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ORIGINAL RESEARCH

Therapeutic Targets and Immune Mechanisms of Yinghuang Decoction in Sepsis: Integrating Network Pharmacology, Molecular Docking, and Pharmacokinetic Approaches

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Background: Yinghuang Decoction is an herbal formula that is used for the treatment of sepsis. This study used network pharmacology and molecular docking methods to explore the potential mechanism of Yinghuang Decoction against sepsis.

Methods: The active ingredients, target genes, and sepsis-related differentially expressed genes (DEGs) were acquired from the public database. The intersection genes were obtained, and the function enrichment analysis was performed. Next, the herbs-active ingredients-genes-disease and protein-protein interaction networks were constructed using Cytoscape v3.7.2. Subsequently, the hub genes were identified using the CytoHubba plugin. The immune cell levels were evaluated by the single-sample Gene Set Enrichment Analysis (ssGSEA). Furthermore, molecular docking was carried out. Finally, the pharmacokinetics and toxicity of active ingredients were predicted.

Results: A total of 7 hub genes (ESR1, PTGS2, CACNB4, KCNMA1, GMPS, AHR, PRKCA) and 11 active ingredients were obtained. These hub genes were significantly correlated with immune cells that are significantly dysregulated in sepsis, such as immature B cells. Among them, three hub genes (CACNB4, GMPS, and PRKCA) exhibited relatively stable diagnostic performance for sepsis (AUC above 0.7). Four active ingredients, linoleic acid, palmitic acid, kaempferol, and afzelin, had good binding affinities with ESR1, PRKCA, and PTGS2, respectively. The four active ingredients met Lipinski's rule principles and were not hepatotoxic or carcinogenic. Real-time qPCR validated the expression of hub genes in sepsis patients, which could reverse after Yinghuang Decoction treatment.

Conclusion: This study exhibited the multiple active ingredients and hub genes of Yinghuang Decoction against sepsis and might offer new insight for advancing its research in sepsis treatment. Due to limited sample size, the expressions of hub genes should be validated in the larger cohorts.

Keywords: Yinghuang decoction, sepsis, network pharmacology, molecular docking

Introduction

Sepsis is a complex disease caused by dysregulation of the host immune response, leading to systemic inflammatory response and organ damage.[1] The global incidence of sepsis is 189 cases per 100,000 person-years, with a mortality rate of 26.7%, which places a heavy burden on public health.[2] In the early phase of sepsis, the pro-inflammatory response was active, involving neutrophils, vascular endothelium, platelets, and complement system.[3] In the later phase of sepsis, immunosuppression predominated due to lymphocyte apoptosis and exhaustion, along with the reprogramming of antigen-presenting cells.[3] Patients with sepsis have a poor prognosis. One-third of patients pass away within the second year after discharge, and one-sixth experience severe and sustained impairment, including functional limitations, cognitive impairment, and mental health problems.[4] Despite some advances in our understanding of the pathology of sepsis, there are shortcomings in sepsis treatment, such as antimicrobial resistance, glucocorticoid

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utilization, and high treatment costs.[5] Given these circumstances, it is crucial to develop an effective medication for treating sepsis.

Traditional Chinese medicine (TCM), with a history spanning thousands of years, stands as a valuable heritage in human healthcare.[6] It has long been utilized to improve quality of life and treat illnesses, particularly in managing inflammatory conditions like sepsis.[7] Based on TCM principles, the main approach to treat sepsis involves clearing heat and toxins, purging internal organs heat, promoting blood circulation, eliminating stasis, fortifying the body, and solidifying detoxification.[6] Yinghuang Decoction, a TCM formula, has demonstrated significant efficacy in treating gastrointestinal disorders and inflammatory markers in patients with sepsis.[8] Additionally, its combination with other TCM treatments can effectively improve sepsis-associated acute lung injury (ALI).[9] The basic formula of Yinghuang Decoction consists of seven herbs, including rheum (da huang), taraxacum officinale (pu gong ying), immature tangerine peel (qing pi), angelica sinensis (dang gui), radix paeoniae rubra (chi shao), radix paeoniae alba (bai shao), and ligusticum chuanxiong (chuan xiong).[8] Rheum purges intestinal heat and toxic accumulation and resolves blood stasis.[10] Taraxacum officinale clears heat and promotes detoxification; when combined with rheum, their synergistic effect enhances detoxification.[11] Immature tangerine peel promotes the movement and dispersal of Qi. Additionally, the other four herbs invigorate and harmonize the blood, aiding in the resolution of stasis. Therefore, based on TCM theory and clinical experience, Yinghuang Decoction can promote bowel clearance to purge heat, invigorate blood circulation, eliminate toxins, and facilitate the recovery of Qi and blood, which align with the TCM syndrome patterns commonly observed in sepsis.[12] Previous studies showed that rheum and the components of angelica sinensis could against sepsis in mice models.[13,14]

Network pharmacology and molecular docking are widely employed to explore potential mechanisms underlying TCM in treating diseases, including sepsis.[15,16] Although Yinghuang Decoction has been applied in clinical practice for sepsis and has shown promising therapeutic effects, its underlying pharmacological mechanisms remain unclear. To date, few studies have systematically explored the active compounds, molecular targets, and biological pathways associated with its efficacy. This knowledge gap limits the modernization and broader application of this traditional formula. Therefore, this study aimed to use network pharmacology and molecular docking approaches to investigate the active ingredients, target genes, and molecular biological processes involved in treatment of sepsis by Yinghuang Decoction. Furthermore, pharmacokinetics, drug-likeness, and toxicity were also predicted for the selected active ingredients. These findings might offer new insight for advancing the research of Yinghuang Decoction for sepsis treatment.

Material and Methods

Screening Active Ingredients and Respective Target Genes of 7 Herbs in Yinghuang Decoction

The Encyclopedia of Traditional Chinese Medicine (ETCM), is a comprehensive and standardized database of herbs (http://www.tcmip.cn/ETCM/).[17] The ETCM database includes 403 herbs, 3,962 herbal formulations, 7,274 herbal ingredients, 2,266 validated or predicted drug targets of TCM, and clinical information on 3,027 diseases.[17] The components of Chinese herbs recorded in the ETCM database were manually collected based on *The Pharmacopoeia of the People's Republic of China* (2020 edition) and other published literature sources.[18] In the initial stage, all active ingredients and their respective target genes in 7 herbs were obtained from the ETCM database without applying activity thresholds, to ensure inclusivity of potentially bioactive components.

Identification of the Differentially Expressed Genes (DEGs)

The keywords "Sepsis" and "Homo sapiens" were utilized to retrieve sepsis datasets from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The following inclusion criteria were used: (1) the sample size was not less than five; (2) the control group was available in the dataset; (3) samples were obtained from humans rather than cell lines or animals. Finally, two datasets, GSE185263 (GPL16791) and GSE95233 (GPL570), were selected. The GSE185263 was considered as the training set in this study, including blood samples from 44 healthy controls and 348 sepsis patients. Based on the Sequential Organ Failure Assessment (SOFA) score 24 hours after admission, sepsis

patients were categorized into mild (0–2, n = 195), moderate (3–8, n = 112), and severe (≥9, n = 38) groups. The GSE95233 served as the validation set, and we selected blood samples from 22 healthy individuals and 51 sepsis patients on day 1. The gene expression matrix files were downloaded, and the gene probes were converted to gene symbols, subsequently computing the mean values for multiple probes corresponding to the same gene. The "limma" package in R was used to perform differential expression analysis. False discovery rate (FDR) <0.05 and $|\log_2$ foldchange (FC)| >1 were the filtering criteria to obtain sepsis-related DEGs. The volcano plot and heatmap visualized results.

Identification of Intersection Genes, Function Enrichment and Herbs-Active Ingredients-Genes-Disease Network Analysis

The target genes intersected with sepsis-related DEGs to obtain the intersection genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to investigate the function of intersection genes using the DAVID database (https://david.ncifcrf.gov/). Terms with FDR <0.05 were considered significant. Cytoscape v3.7.2 was utilized to construct the herbs-active ingredients-genes-disease network in Yinghuang Decoction, active ingredients, intersection genes, and sepsis.

Construction of Protein-Protein Interaction (PPI) Network and Screening of Hub Target Genes

The PPI networks for intersection genes were constructed with STRING database (https://cn.string-db.org/) and visualized via Cytoscape. CytoHubba, a Cytoscape plugin, was utilized to rank the network nodes based on their inherent network characteristics. Nine algorithms in CytoHubba were selected to screen hub genes, including Degree, Edge Percolated Component (EPC), Maximum Neighborhood Component (MNC), Density of Maximum Neighborhood Component (DMNC), Maximal Clique Centrality (MCC), Stress, Closeness, Radiality, and Betweenness.[19] The "UpSet" package in R was used to screen the top 30 node genes based on algorithmic scores to obtain the hub genes.

Immune Microenvironment Analysis

The single-sample Gene Set Enrichment Analysis (ssGSEA) was employed to calculate the enrichment score to quantify the relative abundances of immune cells. The gene panels of human immune cell types were obtained from Charoentong et al's study.[20] The difference in the abundance of immune cells between controls and sepsis patients was compared by the Wilcox test. Additionally, the association between differential immune cells and hub genes was investigated using Pearson's correlation analysis. As this method assesses linear correlations, it does not infer causality.

Receiver Operating Characteristic (ROC) Analysis

The ROC analysis was performed using the "pROC" package (V 1.15.3) in R. The area under the curve (AUC) was calculated to evaluate the accuracy of hub genes in distinguishing sepsis patients from controls. The expression levels of hub genes in sepsis patients in two datasets were displayed in the box plot.

Molecular Docking

Molecular docking is a computational method that simulates the position of small molecules (ligands) within the binding site of larger molecular targets (receptors).[21] It helps to identify the optimal binding positions by minimizing binding energy.[21] The 3D structures of target proteins were downloaded from the RCSB Protein Data Bank (PDB) database (http://www.rcsb.org/pdb/home/home.do).[22] Then, the protein receptor was processed to remove water molecules and small molecule ligands by the PyMol software (http://www.pymol.org/pymol), and hydrogenation and other pretreatments were performed using AutoDockTools.[23,24] The structures of active ingredients were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).[25] Compared to in silico predicted 3D structures generated from SMILES strings, the conformers provided by PubChem are computed using standardized protocols, such as MMFF94s force field and energy minimization, which offer more chemically reasonable starting structures for molecular docking.[26] Similarly, the active ingredients were treated by removing water molecules, hydrogenation, and other pretreatments by the

AutoDockTools. The molecular docking was performed using the AutoDockTools. The most active ligand molecular and target genes were selected based on their binding affinities. This study considers better binding activity with an energy of less than −5.0 kJ/mol (= −1.19423 kcal/mol).[27] Finally, the docking results were outputted by the PyMol software.

Pharmacokinetic, Drug-Likeness, and Toxicity Prediction

The absorption, distribution, metabolism, and excretion (ADME) properties of selected active ingredients were predicted using the SwissADME (http://www.swissadme.ch/) database.[28] The pharmacokinetic properties were evaluated based on Lipinski's "Rule of Five", which analyses the biochemical characteristics of drugs that may affect their uptake and permeation across cell membranes. Lipinski's rule states that compounds exhibiting drug-like properties should meet at least three of the following criteria: molecular weight (MV) ≤500 Daltons (Da), lipophilicity (LogP) ≤5, number of hydrogen bond donors ≤5, acceptors ≤10, and 130 <Molar refractivity (MR) <140.[29] Furthermore, the toxicity predictions were performed using the ADMETlab 2.0 (https://admetmesh.scbdd.com/) database.[30] In addition, the ADMETlab 2.0 database was also utilized to predict the solubility (LogS) of active ingredients, which is a determinant of absorption and bioavailability. A value of LogS ≥-4 indicates favorable solubility.[31]

Real-Time qPCR

We collected 14 blood samples from healthy controls (n = 7), sepsis patients (n = 3), and sepsis patients treated with Yinghuang Decoction (n = 4). Patients with sepsis were included based on the international consensus definition.[32] Sepsis was defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection, identified clinically as an acute increase of ≥2 points in the total SOFA score. The exclusion criteria were: a history of malignancy or other severe systemic conditions (eg, major trauma, non-infectious shock, or acute pancreatitis), and prior chemotherapy or radiotherapy. Inclusion criteria for the healthy control group were as follows: 1) individuals undergoing routine physical examination who were frequency-matched to the sepsis group by sex and group-matched by age (age difference within 5 years); 2) no history of any illness two weeks before or after sample collection. Exclusion criteria were: 1) use of glucocorticoids within two weeks prior to sample collection; 2) presence of any febrile illness or any acute or chronic condition associated with inflammation within two weeks before sampling.

Patients were randomly assigned to the treatment or control group according to a random number table and the order of clinical visits. The treatment group received intervention with the Yinghuang Decoction, which consisted of: rheum (9 g, 24012771), taraxacum officinale (12 g, 23091861), immature tangerine peel (6 g, 230526F1), angelica sinensis (9 g, 24042461), radix paeoniae rubra (12 g, 24031971), radix paeoniae alba (12 g, 24020381), and ligusticum chuanxiong (9 g, 24013182). All herbal ingredients were sourced from granule preparations procured by the TCM Pharmacy of Hebei Provincial Hospital of TCM from Shineway Pharmaceutical Group (Hebei, China). The prescription was taken once daily, dissolved in hot water, and administered in two divided doses (morning and evening) for 3 consecutive days. Clinical information of subjects is shown in Supplemental Table S1. Real-time qPCR was performed to validate the expression of hub genes. Briefly, total RNAs were extracted from blood samples using HiPure Liquid RNA Kit (Magen, Cat#R4163-02) and reversed to complementary DNAs using FastKing RT Kit (With gDNase, TIANGEN, Cat#KR106). SuperReal PreMix Plus (SYBR Green, TIANGEN, Cat#FP205) was employed to perform real-time qPCR. The relative expression of hub genes was calculated using the $2^{-\Delta\Delta t}$ method. Primer sequences are shown in Supplemental Table S2. *T*-tests were used to compare the differences between groups using GraphPad Prism (v10.1.2, USA, www.graphpad.com).

Results

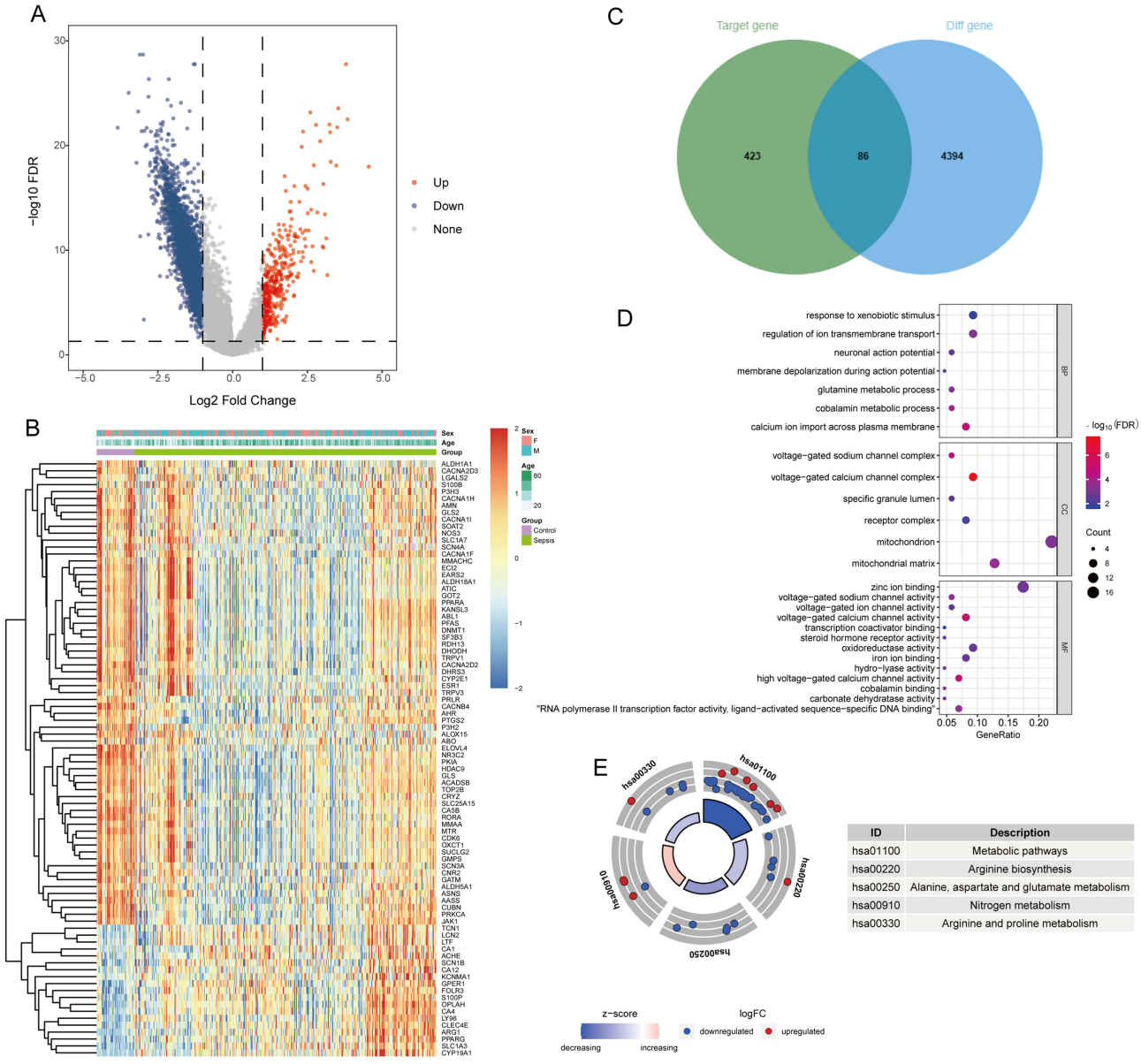
Identification of Active Ingredients in Yinghuang Decoction and Target Genes

In total, 281 active ingredients in Yinghuang Decoction and 509 target genes were detected using the ETCM database. The amount of the active ingredients and corresponding target genes were as follows: rheum had 88 active ingredients and 145 target genes; taraxacum officinale had 2 active ingredients and 25 target genes; immature tangerine peel had 18 active ingredients and 61 target genes; angelica sinensis had 65 active ingredients and 265 target genes; radix paeoniae

rubra had 20 active ingredients and 105 target genes; radix paeoniae alba had 54 active ingredients and 300 target genes; ligusticum chuanxiong had 72 active ingredients and 263 target genes.

Identification of the Intersection Genes and Function Enrichment Analysis

Compared with the healthy control group, 4480 DEGs were detected in sepsis patients, including 412 up-regulated and 4068 down-regulated genes (Figure 1A and B). Next, the 509 target genes in Yinghuang Decoction and 4480 DEGs were intersected, and 86 intersection genes were acquired (Figure 1C). GO analysis showed that these genes are mainly involved in calcium ion import across plasm membrane, regulation of ion transmembrane transport, neuronal action potential, and glutamine and cobalamin metabolic process (Figure 1D). KEGG enrichment analysis exhibited that these genes were mainly involved in amino acids metabolic pathways, such as arginine biosynthesis, alanine, aspartate and glutamate metabolism, nitrogen metabolism, and arginine and proline metabolism (Figure 1E).



ID	Description
hsa01100	Metabolic pathways
hsa00220	Arginine biosynthesis
hsa00250	Alanine, aspartate and glutamate metabolism
hsa00910	Nitrogen metabolism
hsa00330	Arginine and proline metabolism

Figure 1 Screening of intersection genes. (**A** and **B**) Volcano plot (**A**) and heatmap (**B**) showed the DEGs between sepsis and healthy control groups in GSE185263 dataset. (**C**) Venn diagram of DEGs and target genes in Yinghuang Decoction. (**D** and **E**) GO (**D**) and KEGG (**E**) enrichment analysis of intersection genes.

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Construction of Herbs-Active Ingredients-Genes-Disease Network

The herbs-active ingredients-genes-disease network was constructed among 7 herbs, 86 intersection genes, 135 corresponding active ingredients, and sepsis using Cytoscape v3.7.2 (Figure 2A). In this network, we identified 12 target genes with the count of interaction with active ingredients ≥12, including AHR, DNMT1, ESR1, JAK1, SF3B3, PTGS2, CA1, CA12, CA4, CA5B, LCN2, and PRKCA (Figure 2B). In addition, the 11 active ingredients that interacted with ≥10 target genes were identified, including myristic acid (MA), lignoceric acid, palmitic acid, L-Î′-amino-Î″-hydroxyvaleric acid, kaempferol, afzelin, linoleic acid, procyanidin b2 3,3′-di-o-gallate (PCB2DG), procyanidin b1 3′-o-gallate, casuariin, and 5-desgalloylstachyurin (Figure 2C).

Identification of Hub Genes

In the PPI network, a total of 189 interaction pairs were detected among 86 hub genes (Supplemental Figure S1). Among them, 23 pairs exhibited interaction scores above 0.9. Moreover, ESR1 interacts with the most genes, comprising 15 genes. Then, the intersection of the top 30 genes among nine algorithms was taken as the hub genes, and finally, 7 hub genes were obtained, including ESR1, PTGS2, CACNB4, KCNMA1, GMPS, AHR, and PRKCA (Figure 3A).

Then, 6 interaction relationships were detected in 7 hub genes. Among these, AHR and ESR1 had the highest interaction scores of 0.998 (Figure 3B). Next, the Pearson correlation analysis was performed among 7 hub genes in the GSE185263 dataset (Figure 3C). Apart from KCNMA1 and CACNB4, which show no significant correlation with ESR1, all other gene pairs exhibited a notable positive correlation. Furthermore, there are four gene pairs with correlations above 0.7, including GMPS and PRKCA (R = 0.79), GMPS and ESR1 (R = 0.75), GMPS and AHR (R = 0.73), and PRKCA and AHR (R = 0.71).

Immune Microenvironment Analysis

The abundance of 23 immune cells was analyzed in the GSE185263 and GSE95233 datasets, with 14 immune cells showing differences between sepsis patients and controls (Figure 4A and B). Specifically, the sepsis patients had much higher levels of activated dendritic cells, gamma delta T cells, macrophages, mast cells, neutrophils, plasmacytoid dendritic cells, regulatory T cells, and type 17 T helper cells compared with controls in both two datasets. Conversely, the levels of activated B cells, activated $CD8^+$ T cells, eosinophils, immature B cells, natural killer T cells, and type 1

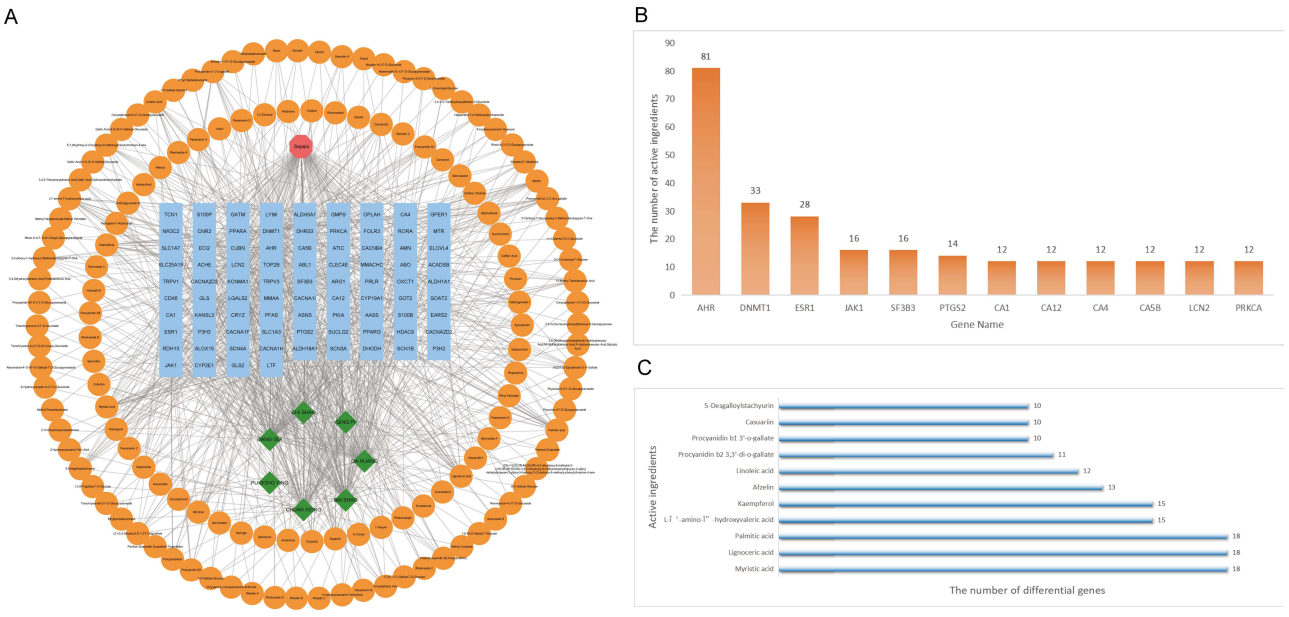

Figure 2 Drug-active ingredients-genes-disease network. (**A**) The drug-active ingredients-genes-disease network, blue nodes indicated intersection genes; greens nodes showed herbs; red node meant sepsis; saffron nodes represented active ingredients. (**B**) A histogram depicted the target genes with the count of active ingredients ≥12. (**C**) A histogram showed the active ingredients with the count of active genes ≥10.

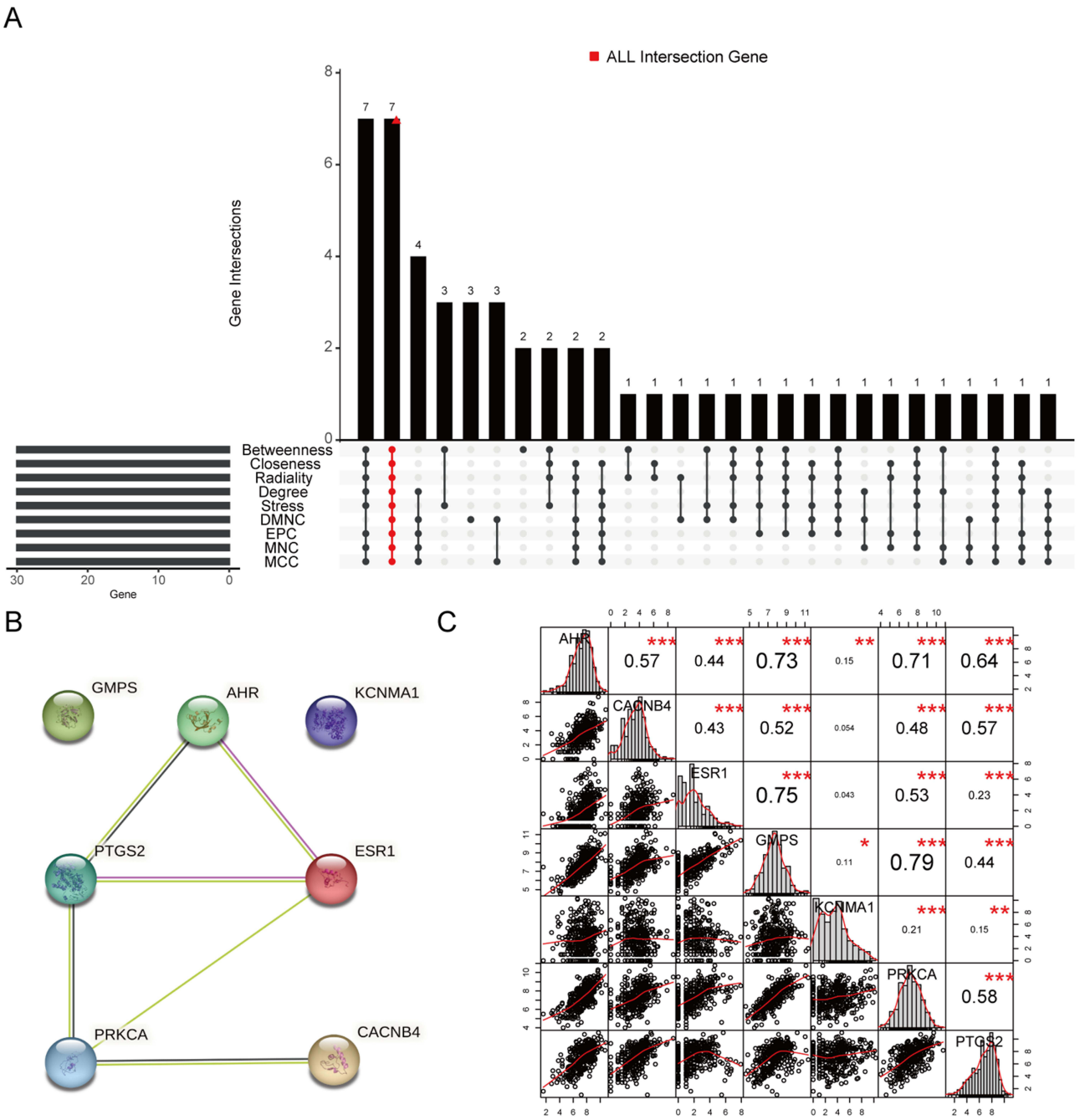

Figure 3 Identification of the hub genes. (**A**) Upset plot of top 30 nodes in nine algorithms. (**B**) PPI network of hub genes. (**C**) Pearson correlation analysis of hub genes. * P < 0.05; ** P <0.01; *** P <0.001.

T helper cells were significantly lower than controls in the two datasets. Correlation analysis was evaluated between 14 immune cells and 7 hub genes (Figure 4C). Macrophages and ESR1 had the highest negative correlation of −0.55. Immature B cells showed a significant correlation with 7 hub genes.

Evaluation of the Diagnostic Value of Hub Genes in Sepsis

In both two datasets, 7 hub genes showed consistent expression trends (Figure 5). Notably, CACNB4, GMPS, PRKCA, and PTGS2 significantly decreased in sepsis patients, while KCNMA1 expression in the sepsis group was significantly increased (Figure 5A and B). Furthermore, we assessed whether sepsis severity could influence the expression of the hub

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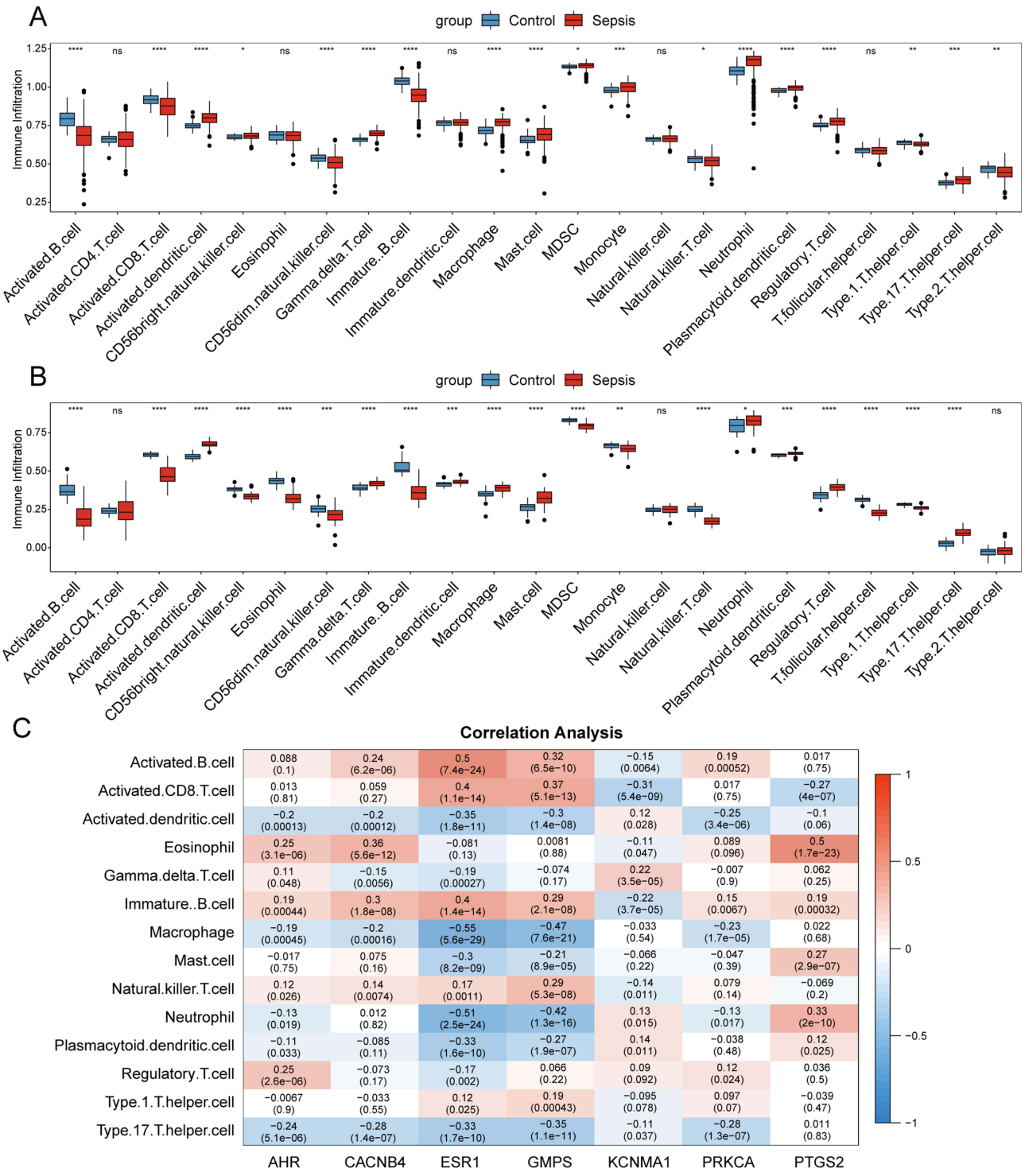

Figure 4 Immune infiltration analysis of sepsis in GSE185263 and GSE95233 datasets. (**A** and **B**) Box plots exhibited the comparison of immune infiltration level between the sepsis and healthy control samples in GSE185263 dataset (**A**) and GSE95233 dataset (**B**). (**C**) Correlation analysis of hub genes and immune cells. * P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001; ns, not significant.

genes (Supplementary Figure 2). The results showed that the expression of these genes differed significantly from controls across all sepsis severity groups, including mild, moderate, and severe sepsis groups. The ROC results indicated that, except for KCNMA1, the AUC values of six genes were more outstanding than 0.7 in the training set (Figure 6A).

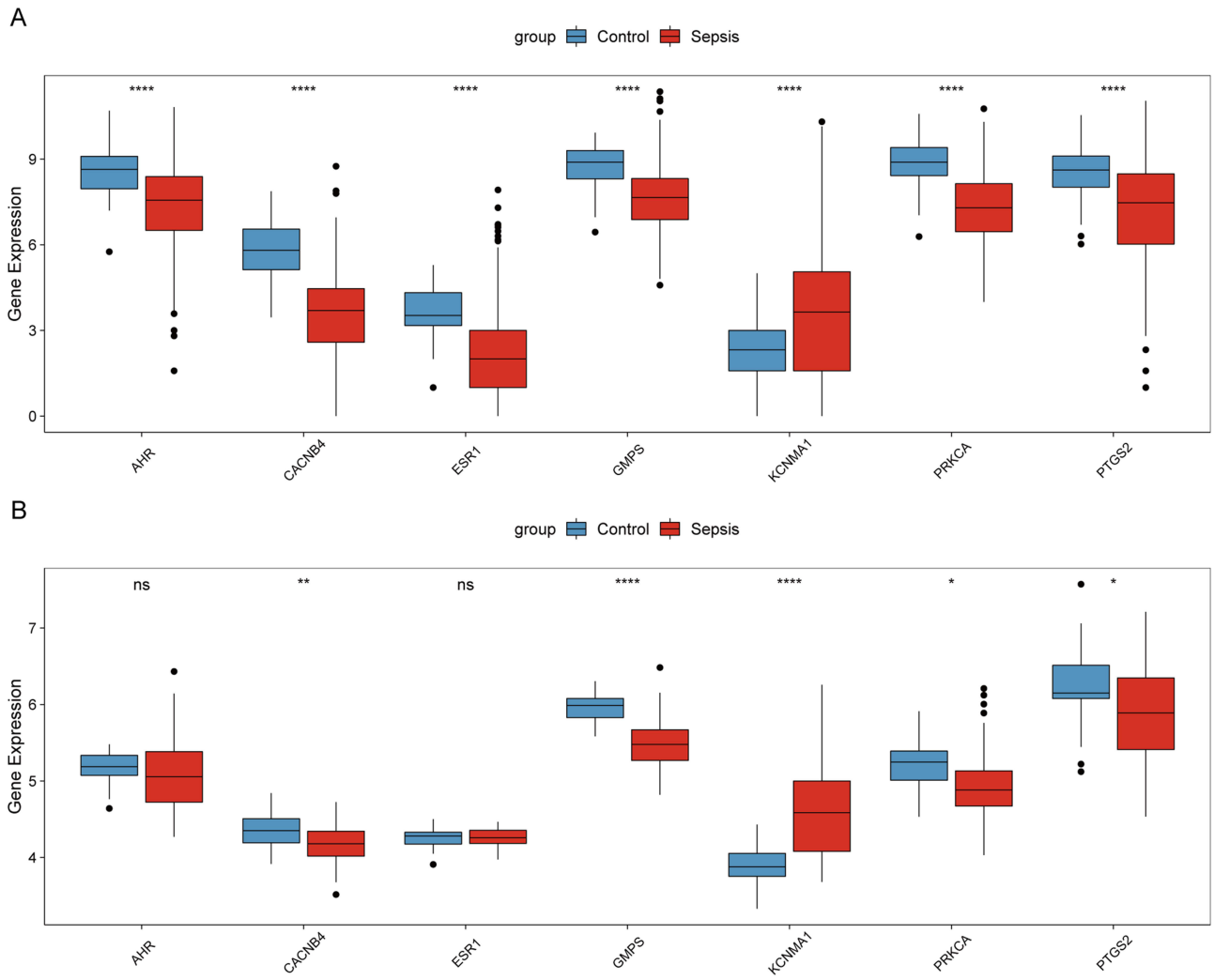

Figure 5 The expression levels of hub genes in GSE185263 (**A**) and GSE95233 (**B**) datasets. * P <0.05; ** P <0.01; **** P <0.0001; ns, not significant.

In the GSE95233 dataset, the AUC values of CACNB4, GMPS, KCNMA1, and PRKCA exceeded 0.7 (Figure 6B). Notably, the AUC values of CACNB4, GMPS, and PRKCA were over 0.7 in both two datasets.

Molecular Docking

Among 7 hub genes that interacted with ≥12 active ingredients and 11 active ingredients that interacted with ≥10 target genes, three hub genes (ESR1, PRKCA, and PTGS2) and four active ingredients (linoleic acid, palmitic acid, kaempferol, and afzelin) with 3D structure available were selected for molecular docking. Three hub genes showed good interaction of free binding energy with the selected active ingredients. Results showed that the binding energies of the four active ingredients ranged from −6.89 to −5.4 kcal/mol. The docking images of the hub genes and active ingredients are displayed in Figure 7. Among them, kaempferol had the lowest binding energy score of −6.89 kcal/mol with PRKCA by binding Clu 481 and Val 420 amino acid residues with 4 hydrogen bonds (Figure 7B).

ADEM, Drug-Likeness, and Toxicity Prediction

The gastrointestinal absorption and brain penetration of four active ingredients were predicted by the brain or intestinal estimated permeation method (BOILED-Egg) (Figure 8). In this model, ingredients in the yellow region might have a high blood-brain barrier (BBB) permeation. In contrast, active ingredients in the white region might tend to be absorbed by the gastrointestinal system (GI).[33] The results showed that palmitic acid ($C_{16}H_{32}O_2$), linoleic acid ($C_{18}H_{32}O_2$), and

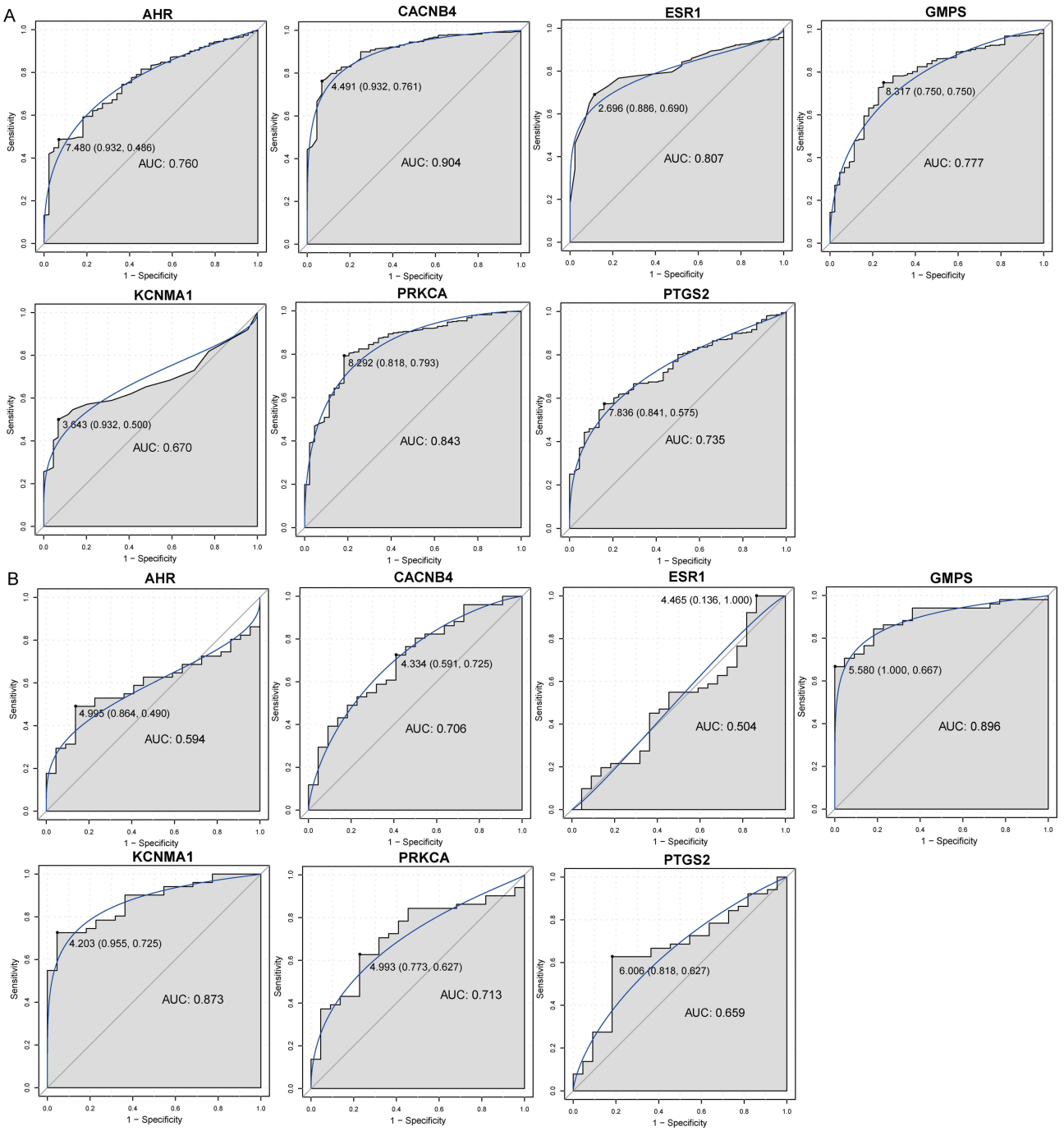

Figure 6 ROC analysis of seven hub genes in GSE185263 (**A**) and GSE95233 (**B**) datasets. The black dot on the ROC curve represents the optimal cutoff point, with the corresponding sensitivity and false positive rate (1–specificity) values shown in parentheses.

kaempferol ($C_{15}H_{10}O_6$) might have good GI absorption, and palmitic acid and linoleic acid exhibited high BBB permeation (Figure 8). However, afzelin ($C_{21}H_{20}O_{10}$) may be malabsorbed in the GI tract (Figure 8). As these ingredients are considered for oral delivery, the ADEM features were calculated, and the results are displayed in Table 1. Out of the four active ingredients, kaempferol did not violate Lipinski's "rule of five", and other active ingredients satisfied the three Lipinski's rule principles. Furthermore, the LogS of kaempferol and linoleic acid were −3.624 and −5.23, respectively, indicating that kaempferol has good solubility, but linoleic acid had relatively low solubility compared to other molecules (Table 1).

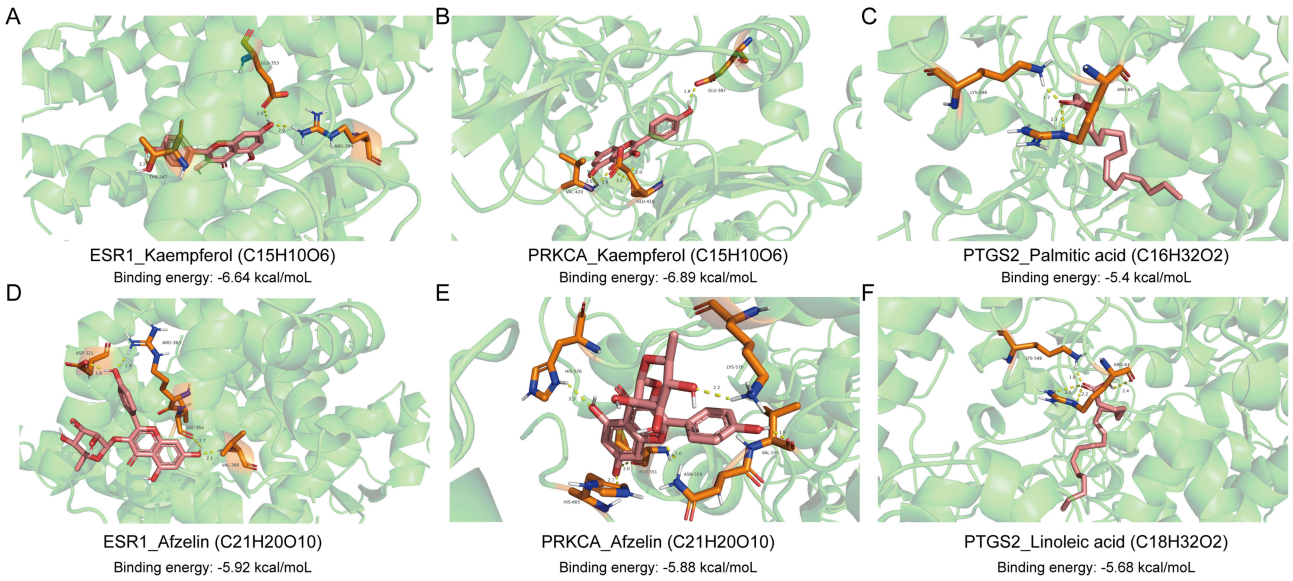

Figure 7 Results of molecular docking. (**A**) ESR1 and kaempferol molecular docking. (**B**) PRKCA and kaempferol molecular docking. (**C**) PTGS2 and palmitic acid molecular docking. (**D**) ESR1 and afzelin molecular docking. (**E**) PRKCA and afzelin molecular docking. (**F**) PTGS2 and linoleic acid molecular docking. Key interacting residues (eg, ARG-194 and THR-347) are highlighted and labeled. Hydrogen bonds are depicted as yellow dashed lines, with corresponding bond distances (in Å) indicated.

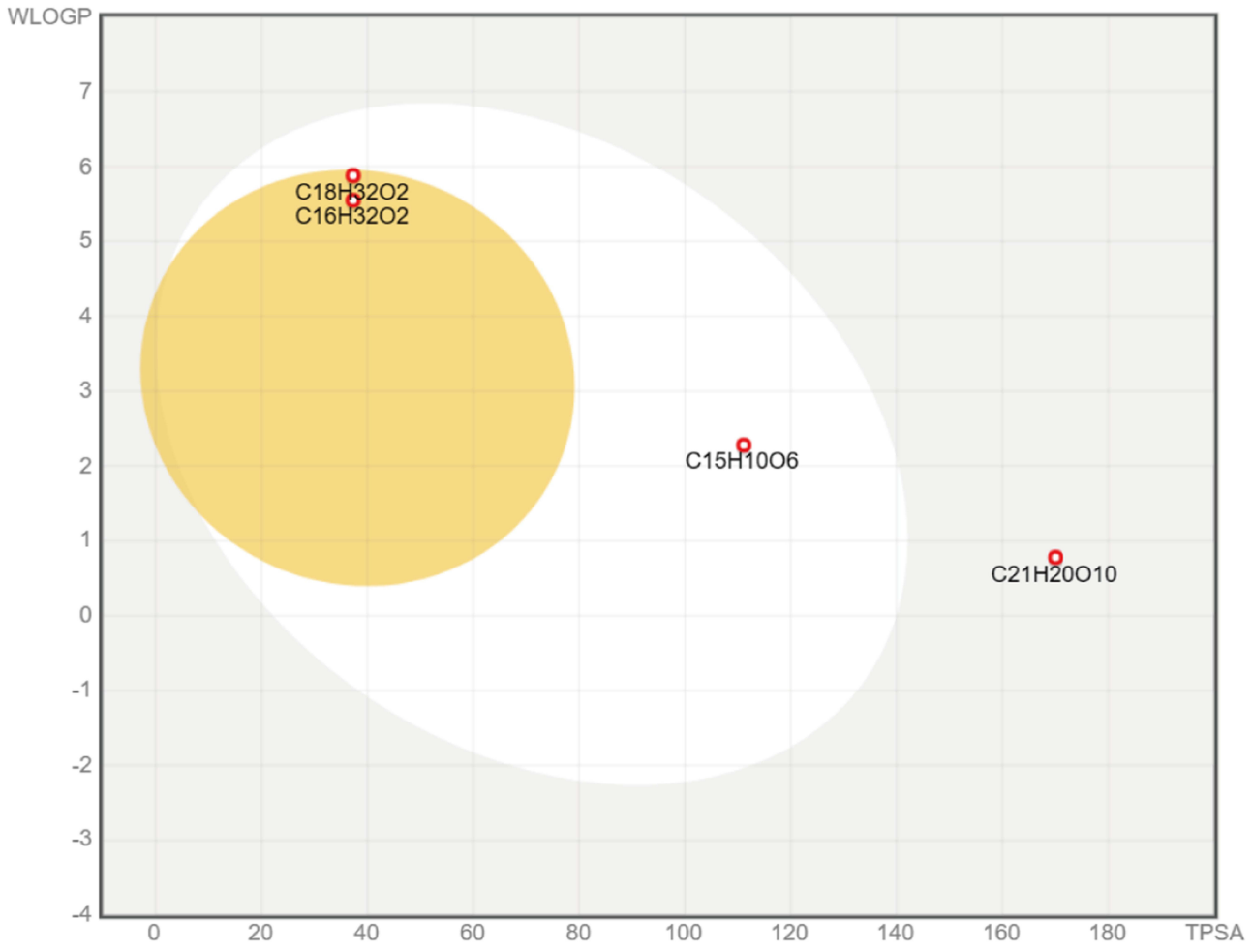

Figure 8 BOILED-Egg model of the four active ingredients. The passive intestinal absorption and brain penetration of compounds were predicted using the parameters WLOGP (octanol-water partition coefficient) and TPSA (topological polar surface area). Compounds located within the white area are predicted to have high human intestinal absorption, while those within the yellow region are additionally predicted to cross the blood–brain barrier.

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Table 1 The ADEM Features of Four Active Ingredients

Molecule	MW (<500)	H-Bond Acceptors	H-Bond Donors	Log P	Lipinski #Violations	GI Absorption	BBB Permeant	Log S
Kaempferol	286.24	6	4	1.58	0	High	No	−3.624
Palmitic acid	256.42	2	1	5.2	1	High	Yes	−5.223
Linoleic acid	280.45	2	1	5.45	1	High	Yes	−5.23
Afzelin	432.38	10	6	0.6	1	Low	No	−4.013

Abbreviations: MV: Molecular Weight; Log P: Lipophilicity; BBB: Blood Brain Barrier; Log S: Solubility.

Additionally, the results of toxicity predictions are presented in Table 2. All the ingredients were not hepatotoxic or carcinogenic. Kaempferol exhibited three toxicological endpoints, inducing drug-induced liver injury (DILI), skin sensitization, and eye irritation (EI). Palmitic acid and linoleic acid showed the same toxicological endpoint: skin sensitization, eye corrosion (EC), EI, and respiratory toxicity. Afzelin presented two toxicological endpoints: DILI and ames toxicity.

Expression Validation of the Hub Gene

We examined the expression of hub genes in sepsis patients and in patients treated with Yinghuang Decoction. The results revealed significant differences in the expression levels of AHR, GMPS, KCNMA1, and PRKCA between the sepsis group and the control group (Figure 9). Additionally, while not statistically significant, the expression trends of CACNB4, ESR1, and PTGS2 in sepsis were consistent with findings in public databases. Notably, the expression of these genes was reversed following treatment with Yinghuang Decoction. Overall, these genes are abnormally expressed in sepsis and may represent potential targets for treatment using Yinghuang Decoction.

Discussion

Sepsis is a disease characterized by the body's immune response imbalance to an infection, ultimately resulting in organ dysfunction.[34,35] This study investigated the potential role of Yinghuang Decoction in treating sepsis through network pharmacology and molecular docking methods.

This study acquired 281 active ingredients in Yinghuang Decoction and 509 corresponding target genes. Furthermore, 7 hub genes (ESR1, PTGS2, CACNB4, KCNMA1, GMPS, AHR, and PRKCA) were obtained. Except for KCNMA1, the expression levels of the other 6 hub genes were decreased in sepsis patients in the training dataset. Estrogen receptor

Table 2 The Toxicity Prediction of Four Active Ingredients

	Kaempferol	Palmitic Acid	Linoleic Acid	Afzelin
hERG Blockers	0.07	0.056	0.009	0.018
H-HT	0.098	0.026	0.013	0.106
DILI	0.979	0.043	0.009	0.978
Ames Toxicity	0.672	0.005	0.013	0.782
ROA	0.156	0.029	0.01	0.107
FDAMDD	0.109	0.015	0.017	0.045
Skin Sensitization	0.856	0.899	0.961	0.063
Carcinogenicity	0.097	0.064	0.153	0.218
EC	0.009	0.977	0.984	0.003
EI	0.929	0.978	0.98	0.031
Respiratory Toxicity	0.09	0.891	0.712	0.041

Abbreviations: H-HT, Human Hepatotoxicity; DILI, Drug Induced Liver Injury; ROA, Rat Oral Acute Toxicity; FDAMDD, Maximum Recommended Daily Dose; EC, Eye Corrosion; EI, Eye Irritation.

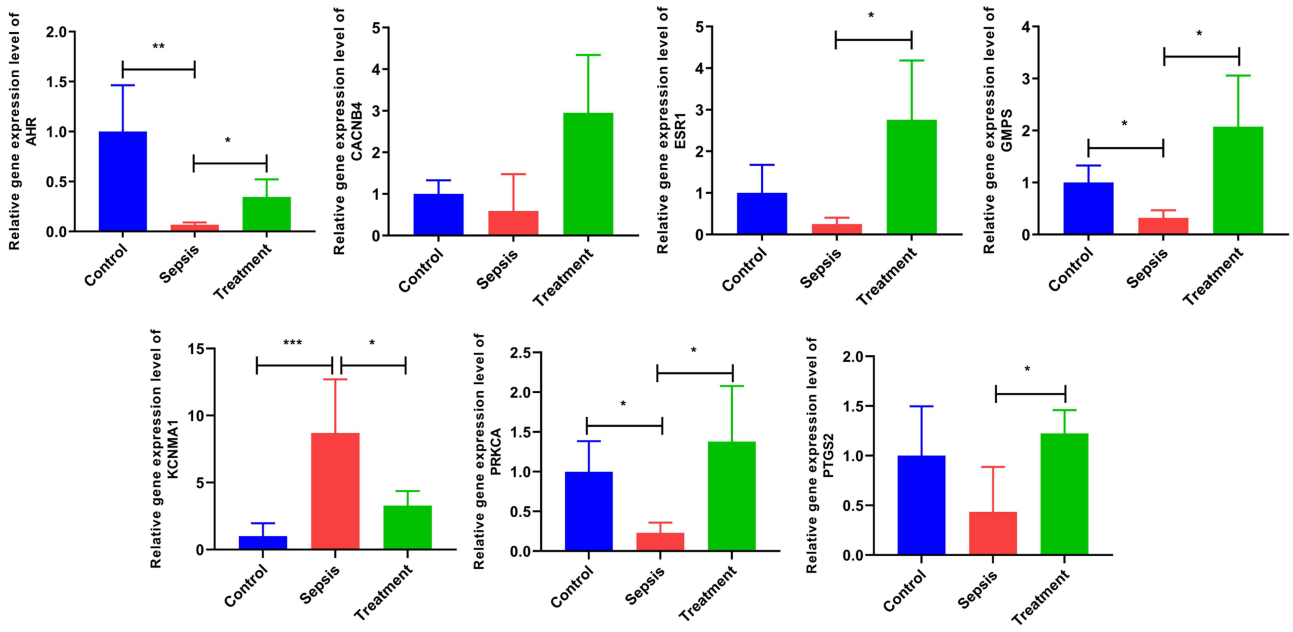

Figure 9 Expression validation of the hub genes. Comparison of mRNA levels among the control, sepsis, and treatment groups (sepsis patients treated with Yinghuang Decoction). * P <0.05; ** P <0.01; *** P <0.001.

1 (ESR1) and its encoded protein are essential for cell growth and survival.[36] It could be a potential target of acacetin and related to sepsis.[37] Prostaglandin endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2 (COX-2), was increased in sepsis-induced cardiac injury[38] and brain tissues in sepsis-associated encephalopathy model.[39] Potassium calcium-activated channel subfamily M alpha 1 (KCNMA1), which encodes the $K_{Ca}1.1$ channel, may contribute to impaired renal blood flow due to its abnormal function in the renal vascular bed during sepsis in the cecal ligation and puncture (CLP) model.[40] Aryl hydrocarbon receptor (AHR), a transcription factor, was significantly downregulated in sepsis-related mouse liver, which might be caused by proinflammatory cytokines TNF-α and IL-1β.[41] Song et al research showed that sinomenine improves septic ALI in an animal model by regulating gut homeostasis through the AHR/Nrf2 pathway.[42] The Protein kinase C isozyme alpha (PRKCA) overexpression alleviated lung injury by promoting mitophagy and suppressing the inflammatory response, reducing ROS production, and inhibiting cell apoptosis in sepsis-induced ALI.[43] Although the roles of Guanine monophosphate synthase (GMPS) and Calcium channel beta 4 (CACNB4) in sepsis have not been reported, previous studies have highlighted their regulatory roles in cancer cell growth.[44,45] ROC analysis showed that several hub genes, including CACNB4, GMPS, and PRKCA, demonstrated fair diagnostic performance (AUC > 0.7) in distinguishing sepsis from healthy controls. These findings suggest that these hub genes may play a significant role in sepsis and could be important targets in the treatment of sepsis with Yinghuang Decoction.

We found that most immune cells are dysregulated in sepsis patients, such as activated B cells, eosinophils, activated $CD8^+$ T cells, and neutrophils. Previous studies have reviewed the host response during sepsis, which is characterized by the coexistence of excessive inflammation and immune suppression.[3,46] Immune suppression is manifested by the apoptosis of B cells and $CD8^+$ T cells, as well as an increase in regulatory T cells.[47] The suppression of innate response activator-B cells could damage bacterial elimination, trigger a cytokine storm, and lead to septic shock.[48] Persistent peripheral eosinophils in sepsis patients are linked to poor outcomes,[49] and eosinophils may serve as a diagnostic marker upon ICU admission.[50] Suppressing macrophage phagocytosis using interleukin-17D exacerbated sepsis.[51] Neutrophils play intricate roles in sepsis as the most abundant innate immune cells, aiding pathogen clearance early on, but their high activation triggers the release of inflammatory mediators and tissue damage during severe sepsis.[52] We also observed correlations between the hub genes and several immune cells, suggesting their potential role in immunomodulation. AHR can regulate LPS-induced macrophage inflammation and antimicrobial activity.[53] ESR1 regulates the production of pro-inflammatory cytokines in neutrophils and macrophages during inflammatory diseases.[54] KCNMA1 may be involved in

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the regulation of potassium channels in macrophages, thereby mediating the extracellular ATP signal and regulating the inflammatory response.[55] While these findings support the biological plausibility of immune involvement, the observed associations do not imply direct causation. Further functional studies are needed to clarify whether Yinghuang Decoction modulates immune responses through these targets in the context of sepsis.

Based on the herb-ingredient-target-disease network analysis, all the herbs in Yinghuang Decoction have the potential to act on various targets through their active ingredients to treat sepsis. Molecular docking showed that 4 main active ingredients (palmitic acid, kaempferol, afzelin, and linoleic acid) that interacted with ≥10 target genes have good binding ability with ESR1, PRKCA, and PTGS2. Palmitic acid is associated with the highest number of target genes in the herb-ingredient-target-disease network, which suggests it could be a crucial ingredient in the treatment of sepsis with Yinghuang Decoction. Palmitic acid is a saturated fatty acid, and dietary palmitic acid has been shown to mediate innate immune memory during endotoxemia. It may induce excessive inflammation and infection clearance, with this immune memory response being plastic.[56] Kaempferol, a flavonoid, has been reported to possess anti-inflammatory and anti-oxidant properties, and it could alleviate ALI in septic mice.[57] Kaempferol could alleviate the inflammatory response and endothelial barrier damage in LPS-induced sepsis through the SphK1/S1P pathway.[58] Linoleic acid protects against IFN-γ-induced hepatocyte injury[59] and is associated with the outcome in septic rats.[60] Afzelin reduces pro-inflammatory factor levels and enhances mitochondrial biogenesis to prevent liver damage in a fulminant hepatic failure model.[61] Overall, the active ingredients in Yinghuang Decoction may exert their potential effects by targeting hub genes to treat sepsis, possibly through inflammatory and immune mechanisms. Drug-likeness and toxicity prediction analysis showed that these active ingredients had drug-likeness and lacked hepatotoxic and carcinogenicity.

This study's strength lies in its integration of multiple bioinformatics approaches, such as network pharmacology, immune infiltration correlation analysis, molecular docking, and pharmacokinetic predictions, to thoroughly elucidate the potential mechanisms of Yinghuang Decoction in treating sepsis. We preliminarily identified key targets and active ingredients, providing a theoretical foundation for further experimental validation. However, several limitations should be acknowledged. First, the analyses are heavily reliant on existing databases and predictive algorithms, which may not fully capture the dynamic pharmacological interactions occurring in vivo. Second, docking analysis was limited to compounds with available 3D structural data, potentially excluding bioactive ingredients lacking such data. Third, while the qPCR validation supported the expression trends of hub genes, the small sample size may have reduced the statistical power, warranting further validation in larger cohorts. Moreover, network pharmacology cannot establish causality or quantify the contribution of individual compounds, which requires follow-up experimental investigations. Future studies will focus on confirming the pharmacological activity and biological relevance of the predicted targets and compounds in sepsis models. Lastly, although we stratified sepsis patients by severity, this study did not distinguish molecular subtypes with different sources of infection or immune-inflammatory profiles. Future research should explore whether Yinghuang Decoction exerts consistent therapeutic effects across diverse sepsis subtypes to assess its broader applicability.

Conclusion

In this study, we explored the pharmacological and molecular mechanisms of Yinghuang Decoction in treating sepsis using public databases. We identified 7 hub genes that might participate in the treatment of sepsis with Yinghuang Decoction. These hub genes were significantly associated with certain immune cells that are notably dysregulated in sepsis. Furthermore, 4 active ingredients, linoleic acid, palmitic acid, kaempferol, and afzelin, demonstrated good binding affinities with ESR1, PRKCA, and PTGS2. These ingredients had drug-likeness and were not associated with hepatotoxic or carcinogenicity. Overall, this study preliminarily identified potential target genes and active compounds that may underlie the therapeutic effects of Yinghuang Decoction in sepsis. Our findings provide an exploratory molecular basis for future comprehensive studies.

Data Sharing Statement

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

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Hebei Provincial Hospital of TCM (HBZY2022-KY-113-01). Written informed consent was obtained from every participant.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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