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Network pharmacology and molecular docking for exploring the mechanism of *Hedyotis diffusa* against hepatitis B virus infection

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Abstract: **Background**: Hepatitis B virus (HBV) chronically infects nearly 250 million people and causes nearly 700 000 deaths annually. *Hedyotis diffusa* (HD) is widely used in traditional Chinese medicine for HBV-related liver diseases, yet its systemic mechanism remains unclear. **Objectives**: To elucidate the anti-HBV mode of action of HD by integrating network pharmacology with molecular docking. **Methods**: Three databases, including TCMSP, TCMID and Chemical database were used to collect ingredients. PubChem database was used to collect the known targets and Pharmmapper database for the prediction of chemicals. DisGeNET database, PharmGKB and Drugbank were used to get disease-related targets. Mapping all candidate targets transformed into gene symbols to disease-related targets. Biological analysis contains Gene Ontology analysis and enrichment analysis of KEGG signaling pathway analyzed on DAVID 6.8 database. **Results**: One hundred and forty-five putative HD–HBV targets emerged; 30 met hub criteria, with AKT1, ALB and GAPDH highest-ranked. GO terms centred on negative regulation of apoptosis, enzyme binding and MAP-kinase activity. Fifty-eight KEGG pathways were enriched, foremost TNF, FoxO, estrogen, prolactin and ErbB signalling. The ingredient–target–pathway network implicated ferulic acid, coumarin and digitolutein as core components. Ferulic acid docked favourably to all seven selected kinases (−7.1 to −5.3 kcal mol^{-1}), suggesting direct modulation of viral entry (EGFR), replication (AKT1) and immune-evasion (MAPK) checkpoints. **Conclusion**: This study elucidates the multi-component, multi-target and multi-pathway mechanisms of HD in treating HBV infections, thereby providing an efficient and systematic preliminary direction for elucidating the complex actions of traditional Chinese medicine (TCM) formulations and guiding subsequent experiments.

Keywords: Hepatitis B virus; *Hedyotis diffusa*; Mechanism; Network pharmacology; Molecular docking

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INTRODUCTION

Hepatitis B virus (HBV), one of the common DNA viruses, causes several liver diseases including fibrosis, Chronic Hepatitis B and hepatocellular carcinoma (Al-Juboori *et al*., 2023; Yu *et al*., 2015). Nearly 250 million individuals were chronically infected with HBV worldwide (Mingjuan *et al*., 2021). Approximately 700 thousand Hepatitis B virus (HBV)-related mortalities occur worldwide annually (Aparna *et al*., 2015). HBV-related liver diseases have become a global burden and public health threat.

Traditional Chinese medicines (TCM) have been used to treat hepatitis B virus (HBV) infections for a long time. Among them, HD was widely used for the clinical treatment of inflammatory diseases like Hepatitis B according to an ancient book named Guang Xi Zhong Yao Zhi. HD is mainly derived from the dried whole herb of *Oldenlandiae diffusa Roxb.* Several studies showed that HD had a positive influence on HBV infections. Huang K.C. et al. found that HD could reduce the incidence of chronic hepatitis in breast cancer patients (Chin *et al*., 2017). Clinical application of *hedyotis diffusa* in patients with chronic hepatitis B could significantly diminish the risk of hepatocellular carcinoma (Xiaoke *et al*., 2020). Despite its strong effects against HBV infections, it is urgent to explore its underlying mechanism. Naturally derived products from plants show potential in the treatment of HBV and HCV by inhibiting viral replication and key steps in the viral life cycle (Y. *et al*., 2023; Yeluri *et al*., 2023). Hedyotis diffusa (Willd.), also known as Oldenlandia diffusa (Willd.) Roxb., is an annual, sprawling herbaceous plant that belongs to the Rubiaceae family (Jun *et al*., 2011; Song *et al*., 2019). It is native to tropical and subtropical regions of Asia, including China, India, Nepal and Southeast Asian countries (Lin *et al*., 2013; Shao *et al*., 2011). In Traditional Chinese Medicine (TCM), Hedyotis diffusa is an extensively used herb with anti-inflammatory and anti-tumor properties (Feng *et al*., 2022; R. *et al*., 2016; Yi *et al*., 2022). Modern phytochemical research has identified hundreds of compounds from Hedyotis diffusa, including anthraquinones, iridoids and their glycosides, triterpenes and steroids, flavonoids and phenolic acids (R. *et al*., 2016; Yi *et al*., 2022).

The clinical application of TCM often involves multiple targets and pathways. Traditional pharmacology struggles to comprehensively, effectively and scientifically analyze the mechanisms of HD in treating HBV infections due to its complex active ingredients. Network pharmacology, leveraging computer technology and extensive public

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databases, can address these limitations. This approach has been successfully applied to elucidate the mechanisms of TCM in treating various diseases (Meng *et al*., 2020; Yonghui *et al*., 2020).

In this study, we employed network pharmacology and molecular docking to explore the potential mechanisms of HD against HBV infections. We screened the active components of HD from three Chinese public databases, identified potential anti-HBV targets, constructed an ingredient-target-pathway network and validated the binding potential between active ingredients and targets through molecular docking. The workflow of this study is illustrated in fig. 1. By screening the TCMSP, TCMID and chemical databases, active ingredients (Ingredients) were obtained. Subsequently, through screening with the PubChem database and prediction with the Pharmmapper platform, the ingredients were converted into targets (Targets) and standardized to chemical-related targets in gene symbol format through the Uniprot database. Meanwhile, disease-related targets associated with HBV infection were screened from the DisGeNET, PharmGKB and Drugbank databases. The intersection of the two sets of targets yielded putative targets, which were then used to construct a protein-protein interaction network (PPI network) and an ingredient-target-pathway network (I-T-P network). Topological analysis was performed to identify hub targets and key chemicals. Finally, gene ontology (GO) and KEGG pathway enrichment analyses were conducted on the hub targets and molecular docking was performed to validate the key chemicals.

MATERIALS AND METHODS

Active ingredients and potential targets

Three Chinese databases contain Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (Jinlong *et al*., 2014), Traditional Chinese Medicines Integrated Database (TCMID)(Xue *et al*., 2013) and Chemical database (http://www.organchem.csdb.cn/scdb/default.asp) were used to obtain the chemicals in HD. After deleting the same chemicals and chemicals without three-dimension conformations from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and TCMSP, the remaining were selected via Lipinski's five of rules (Molecular weight < 500 daltons, LogP < 5, H-bond donors < 5, H-bond acceptors < 10 and rotatable bonds < 10), which directly get from TCMSP or SwissADME (http://www.swissadme.ch/).

Chemical-related targets were divided into known and predicted categories. The known chemical-related targets were acquired from PubChem database. Predicted targets were obtained using the online platform PharmMapper using chemical-related conformations. All conformational data were saved, including fit score, z score, protein name and other important information. Targets of prediction were screened out with a z score > 0. Chemical-related targets should be *Homo sapiens*, none repetitions and standard ones and finally transformed into official gene symbols through a protein database named Uniprot. To minimize false positives and negatives in target prediction, we applied a multi-step filtering strategy. Only targets with a PharmMapper z-score > 0 were retained and predictions were cross-referenced with known compound-target interactions from the PubChem database when available. Additionally, targets without human gene symbols or with low prediction confidence (i.e., low fit score or absence in literature-supported associations) were excluded. While only one predictive tool (PharmMapper) was used due to resource constraints, this approach provides a conservative and reproducible target set suitable for hypothesis generation.

Although the use of a single predictive tool (PharmMapper) may limit the breadth of target prediction, it was selected for its accessibility, compatibility with 3D molecular structures and previous validation in TCM network pharmacology studies. Future studies will incorporate additional tools (e.g., SwissTargetPrediction, TargetNet) to enhance robustness.

Disease-related genes and putative targets

Three databases including Drugbank, PharmGKB and DisGeNET were used to collect hepatitis B infection-associated genes, which were finally turned into official gene symbols. Mapped them to chemical-related targets and extracted the same ones as the putative targets.

Protein-protein interaction network construction

To further understand the potential mechanisms of HD, we constructed the PPI network using potential targets of HD against HBV infections. STRING database (https://string-db.org/) was used to construct a network with protein-protein (PPI) interactions among proteins. The PPI network diagram was generated using Cytoscape version 3.7 using the network relationship results from STRING.

The ingredient-target-pathway network was established by connecting the herbal compounds, key targets and pathways. The network was visualized by Cytoscape software (version 3.7). According to the Degree and Betweenness centrality and Closeness centrality, three indicators assess the importance of nodes and get the topological features of the network. The higher these quantitative values of the nodes are, the more crucial the nodes will be.

Key targets selections

Key targets selections were based on the topological analysis with three core parameters like Degree, Betweenness Centrality and Closeness Centrality. Topological analysis was performed on Cytoscape software in this study. The core parameters of key targets were larger than the mean of the corresponding parameters in the putative targets.

Gene ontology (GO) and KEGG pathway enrichment analysis

DAVID 6.8 database (https://david.ncifcrf.gov/) was applied for GO enrichment analysis and KEGG pathway enrichment analysis to understand the biological roles of the potential anti-virus mechanism of HD. Three parts including biological process, molecular functions and cellular components make up GO enrichment analysis. For screening significant biological functions and pathways, the FDR value was less than 0.01.

Molecular docking validation

Molecular docking was a common way to validate the binding ability between the ligands and receptors. In this study, small molecules were ligands and the proteins were receptors. Autodock Vina is a relatively accurate molecular docking tool, which is faster than another tool Autodock.

A binding energy threshold of ≤ −5.0 kcal/mol was set as the cutoff for defining potential binding interactions, based on previously reported standards for weak-to-moderate binding affinity. To assess docking reliability, control ligands with known binding affinities were included in the docking protocol. All docking simulations were repeated three times to ensure reproducibility.

The chemical structures of ferulic acid was downloaded via TCMSP (https://tcmspw.com/molecule.php?qn=360), then transformed into the PDB format through Openbabel 3.1.1. The conformations of *SRC* (PDB ID:1Y57), *MAPK14* (PDB ID:6SFO), *EGFR* (PDB ID: 6DUK), *MAPK1* (PDB ID: 6G54), *AKT1* (PDB ID: 1UNQ), *MAPK8* (PDB ID:4G1W) and *MAPK3* (PDB ID: 4QTB) were obtained from RSCB PDB (https://www.rcsb.org/). Proteins were prepared by deleting solvents and ligands and adding hydrogen, computer charge, etc. using Pymol and Autodock 4.2.

Although this study is limited to in silico analyses using publicly available databases, we acknowledge that computational predictions alone cannot confirm biological activity. Due to resource constraints, experimental validation was not performed in this phase. However, this bioinformatics-driven approach provides a cost-effective and systematic framework for hypothesis generation, which can guide future in vitro and in vivo studies.

RESULTS

Active ingredients and their targets

According to the methods of screening bioactive chemicals, we finally screened out a total of 15 active ingredients from HD. All the active ingredients are listed in table 1. Meanwhile, 165 known targets and 405 predictive targets for active ingredients were collected in this study. After deleting overlapped data, a total of 532 effective targets were finally picked out.

Disease-related targets and putative targets for treatments

There was a total of 1473 targets related to HBV infection found in three databases. 145 putative targets for HD against HBV infections were acquired from the intersection of disease-related targets and chemical-related targets. The Venn diagram of putative targets was listed in fig. 2 using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Key targets selection

After topological analysis, 30 key targets were finally found. All key targets and the corresponding topological parameters were presented in table 2. Among them, the top 3 targets with the highest values of Degree Closeness Centrality and Betweenness Centrality were *ALB, GADPH and AKT1*.

GO and KEGG pathway enrichment analyses

According to the results of DAVID 6.8 database, 25 biological processes, 9 molecular functions and 4 cellular components were found in this research. Among them, the top 3 most significant biological processes were negative regulation of apoptotic process, response to drug and positive regulation of smooth muscle cell proliferation, the top 3 most significant cellular components were protein complex, caveola and perinuclear region of cytoplasm, the top 3 most significant molecular functions were enzyme binding, nitric-oxide synthase regulator activity and MAP kinase activity. All the results of GO enrichment analysis showed in fig. 3 using R software. Among them, biological processes were just listed the top 10 most significant.

KEGG pathway enrichment analysis showed that 58 pathways were involved with HD in the treatment for HBV infections. Among them, the top 10 most significant pathways were TNF signaling pathway, Proteoglycans in cancer, Prolactin signaling pathway, Pathways in cancer, Estrogen signaling pathway, Pancreatic cancer, Bladder cancer, FoxO signaling pathway, ErbB signaling pathway, Progesterone-mediated oocyte maturation. Fig. 4 showed the top 10 most significant pathways using R software.

Protein-protein interaction and ingredient-target-pathway network

Eventually, 145 potential targets were imported into STRING database. Fig. 5 was the PPI network diagram in this study. A total of 143 nodes and 1692 edges were found in Cytoscape. Among them, *REG1A* and *CA12* were not included. It was indicated that *REG1A* and *CA12* didn't take part in the interactions with the other proteins. The targets with the largest Degree values were green ones, including *GADPH, ALB, AKT1, MAPK3, EGFR, MAPK1, SRC, CASP3, MAPK8, ESR1, HSP90AA1, MMP9, PTGS2, IGF1 and ERBB*2. Fig. 6 showed the ingredient-target-pathway network (I-T-P). Meanwhile, we further investigated the I-T-P network with the topological analysis.

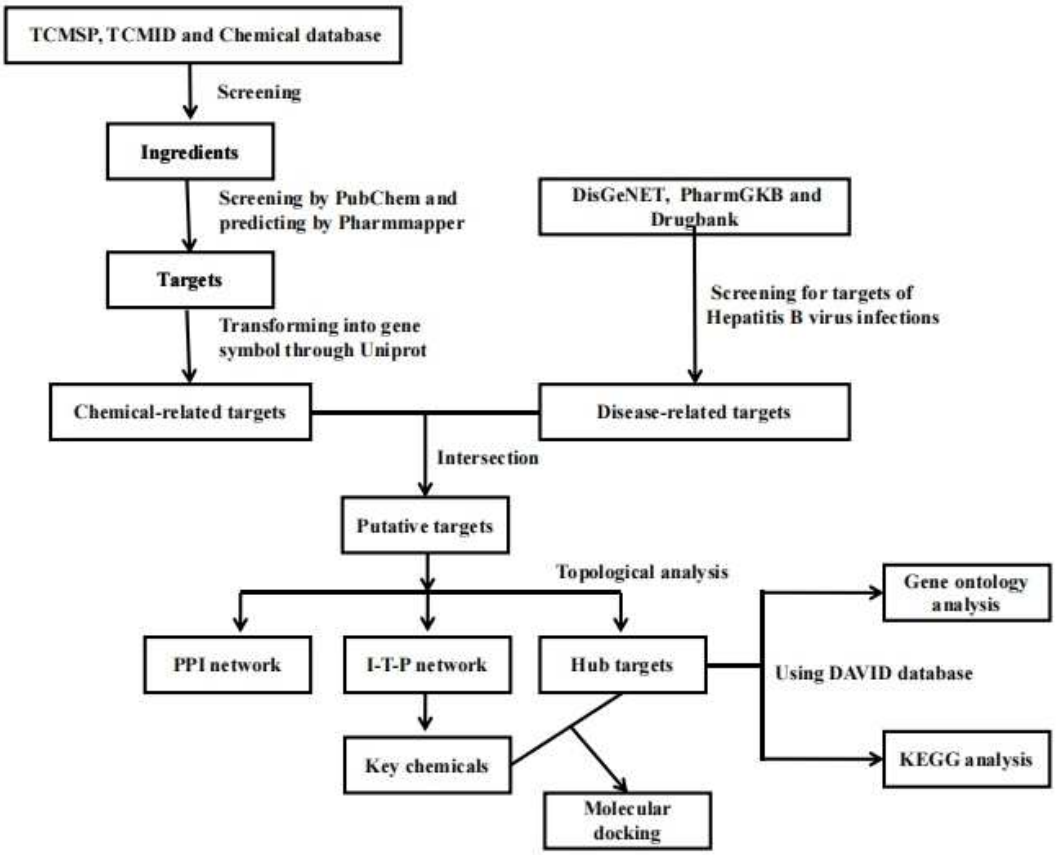

Fig. 1: The workflow of the study

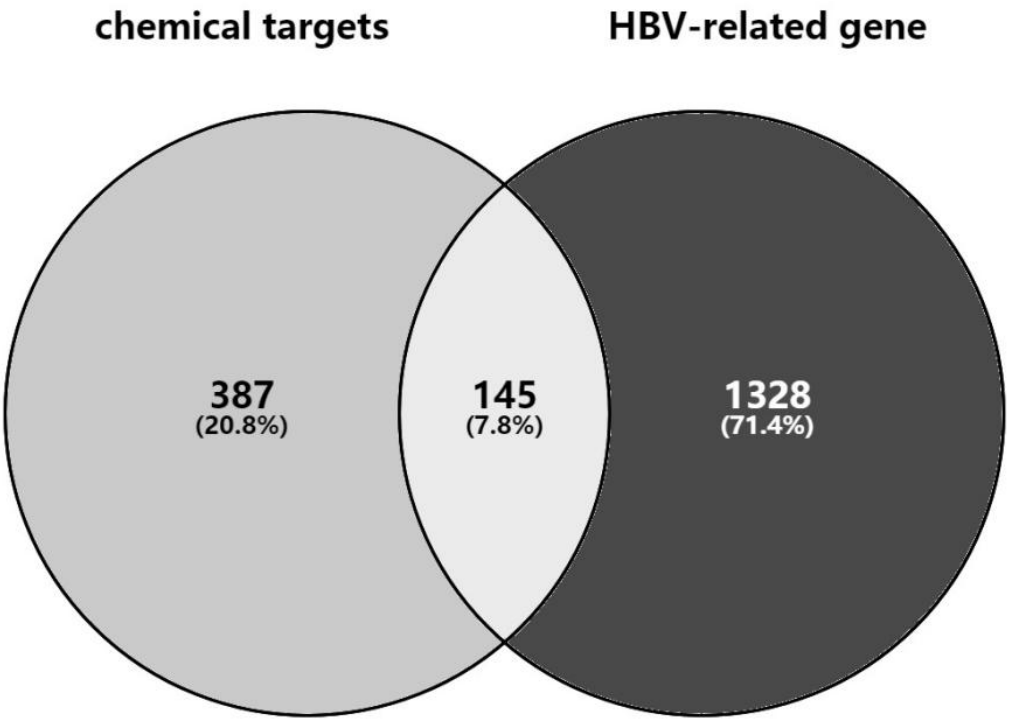

Fig. 2: The Venn diagram of putative targets

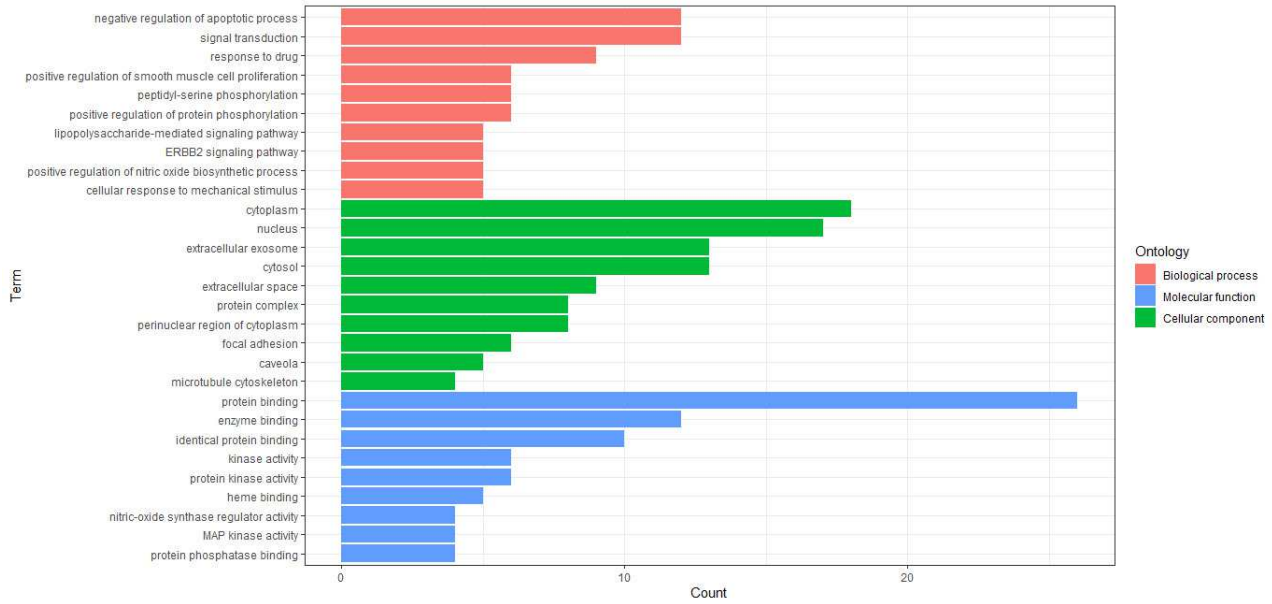

Fig. 3: Results of GO enrichment analysis

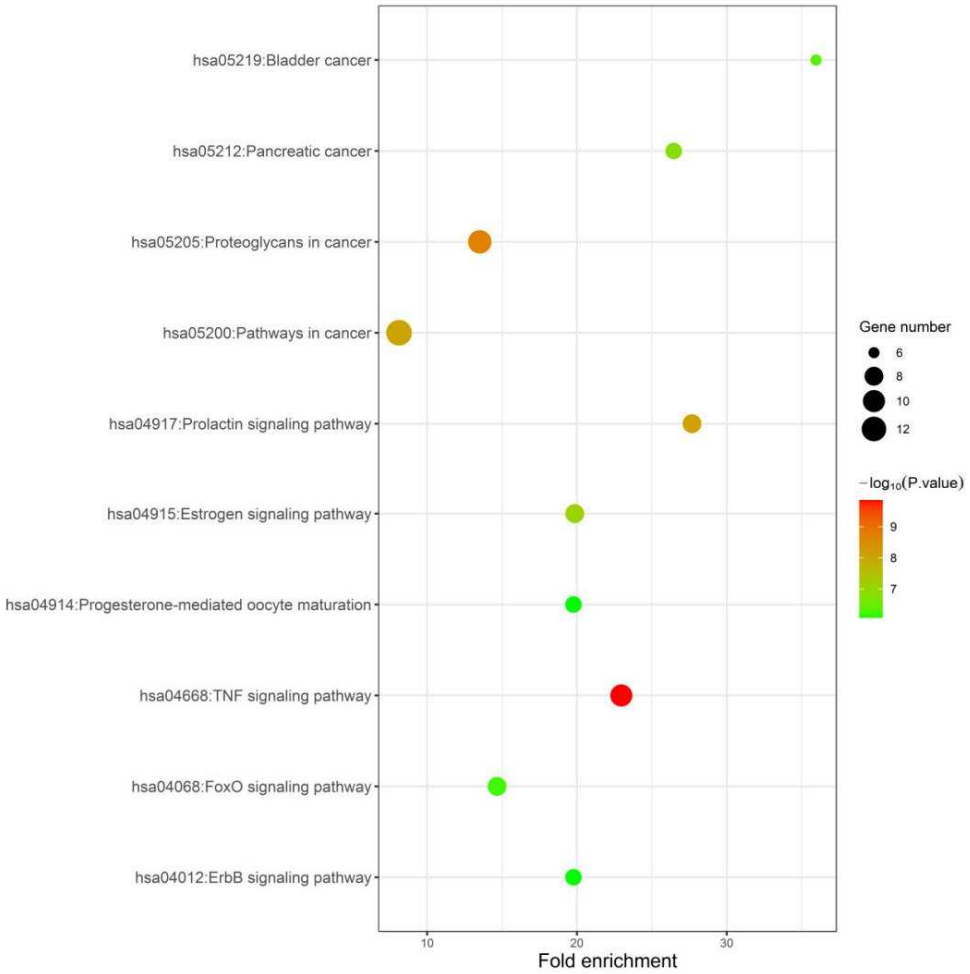

Fig. 4: The top 10 most significant pathways

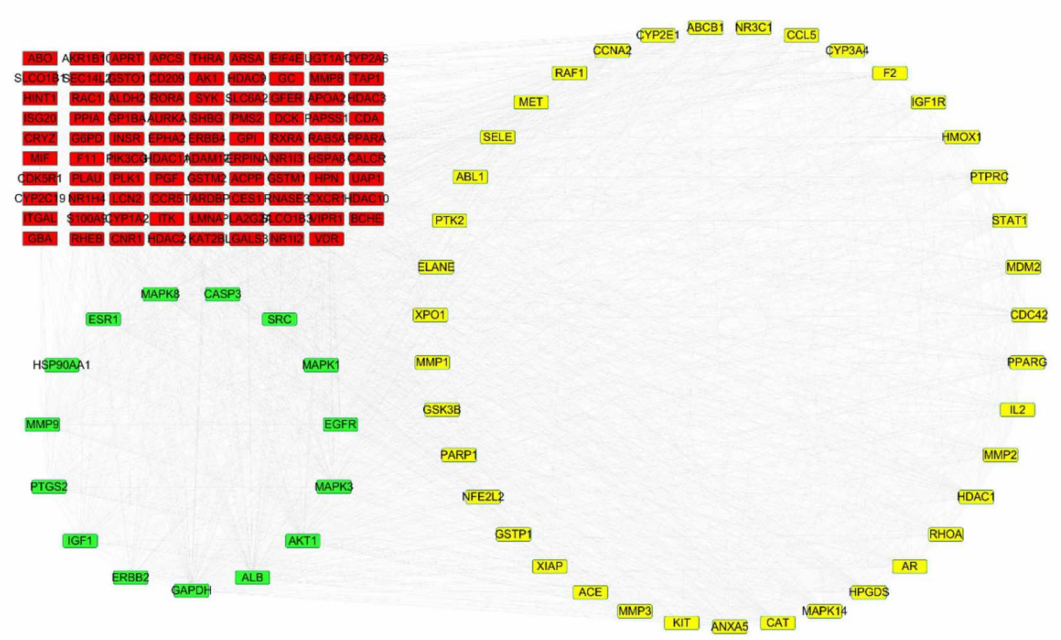

Fig. 5: The PPI network diagram

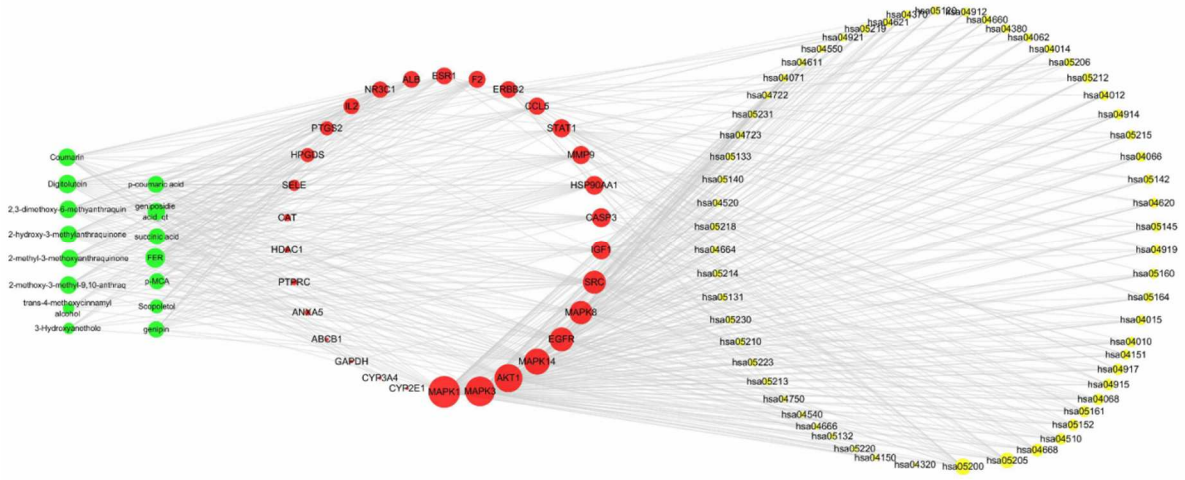

Fig. 6: Ingredient-target-pathway network (I-T-P)

Table 1: The active ingredient in *Hedyotis diffusa*

Number	Chemical names	Weight (g/mol)
1	2,3-Dimethoxy-6-methyanthraquinone	282.31
2	2-Hydroxy-3-methylanthraquinone	238.25
3	2-Methoxy-3-methyl-9,10-anthraquinone	252.28
4	2-Methyl-3-methoxyanthraquinone	252.26
5	3'-Hydroxyanethole	164.22
6	Coumarin	146.14
7	Digitolutein	268.28
8	Ferulic acid	194.2
9	Genipin	226.25
10	Geniposidie acid_qt	212.22
11	p-Coumaric acid	164.17
12	p-MCA	178.2
13	Scopoletol	192.18
14	Succinic acid	118.1

Table 2: The topological parameters of 30 key targets

Gene name	Betweenness centrality	Closeness centrality	Degree
ALB	0.125	0.724	92
GAPDH	0.114	0.732	92
AKT1	0.059	0.700	83
EGFR	0.039	0.645	69
SRC	0.034	0.640	67
MAPK1	0.033	0.640	68
CAT	0.032	0.587	47
MAPK3	0.027	0.654	72
HSP90AA1	0.026	0.607	58
STAT1	0.025	0.557	38
HDAC1	0.022	0.566	41
ESR1	0.021	0.612	58
ERBB2	0.019	0.599	53
MAPK8	0.018	0.626	62
ANXA5	0.018	0.584	48
F2	0.018	0.548	35
MMP9	0.017	0.599	54
CASP3	0.014	0.626	63
HPGDS	0.014	0.573	45
CYP3A4	0.012	0.540	34
PTGS2	0.011	0.599	53
CCL5	0.010	0.536	32
ABCB1	0.010	0.536	32
MAPK14	0.009	0.577	45
IGF1	0.009	0.592	53
PTPRC	0.009	0.550	37
CYP2E1	0.009	0.526	31
IL2	0.008	0.568	41
NR3C1	0.008	0.544	32
SELE	0.008	0.534	30

Table 3: The lowest binding affinity between ferulic acid and other seven proteins.

Protein	PDB ID	Binding energy kcal/mol
SRC	1Y57	-6.1
MAPK14	6SFO	-7.1
EGFR	6DUK	-5.9
MAPK1	6G54	-5.7
AKT1	1UNQ	-5.2
MAPK8	4G1W	-5.9
MAPK3	4QTB	-6.8

Topological parameters of ferulic acid (FER), coumarin and digitolutein, considered as hub ingredients, were larger than mean of the corresponding topological parameters. According to the topological analysis with the same principles, *SRC, MAPK14, EGFR, MAPK1, AKT1, MAPK8 and MAPK3* were considered as key genes.

Molecular docking

The ligands showed reliable binding interactions with the target proteins, when the binding energy between ligands and receptors was less than -5kJ/mol (1.195kcal/mol) (Hao *et al*., 2021). According to table 3, the lowest binding energy between ferulic acid and seven proteins was smaller than the corresponding standard. It is indicated that ferulic acid showed binding affinity to all seven key targets.

DISCUSSION

This study systematically revealed through network pharmacology that the anti-HBV effect of Oldenlandia diffusa (HD) is not dependent on a single component, but rather the result of the synergistic action of its multiple active components (such as ferulic acid, coumarin, digitalutein, etc.). These components form a complex network, acting on multiple key targets such as SRC, MAPK14 and EGFR and synergistically regulate signaling pathways such as TNF and FoxO, thereby reflecting the

holistic therapeutic concept of traditional Chinese medicine of "multi-components - multi-targets - multi-pathways." This provides a modern scientific interpretation of the traditional medicinal value of HD. Network pharmacology elucidates the antiviral mechanisms of traditional Chinese medicine by analyzing its components, targets and signaling pathways (S. *et al*., 2022).

Our I-T-P network identified ferulic acid, coumarin and digitolutein as the topologically dominant compounds. Among them, ferulic acid was predicted to play a central role by simultaneously interacting with seven host proteins (SRC, MAPK14, EGFR, MAPK1, AKT1, MAPK8 and MAPK3) that are known to be hijacked during HBV life-cycle. Molecular-docking results showed consistently favourable binding energies (≤−5.0 kcal/mol) for ferulic acid against all seven targets, supporting the plausibility of these interactions beyond mere computational prediction. In a previous study, ferulic acid was also found to effectively protect the integrity of liver tissues through inhibition of TGF-β1/Smad3 signaling and differential expression of three miRNAs in thioacetamide-induced liver fibrosis rats (A *et al*., 2020). Ferulic acid could ameliorate oxidative stress, liver dysfunction and inflammation to protect liver function in chemicals-induced hepatotoxicity (Fethullah *et al*., 2016; Mozhdeh *et al*., 2020).

Ferulic acid alleviated nonalcoholic fatty liver disease in high-fat diet fed gene knockout mice (Shaoyu *et al*., 2019). Further investigation is needed whether ferulic acid will protect the liver in the treatment of hepatitis B infections. *AKT1* activation of phosphorylation enhanced the oncogenic potential of hepatitis B virus X protein of HBV (Ekta *et al*., 2012). A previous study showed *AKT1* activation could reduce the levels of HBV mRNA and replication of HBV in primary hepatocytes (Siddhartha *et al*., 2015). In addition, HBV-encoded X Protein showed an inhibitory effect on the levels of *EGFR* expression through a micro-RNA in hepatocellular carcinoma cells (Ju *et al*., 2013). Furthermore, the inhibitors of *EGFR* had an obvious suppression of HBV via inhibiting the *STAT3* phosphorylation (Gan *et al*., 2020). Iwamoto, M. *et al* showed an important role of *EGFR* in regulating sodium taurocholate transporting polypeptide internalization, which was a host cell receptor required for HBV entry (Masashi *et al*., 2019). A prior study showed that the impairment of innate immune cells by *MAPK* was a feature in HBV patients with negative HBeAg (Marianna *et al*., 2020). Also, some polymorphisms of *MAPK8* have a positive effect on the risk of low immune responsiveness to the vaccines of hepatitis B (Meng *et al*., 2018). Up-regulation and activation of *SRC* have an effect on the progression of HBV-hepatocellular carcinoma (Gao *et al*., 2021). Total SRC-enhanced by hepatitis B virus X protein is associated with the progression of hepatocarcinogenesis (Liu *et al*., 2018). These findings suggest that HD may treat HBV infections by binding to and regulating these key targets.

First, ferulic acid–AKT1 binding may interrupt HBx-induced AKT1 phosphorylation, thereby attenuating HBV transcription and replication while simultaneously limiting the oncogenic potential of HBx. Second, targeting EGFR is expected to impair HBV entry by interfering with epidermal-growth-factor-mediated internalisation of the sodium taurocholate co-transporting polypeptide (NTCP), a key viral receptor. Third, modulation of MAPK family members (MAPK1/3/8/14) could restore innate immune signalling that is characteristically blunted in HBeAg-negative chronic HBV infection. Finally, the pronounced enrichment of TNF and FoxO pathways suggests that HD may re-establish hepatocyte apoptotic thresholds and counteract oxidative stress elicited by chronic viral exposure. Collectively, these data portray HD as a host-directed modulator that synchronously restricts viral replication, entry and immune evasion. Gene polymorphisms of TNF-alpha affect chronicity and are associated with HBV infections (Borekçi *et al*., 2016; J *et al*., 2011). Increased levels of TNF-alpha in the liver were associated with the corresponding carriers of -863A genotype in the HBV infection (Kummee *et al*., 2010). Significantly downregulating FoxO4 protein was regulated by HBV infections and FoxO4 could suppress HBV core promoter activity through down-regulation of hepatocyte nuclear factor-4α (Li *et al*., 2019). HBV X protein, one of regulatory proteins of the HBV, induced up-regulation of FoxO4, which could strengthen resistance to oxidative stress-induced cell death (Chung *et al*., 2011).

While network pharmacology provides a powerful hypothesis-generating tool, several inherent limitations must be acknowledged. The comprehensiveness and accuracy of underlying databases (e.g., TCMSP, SwissTarget Prediction) influence target prediction reliability. Potential false positives and negatives exist. Analyses rely on existing, often static, data which may not fully capture the dynamic physiological context, such as bioavailability, metabolism and post-translational modifications. Computational predictions, including molecular docking scores, primarily indicate potential binding affinity. In *vitro* and in *vivo* experimental validation is indispensable to confirm these interactions and biological effects. This study avoids overstating its conclusions and emphasizes that these findings constitute a predictive framework rather than definitive proof of efficacy. Our findings highlight multi-target interactions involving key signaling pathways such as TNF and FoxO, offering new insights into how HD may modulate HBV-related pathogenesis beyond direct antiviral effects.

Rather than merely listing previously reported targets, we propose a potential mechanism by which HD may exert its anti-HBV effects through the modulation of host signaling pathways. For instance, the interaction between ferulic acid

and AKT1 may suppress HBV replication by inhibiting HBx-induced phosphorylation events. Similarly, targeting EGFR and MAPK family proteins could interfere with viral entry and immune evasion, suggesting a pleiotropic mode of action that aligns with the holistic principles of Traditional Chinese Medicine.

CONCLUSION

In this present study, there finally found 15 active ingredients obtained from HD, 532 potential targets and 58 signaling pathways involving in the therapy of HBV. It was indicated that multi-ingredient, multi-target and multi-pathway were the most obvious features in the process of HD against HBV. Network pharmacology has great advantages of predicting the potential mechanism, although some limitations also exist in this research, such as the lack of detailed data on bioactive ingredient. The questions mentioned above will be improved or solved, when the study is pushed further. This prospective bioinformatics study holds significant value by leveraging existing computational tools and database resources to offer an efficient and systematic preliminary direction for elucidating the complex actions of traditional Chinese medicine (TCM) formulations and guiding subsequent experiments.

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Author's contribution
This work was completed under the overall supervision and guidance of Wang Yu. Chen Zhe conducted the investigation, performed data analysis, and drafted the manuscript.

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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval
This study involved in silico analysis only and did not require ethical approval as it did not involve human participants or animal experiments.

Conflict of interest
The authors declare that they have no conflict of interest.

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