching results and do not represent experimentally validated binding or functional regulation.

The existence of common targets suggests that icariin may not merely exert a local effect on a certain receptor or pathway, but is more likely to be embedded in this abnormal network jointly composed of endocrine, neuroimmune and cellular stress processes and may exert its effect through multi-target synergistic regulation, which is consistent with the settings of "prediction" and "multi-target mechanisms" in the title (Joshi *et al*., 2025). The clinical heterogeneity of perimenopausal emotional symptoms is obvious and explanation only around monoamine neurotransmitters or a single inflammatory factor is often insufficient; existing studies are more inclined to regard it as a state of systemic imbalance under the background of hormone fluctuations (Williams, 2023). Icariin already has pharmacological evidence such as phytoestrogen-like, anti-inflammatory, antioxidant and neuroprotective effects, but these studies are mostly scattered across different disease models or independent mechanistic levels and lack an integrated explanation centered on perimenopausal depressive-like symptoms (Li *et al*., 2022). Through the joint mapping of drug targets with the perimenopausal module and the depression

module, the relationship between icariin and the target disease could be transformed into an analyzable and traceable network relationship, advancing its potential effect from empirical speculation to a testable mechanistic hypothesis at the pathological network level, which is exactly the important research value of the results in this section.

The PPI network exhibited a clear centralization pattern, indicating that the common targets of icariin and perimenopausal depressive-like symptoms were not isolated from each other, but were clustered around a few regulatory nodes with hub status. Although AKT1 ranked first by MCC, ESR1 was also located in the core region and was emphasized here because it provides a more direct link to the endocrine background of perimenopause, suggesting that estrogen signaling imbalance remains the most specific upstream link in this pathological state and also establishing a more direct connection between the potential effects of icariin and the endocrine background of perimenopause. TNF, IL6 and PTGS2 constituted an inflammatory effector module, reflecting that neuroinflammation may be an important mediator in the transformation of hormone fluctuations into emotional and behavioral abnormalities (Yu *et al*., 2025). AKT1, MAPK1, EGFR and SRC were concentrated in the signaling transduction and cell survival regulation network, suggesting that icariin may improve the adaptability of neurons under stress conditions by affecting protein phosphorylation cascades, receptor downstream transduction and processes related to synaptic plasticity (Li *et al*., 2023). TP53 and CASP3 pointed to the links of apoptosis and stress injury, indicating that perimenopausal depressive-like symptoms are not only fluctuations in neurotransmitter levels, but are also accompanied by abnormalities in cell fate regulation (Wang Z *et al*., 2025). Such a core target composition is basically consistent with previous understanding of perimenopausal mood disorders, namely; the abnormal estrogen receptors, elevated inflammatory factors, impaired PI3K-Akt and MAPK signaling and enhanced apoptosis are all involved in the occurrence and development of the disease (Sun *et al*., 2024). Compared with previous studies that mostly focused on a single molecule or a single pathway, this network structure better explains the complexity of perimenopausal depressive-like symptoms and also supports that icariin may exert an intervention effect through the combined actions of estrogen regulation, anti-inflammatory effects and cell protection.

The enriched terms and the integrated network jointly suggested a potentially interconnected pattern of action by which icariin intervenes in perimenopausal depressive-like symptoms. The biological processes were mainly concentrated in regulation of protein phosphorylation, regulation of apoptosis, steroid hormone response and oxidative stress response; the cellular components were located in membrane rafts, membrane microdomains and receptor complexes; and the molecular functions were biased toward transcription factor binding and protein kinase binding, indicating that the related targets were mainly distributed at key interfaces of receptor activation and signal transduction rather than limited to single terminal effector molecules. KEGG analysis further highlighted estrogen, PI3K-Akt, MAPK, TNF, apoptosis and neurotrophin pathways. Their partial overlap and crosstalk suggest that the potential effects of icariin may be functionally coupled around a few key nodes (Di *et al*., 2024). ESR1 and membrane receptor-related targets may represent a regulatory module associated with phytoestrogen-like effects (Zhou *et al*., 2021). AKT1, MAPK1, EGFR and SRC may represent signaling transduction- and cell survival-related modules and may help maintain neural network stability by regulating stress adaptation, synaptic plasticity and cell survival (Gu *et al*., 2023). TNF, IL6, PTGS2, TP53 and CASP3 may be associated with effector processes such as inflammatory amplification, apoptosis activation and neural injury (Dai *et al*., 2021). In molecular docking, icariin showed good binding tendencies with ESR1, PTGS2 and MAPK1 and could form stable hydrogen bonds and hydrophobic or π interactions within the active pockets of ESR1, AKT1, MAPK1 and PTGS2 (Zhang *et al*., 2022). This does not mean that the mechanism has been proven, but it provides structural feasibility support for the network prediction. Previous studies on icariin have mostly focused separately on a single aspect of phytoestrogen-like, anti-inflammatory, or neuroprotective effects (Bi *et al*., 2022). The value of the present study lies in placing these dispersed effects into the pathological context of perimenopausal hormone fluctuations and proposing a framework in which estrogen signaling regulation, inflammation inhibition and neuroplasticity protection may be coupled, which is consistent with the multisystem nature of perimenopausal depressive-like symptoms.

Limitations

This study still has practical limitations. All evidence was derived from public databases and algorithmic predictions, and the results were affected by database coverage, search term settings, and annotation updates. In addition, because no tissue-specific, brain-expression- or hormone-regulation-based prior filtering and no unified cross-database numerical cutoff were applied at the target retrieval stage, some weak or indirect associations may have been retained despite subsequent standardization, intersection screening and high-confidence PPI filtering. In particular, "perimenopausal depressive-like symptoms" is not a standard disease ontology term and could only be defined through the intersection of the perimenopausal module and the depression module, which inevitably caused information compression and omission of some related genes. The PPI network and MCC ranking reflect

topological importance against the background of known interactions and cannot be directly equated with true expression levels in specific brain regions and within specific time windows during perimenopause, nor can they independently determine causal status. GO and KEGG enrichment can only indicate pathway aggregation and cannot provide the direction of activation or inhibition. Molecular docking only provides the possibility of binding at the static structural level and still cannot replace the evaluation of oral bioavailability, pharmacokinetics, metabolic stability, blood-brain barrier permeability, receptor selectivity and *in-vivo* pharmacodynamic effects. Due to a lack of validation from cell experiments, animal models and clinical samples, the conclusions of the study are limited to mechanistic predictions. In the future, targeted validation should preferably be carried out around ESR1, AKT1, MAPK1 and PTGS2 in animal models of perimenopausal depressive-like symptoms and the multi-target synergistic mechanism should be examined in combination with behavior, tissue expression and changes in key pathway proteins, so as to improve the biological interpretability, extrapolation reliability and reproducibility of the conclusions.

CONCLUSION

Icariin may intervene in perimenopausal depressive-like symptoms through multiple molecular events, with ESR1, AKT1, MAPK1 and PTGS2 as key nodes and may form a synergistic regulatory network around estrogen signaling, PI3K/Akt and MAPK cascades, inflammation regulation, oxidative stress response, apoptosis and neurotrophin maintenance. Through these processes, it may potentially exert integrated effects on neuroendocrine disorders, neuroimmune imbalance and impaired neuroplasticity under perimenopausal hormone fluctuations. This predicted mode of action has multi-target and multi-pathway characteristics. Molecular docking further supports the structural feasibility of interactions between icariin and the key targets, suggesting a plausible mechanistic basis for its potential application in perimenopausal depressive-like symptoms.

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Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
Not applicable. This study did not involve human subjects, animal experiments or identifiable personal information, as all data were derived from public databases and computational analyses.

Conflict of interest
The author declares no conflict of interest.

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