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RESEARCH ARTICLE

Exploring the Molecular Mechanisms of *Sophorae tonkinensis Radix et Rhizoma* anti-DHAV-1 by Network Pharmacology Analysis

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A B S T R A C T

The study investigated the bioactive ingredients and the anti-duck hepatitis A virus 1 (DHAV-1) mechanisms of *Sophorae tonkinensis Radix et Rhizoma* (STR) by network pharmacology (NP) and molecular docking (MD). The main bioactive ingredients of the STR were obtained using TCMSP database. Cytoscape 3.8.2 software was used for topology analysis and construction of the STR-active molecule-target interaction network. The STRING database and Cytoscape plotted Protein-protein interaction (PPI) networks. The key targets of STR were analyzed and enriched by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. Finally, the main bioactive ingredients of the STR were verified by MD. The STR-DHAV-1 target network included 13 ingredients and 34 target genes. The key target gene is IL-6. KEGG analysis revealed that the main pathways included AGE-RAGE signaling pathway in diabetic complications, pathways in cancer, and C-type lectin receptor signaling pathway. MD results further verified that the main bioactive components identified in the STR were quercetin, kaempferol and matrine, which had higher binding activities to target. Network pharmacology and molecular docking studies revealed that quercetin, kaempferol and matrine were the main bioactive ingredients of STR and might play a crucial role in potential molecular DHAV-1 therapeutic mechanisms.

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INTRODUCTION

STR is one of the most important medicinal herbs used in traditional Chinese medicine (TCM), which is widely used to clear away heat and toxins. It has antiviral, antibacterial, antitumor, antioxidant, anti-liver injury, anti-inflammatory, analgesic, and immune-enhancing effects (Ding *et al*., 2018). It has been reported that STR significantly inhibits hepatic lipids, liver fibrosis and hepatic inflammation. Additionally, the research found that STR inhibits DHAV-1 (Chen *et al.*, 2015). However, the potential mechanisms of STR against DHAV-1 are unclear.

The STR is a good antipyretic drug and has a good targeted effect on liver heat (Ding *et al*., 2018). Duck viral hepatitis is a highly infectious and fatal disease, which can produce hemorrhagic hepatitis and neurological symptoms after infection, and it belongs to the syndrome of liver heat-generating wind in TCM. DHAV as the cause of duck viral hepatitis is classified into three types I, II, and III (Chen *et al*., 2019). DHAV-1 is a single-stranded positive-sense RNA virus classified in the genus *Avihepatovirus* of the family *Picornaviridae* (Zhang *et al*., 2021b). The size of DHAV-1 genome is about 7.8 kb. DHAV-1 has 3 structural proteins and 9 non-structural proteins. The structural proteins of DHAV-1 include VP0, VP1 and VP3 (Lai *et al*., 2021).

The complexity of the components of TCM increases the blindness of TCM research. The biological characteristics of DHAV-1 are unclear, which makes the research in this area lag behind. NP is a novel method that can clarify the underlying mechanisms of drug actions, including bioinformatics, chemo-informatics, network biology and traditional pharmacology. NP conforms to the integral view of TCM theory and clarifies the complex and multilevel interactions of TCM anti-disease, which fits the characteristics of multi components and targets of TCM (Luo *et al*., 2020). Therefore, the study aims to utilize NP to explore the bioactive ingredients of STR and search for their potential targets and mechanisms in STR anti-DHAV-1 and provide a reference for the research and development of highly effective anti-DHAV-1 drugs.

MATERIALS AND METHODS

Screening of bioactive ingredients of STR: The bioactive ingredients of STR were screened from the TCMSP database (Ru *et al*., 2014). Additionally, the screening criteria for bioactive ingredients are drug-likeness (DL) ≥ 0.18 and oral bioavailability (OB) ≥ 30 % (Zhang *et al*., 2021a).

Construction of bioactive ingredient-target network: The potential targeting genes for the bioactive ingredients of STR were obtained by Related Targets in the TCMSP database. Furthermore, the genes were standardized by the UniProt database (UniProt, 2019). A visual network of a bioactive ingredient-target network was established by the Cytoscape 3.8.2 (Shannon *et al*., 2003).

Determination of DHAV-1-related genes of bioactive ingredients: The common genes of DHAV-1-related genes and bioactive ingredients-targets were obtained from the Venny 2.1.0 by importing all common genes for analysis.

Construction of PPI network: The interaction between the bioactive ingredients of STR and common targets was built using the STRING database (Szklarczyk *et al*., 2021). Using Cytoscape 3.8.2 and the results obtained from STRING to establish PPI networks.

GO and KEGG analysis: The common genes were imported into Metascape and bioinformatics online tools (http://www.bioinformatics.com.cn/) for GO and KEGG analysis (Zhou *et al*., 2019).

Molecular Docking: Three-dimensional (3D) structures of quercetin, kaempferol and matrine were downloaded from TCMSP. The three-dimensional (3D) molecular structure of IL-6 was downloaded from RCSB PDB (Berman *et al*., 2000). Then, the water molecule was removed from the protein structure but the hydrogen molecule was added by AutoDock Tools 1.5.7 (Morris *et al*., 2009). The MD of the ligand quercetin, kaempferol and matrine to the IL-6 active site were obtained from AutoDock Tools 1.5.7. Finally, the conformations of MD were visualized using PyMOL.

RESULTS

Bioactive ingredients of STR: The main bioactive ingredients of STR contain 21 compounds (Table 1). Among the 21 compounds, 3,4,5,6-tetradehydrospartein-2-one, sophoranol, sophoramine, sophojaponicin, subprogenin C, withaferine, etc. are without related targets (the different Mol ID, OB and DL due to the different molecular structure of sophoraol).

The construction of STR- bioactive components-target network: The information of main bioactive components of STR are shown in Table 2. The STR-bioactive components-target network contained 389 edges and 205 nodes (Fig. 1). The size indicates the importance of the node. The node demonstrates the degree of compounds as follows, quercetin followed by kaempferol, formononetin, isorhamnetin, lupiwighteone, etc. respectively, in a descending order.

STR-DHAV-1 intersection targets: The total number of 382 target genes for the main bioactive components of STR were retrieved from the TCMSP database. A total of 3429 DHAV-1-related genes were obtained from our previous experiment (Unpublished). Then, a total of 34 STR-DHAV-1 intersection targets were obtained (Fig. 2).

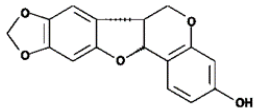
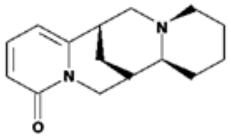
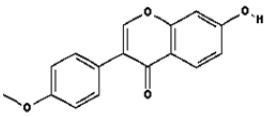
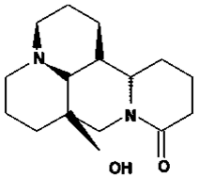
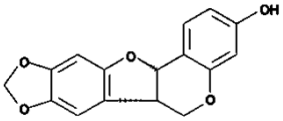
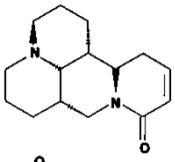
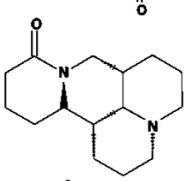
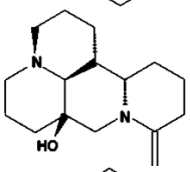
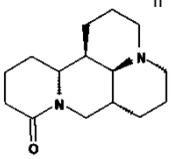
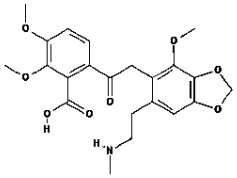
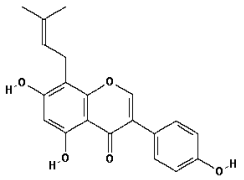
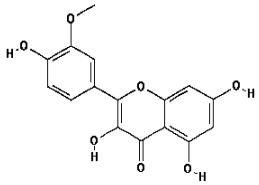
PPI network analysis: The 34 STR-DHAV-1 intersection target genes were analyzed. The PPI network contained 25 nodes and 83 edges (Fig. 3). According to the degree ≥ median (7), we selected 13 more important target genes, including IL-6, VCAM1, PTGS2, MYC, IL-10, CD44, STAT1, PTEN, NFKBIA, NOS2, MMP2, SIRT1 and PLAU, as shown in Table 3. According to the degree ≥ 2-fold median (14), IL-6 is the main target. In addition, the 13 most related STR-DHAV-1 intersection genes were analyzed using the PPI network (Fig. 4). According to the degree of bioactive components, we found three most important compounds, including quercetin, kaempferol and matrine.

GO and KEGG enrichment analysis: The intersecting genes were analyzed using GO enrichment to obtain the function of intersecting genes (Fig. 5). A total of 404 GO biological processes terms were found, out of which 10 most important terms were selected, including regulation of smooth muscle cell proliferation, inorganic substance, hypoxia, and so on. Simultaneously, the enrichment analysis of GO cellular components showed intersecting genes contained membrane raft, membrane micro-domain, plasma membrane raft, predominantly. The genes enriched for molecular function mainly contained oxidoreductase activity, heme binding, tetrapyrrole binding, etc.

Additionally, to further understand the pathway of STR in DHAV-1, the KEGG analysis was structured (Fig. 6). The 10 top-ranking pathways were selected (P<0.05). Meanwhile, the main pathway contained AGE-RAGE signaling pathway in diabetic complications, pathways in cancer, C-type lectin receptor signaling pathway, and PI3K-Akt signaling pathway.

Results of MD: According to the degree in PPI network, we selected IL-6 and 3 top bioactive components to conduct MD verification. The MD analysis showed that quercetin, kaempferol and matrine have a compact binding pattern with IL-6 (Fig. 7). The binding energies of the bioactive components are less than - 5kcal/mol (Table 4).

Table 1: The main bioactive ingredients of STR

Mol ID	Molecule Name	OB (%)	DL	Structure
MOL001484	Inermine	75.18	0.54	
MOL003675	3,4,5,6-tetradehydrospartein-2-one	71.26	0.24	
MOL000392	Formononetin	69.67	0.21	
MOL003663	Sophoranol	67.32	0.28	
MOL003648	Inermin	65.83	0.54	
MOL003627	Sophocarpine	64.26	0.25	
MOL005944	Matrine	63.77	0.25	
MOL003677	Sophoranol	62.77	0.28	
MOL003680	Sophoridine	60.07	0.25	
MOL003633	Oxynarcotine	56.74	0.60	
MOL003656	Lupiwighteone	51.64	0.37	
MOL000354	Isorhamnetin	49.60	0.31	

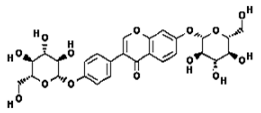
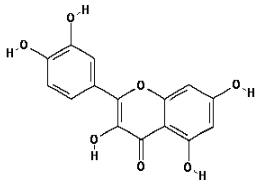
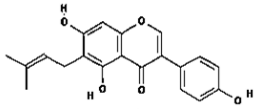
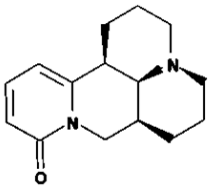
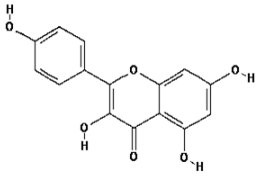
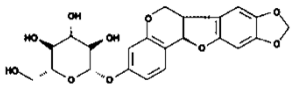
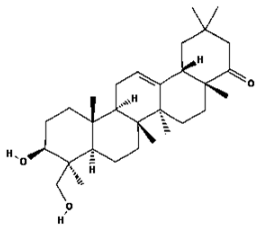
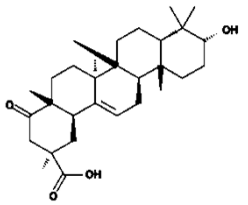
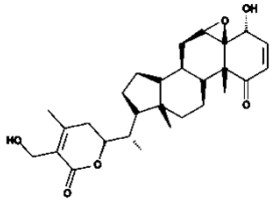
MOL003629	Daidzein-4,7-diglucoside	47.27	0.67	
MOL000098	Quercetin	46.43	0.28	
MOL003673	Wighteone	42.80	0.36	
MOL003676	Sophoramine	42.16	0.25	
MOL000422	Kaempferol	41.88	0.24	
MOL003647	Sophojaponicin	41.51	0.79	
MOL003651	(4aR,6aR,6aS,6bR,8aR,9S,10S,12aR,14bS)-10-hydroxy-2,2,4a,6a,6b,9,12a-heptamethyl-9-methylol-3,5,6,6a,7,8,8a,10,11,12,13,14b-dodecahydro-1H-picen-4-one	37.64	0.75	
MOL003669	Subprogenin C	36.18	0.74	
MOL003644	Withaferine	33.12	0.73	

Table 2: Number information of main bioactive components of STR

Number	Mol ID	Molecule Name
STR1	MOL001484	Inermine
STR2	MOL000354	Isorhamnetin
STR3	MOL003627	Sophocarpine
STR4	MOL003629	Daidzein-4,7-diglucoside
STR5	MOL003633	Oxynarcotine
STR6	MOL003648	Inermin
STR7	MOL003656	Lupiwighteone
STR8	MOL003673	Wighteone
STR9	MOL003680	Sophoridine
STR10	MOL005944	Matrine
STR11	MOL000392	Formononetin
STR12	MOL000422	Kaempferol
STR13	MOL000098	Quercetin

DISCUSSION

DHAV-1 is the main *Avihepatovirus* which can induce highly contagious and acute duck viral hepatitis (Hisham *et al*., 2020). Then, adult ducks infected with DHAV-1 may be manifested as chronic hepatitis, which was recognized as a model for examining the mechanisms of viral hepatitis, liver fibrosis and liver regeneration (Mao *et al*., 2021).

After screening STR, thirteen main bioactive components were obtained. The potential bioactive compounds included quercetin, kaempferol and matrine. Quercetin, an abundant bioflavonoid widely present in many plants,

Table 3: Information of most-related genes and bioactive components

Gene name	Protein name	Degree	Component
IL-6	Interleukin-6	16	sophocarpine, sophoridine, matrine, quercetin
VCAM1	Vascular cell adhesion protein 1	13	kaempferol, quercetin
PTGS2	Prostaglandin G/H synthase 2	11	inermine, isorhamnetin, oxynarcotine, inermin, lupiwighteone, wighteone, formononetin, kaempferol, quercetin
MYC	Myc proto-oncogene protein	11	matrine, quercetin
IL-10	Interleukin-10	11	quercetin
CD44	CD44 antigen	10	matrine
STAT1	Signal transducer and activator of transcription 1-alpha/beta	10	kaempferol, quercetin
PTEN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase	9	quercetin
NFKBIA	NF-kappa-B inhibitor alpha	9	quercetin
NOS2	Nitric oxide synthase, inducible	9	isorhamnetin, lupiwighteone, wighteone, formononetin, kaempferol
MMP2	72 kDa type IV collagenase	8	matrine, quercetin
SIRT1	NAD-dependent deacetylase sirtuin-1	7	formononetin
PLAU	Urokinase-type plasminogen activator	7	quercetin

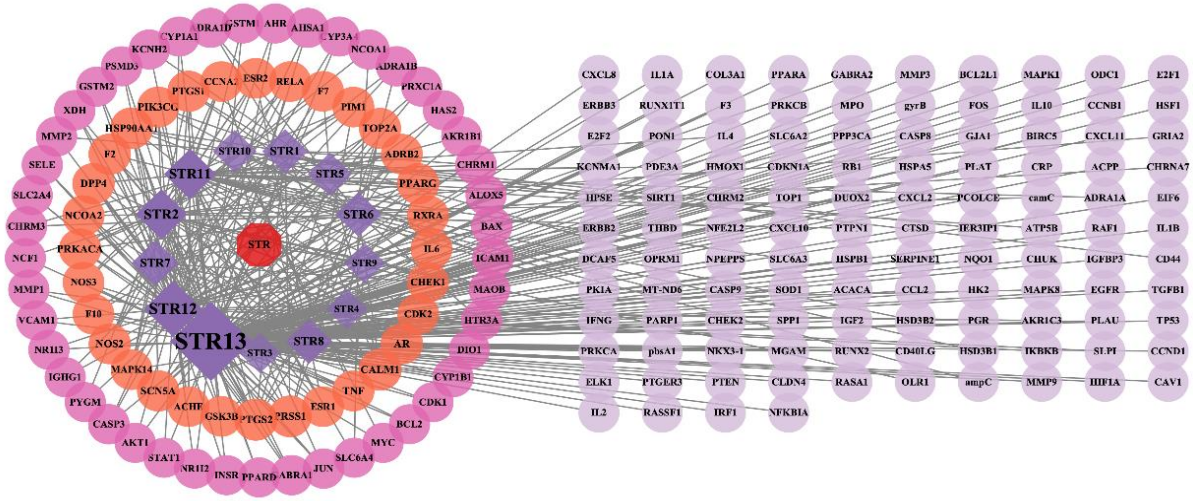


Fig. 1: The STR-bioactive components-target network.

Table 4: The binding energies of the top three compounds (kcal/mol)

Target name	Quercetin	Kaempferol	Matrine
IL-6	-5.02	-5.82	-7.64

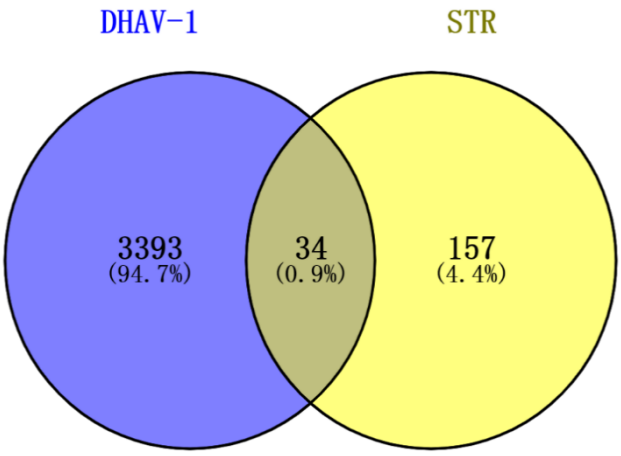


Fig. 2: Venn plot of the STR and DHAV-1 intersection genes.

works as an antioxidant, and has multiple biological functions (Singh *et al*., 2021). Quercetin has been reported as possessing many biological functions, including antiviral, anti-inflammatory, and hepatoprotective (Miltonprabu *et al*., 2017). A previous study also suggests that quercetin is effective against hepatitis C as it decreases hepatitis C-induced reactive oxygen, further clarifying its antiviral ability (Pisonero-Vaquero *et al*., 2014). Moreover, kaempferol, an important bioflavonoid, is a bioactive compound with antitumor, antioxidant, antiviral, antibacterial, etc. qualities (Imran *et al*., 2019). A study has also indicated that kaempferol has a potential as a drug against Hepatitis B (Parvez *et al*., 2021). Furthermore, an inhibition of ALK5 was also reported describing the capacity of kaempferol to attenuate liver fibrosis (Xu *et al*., 2019). Matrine, a quinolizidine alkaloid has been used for the treatment of viral hepatitis in China, having biological functions as antiviral, antitumor and anti-inflammation drug (Yang *et al*., 2012). Another study found that matrine played antiviral activities by inhibiting PRRSV/PCV2 replication and regulating immune functions in mice (Sun *et al*., 2020). It can be concluded that matrine can be a potential candidate as an antiviral drug.

In this work, the PPI network was established based on 34 overlapping genes of STR acting in DHAV-1. Then, a total of 13 targets were regarded as significant based on the degree ≥ 7. IL-6 was the top target, which was regarded as a core gene and may display essential roles in mechanism of STR against DHAV-1. It was obvious that these targets were involved in inflammation, cell regulation, and the immune system. For instance, IL-6, a proinflammatory cytokine, works as a key regulator of innate and adaptive immune responses (Kang and Kishimoto, 2021). Therefore, it can be predicted that quercetin, kaempferol and matrine may suppress DHAV-1 by regulating the immune system and inflammation.

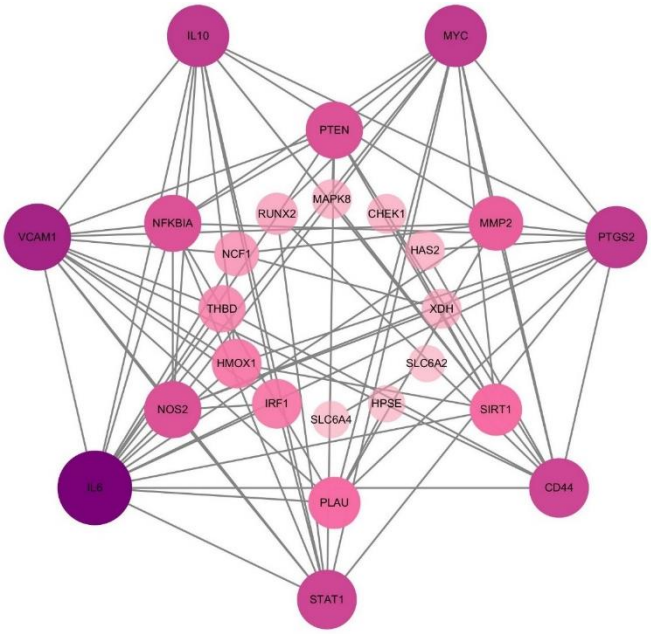


Fig. 3: The PPI network of STR and DHAV-1.

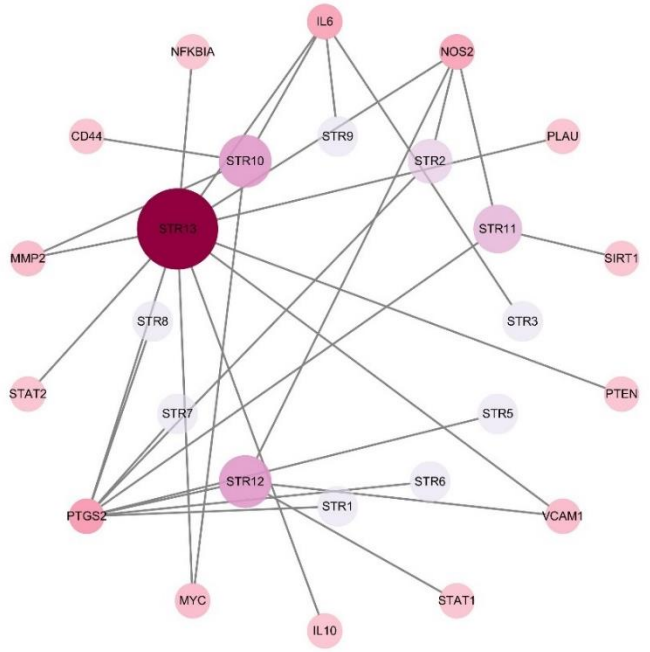


Fig. 4: The PPI network of most related intersection genes.

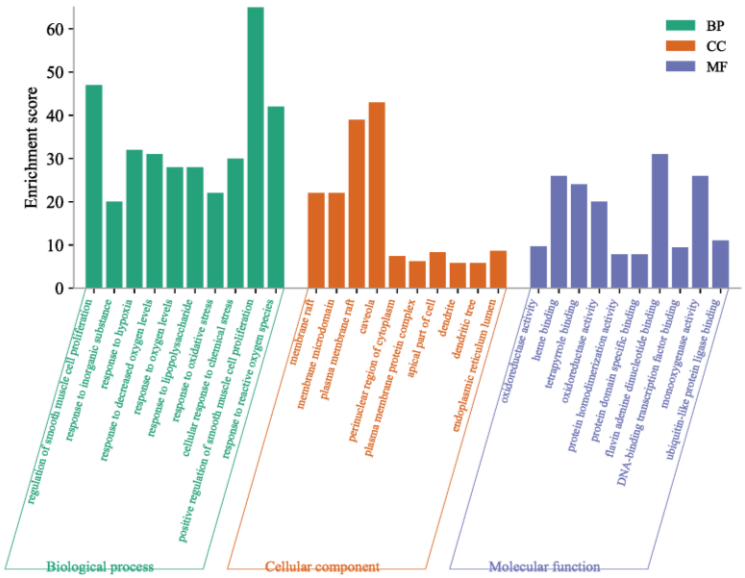


Fig. 5: GO enrichment analysis.

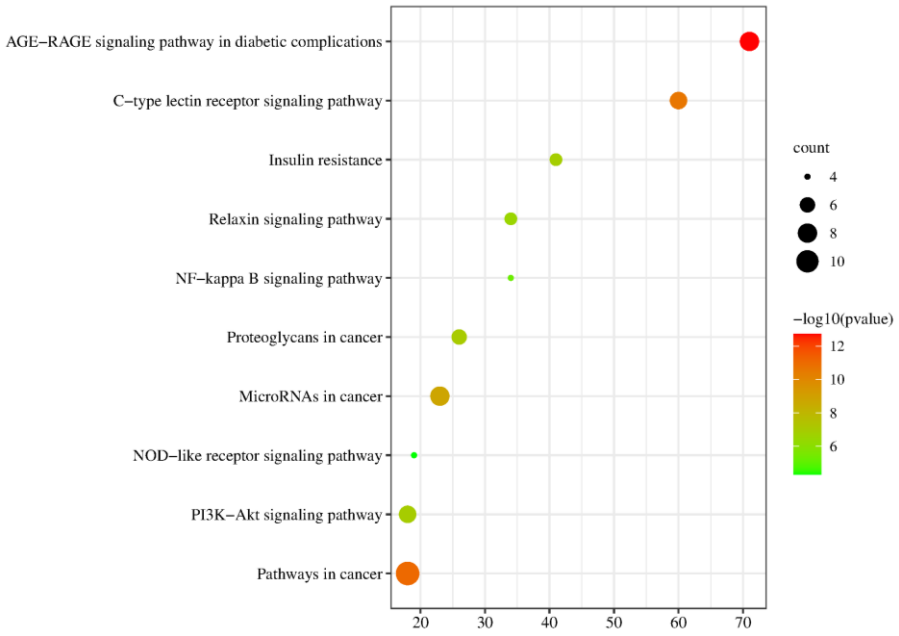


Fig. 6: KEGG enrichment analysis.

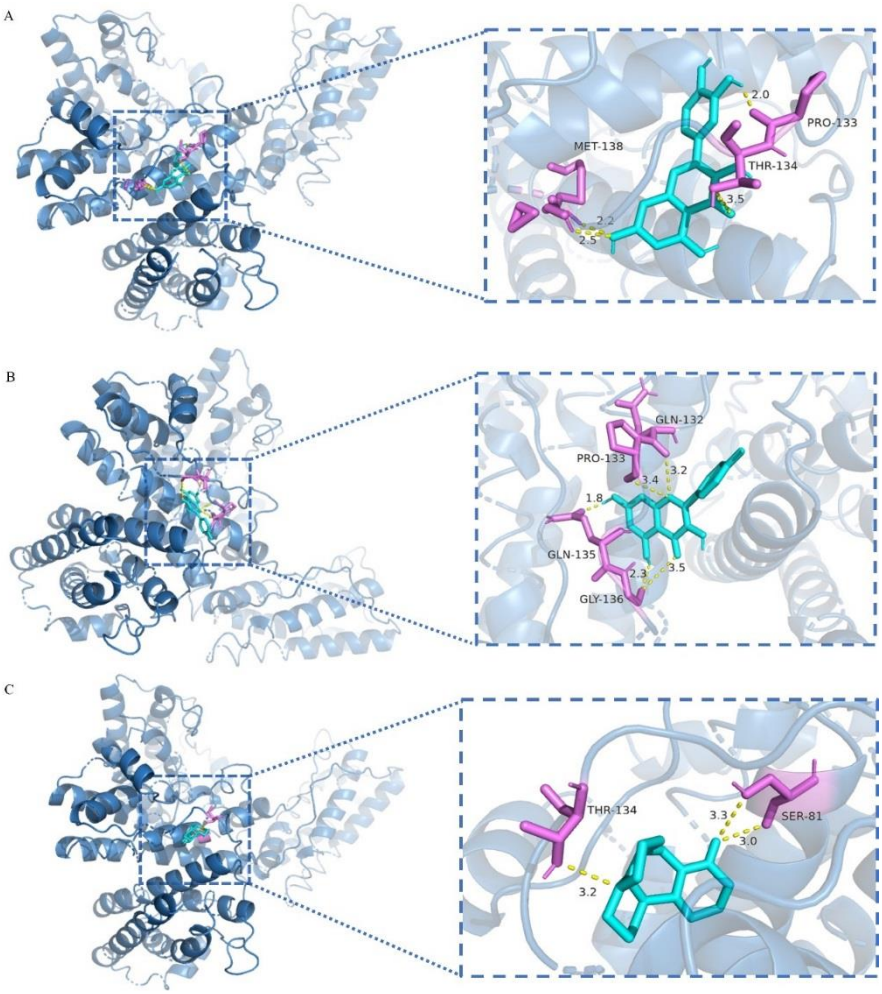


Fig. 7: Molecular docking between IL-6 and three components. (A) Quercetin. (B) Kaempferol. (C) Matrine.

In order to find the mechanisms of STR against DHAV-1, we conducted KEGG and GO analysis. The results of GO analysis provided novel data that the therapeutic effects of STR in DHAV-1 occurred by regulating various biological functions, such as oxidative stress, oxidoreductase activity, oxygen levels, cell proliferation, and ubiquitin-like protein ligase binding, etc. For instance, our previous research found that DHAV-1-infected induced the oxidative stress of duck embryonic hepatocytes (Su *et al*., 2021). It can be inferred that the main bioactive compounds of STR may treat DHAV-1 via inhibiting oxidative stress. In addition, based on KEGG analysis, key targets of STR treating DHAV-1 were closely related to AGE-RAGE signaling pathway in diabetic complications, pathways in cancer, C-type lectin receptor signaling pathway and the PI3K-Akt signaling pathway,

etc. Some pathways were related to immunity and inflammation. Some researchers found that DHAV-1 3C protein could affect the immune system via suppressing the pathway upstream of interferon-β by regulating the activity of interferon regulatory factor 7 (Lai *et al.*, 2021). However, there is no evidence for the therapeutic mechanisms of STR anti-DHAV-1 by these main pathways, though some researchers found that these pathways are associated with liver disease (Garcia-Lezana *et al*., 2021).

To further verify the results of NP, we conducted MD to explore the interaction between bioactive compounds of STR and the key target. MD showed that quercetin, kaempferol and matrine had a good blinding ability to IL-6. Additionally, matrine had higher and more stable binding ability than quercetin and kaempferol. Previous reports indicated that the expression level of IL-6 in DHAV-1 infected ducks was upregulated, and IL-6 was associated with spontaneous viral clearance (Mao *et al*., 2017). A report found that IL-6 is a potential target for antiviral treatment (Tianyu *et al*., 2021), which indicates that the drugs we screened may become the effective anti DHAV-1 drugs. In short, the results of MD further confirmed the accuracy of the NP results.

However, there are some limitations in this study. Firstly, network database is not comprehensive. Secondly, some results need to be verified by further experiments.

Conclusions: In summary, we revealed the potential therapeutic mechanisms of STR anti-DHAV-1 by using NP and MD method. The findings showed that quercetin, kaempferol, and matrine might be potential drugs for the treatment of DHAV-1.

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