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Network pharmacology study of *Phyllanthus niruri* : Potential target proteins and their hepatoprotective activities

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ABSTRACT

This study aims to investigate the potential target proteins, the crucial components of *Phyllanthus niruri*, and the molecular mechanisms involved in hepatoprotective activities using network pharmacology and molecular docking approaches. In addition, the activities of bioactive components obtained were compared to those of *Curcuma longa* and *Curcuma xanthorrhiza*. Nine potential target proteins, namely AKT1, JUN, VEGFA, EGFR, CCND1, SRC, CREB1, MMP2, and RELA, and two crucial components of *P. niruri*, namely ellagic acid and quercetin, which have potential hepatoprotective activities, were identified. This network pharmacology study showed that *P. niruri* affected liver tissues mainly through 10 biological processes and 7 signaling pathways that could be classified into anti-inflammatory and antioxidant activities. The molecular docking study confirmed these activities, demonstrating high binding activity in all ligands and receptors. Among the nine target proteins, CCND1 and RELA were determined as the key targets of *P. niruri* in hepatoprotective activities. We can conclude that *P. niruri* can potentially be a promising new hepatoprotective agent.

INTRODUCTION

In April 2022, at least 169 cases of acute hepatitis of unknown origin had been reported from 11 countries to World Health Organization (WHO) (WHO, 2022). As of 9 May, Indonesia has logged 15 cases (Kiki Siregar, 2022). The clinical syndrome among globally identified cases is acute hepatitis (liver inflammation) with markedly increased hepatic enzymes. Those affected children were between 1 month old and 16 years old. According to the WHO, a worldwide outbreak of severe hepatitis has claimed many lives, including children in Indonesia. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are also important causes of hepatocellular carcinoma (HCC), which has a rising global prevalence and is a fatal and burdensome form of liver cancer. In Asia, liver cancer is the fifth most common cancer and the second most common cause of cancer-related death (Abasseri *et al*., 2023; Liu and Liu, 2022).

In those conditions, hepatoprotective agents are needed to promote liver health. Many herbs have been used to protect liver tissues, such as *Curcuma longa* and *Curcuma xanthorrhiza*. *In vitro* and *in vivo* studies have revealed the mechanisms of action of *C. longa* and *C. xanthorrhiza*. Based on the study results, both herbs have anti-inflammatory and antioxidant effects that are beneficial in maintaining liver function (Oon *et al*., 2015; Salama *et al*., 2013).

In addition to *C. longa* and *C. xanthorrhiza*, *Phyllanthus niruri* (meniran) belongs to the Euphorbiaceae family (Jantan *et al*., 2019) also has anti-inflammatory and antioxidant properties (Bagalkotkar *et al*., 2010), besides immunomodulatory activity (Dirjomuljono *et al*., 2008; Tjandrawinata *et al*., 2017). The previous study demonstrated that the protein fraction of *P. niruri* was effective in protecting liver tissues against oxidative stress in mice. Increased antioxidant defenses are thought to be responsible (Bhattacharjee and Sil, 2006). Tewari *et al*. (2017) and Venkateswaran *et al*. (1987) also described that *P. niruri*

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might have a role in inhibiting the replication of the HBV. In terms of safety, *Phyllanthus* species, including *P. niruri,* were proven to lack adverse reactions. Various clinical studies demonstrated that *P. niruri* has a good safety profile and many potential clinical benefits, including its efficacy in hepatitis (Dirjomuljono and Tjandrawinata, 2011; Tjandrawinata *et al*., 2005).

Therefore, *P. niruri* is suspected of having bioactive compounds in various hepatoprotective-related signaling pathways. However, the chemical constituents, the target proteins, and the signaling pathways responsible for hepatoprotective activities are still unknown. The molecular mechanisms by which *P. niruri* exerts its hepatoprotective effect have never been investigated. Using network pharmacology and molecular docking studies, we could reveal the crucial components, the potential target proteins, and the molecular mechanisms of *P. niruri* involved in hepatoprotective activities, which can be used as a guide to further develop this herb as a new and prospective hepatoprotective drug.

MATERIALS AND METHODS

All data in this research were collected and analyzed from April until June 2022. This study was carried out in several stages; refer to studies of Li *et al*. (2019) and Tjandrawinata *et al*. (2022) (Fig. 1) (Li *et al*., 2019; Tjandrawinata *et al*., 2022; Vengolis, 2013) as follows.

Bioactive components collection and screening

Components of *P. niruri* were obtained from Dr. Duke's phytochemical and ethnobotanical databases (https://phytochem.nal.usda.gov/phytochem/search), Bioinformatic analysis tool for molecular mechanism of traditional Chinese medicine (BATMAN-TCM, http://bionet.ncpsb.org.cn/batman-tcm/), KNApSAcK Core System (http://www.knapsackfamily.com/knapsack_core/top.php), Chemical Entities of Biological Interest (ChEBI, https://www.ebi.ac.uk/chebi/), and literature of the previous study of *P. niruri* phytochemicals (Feng *et al*., 2018; Lem *et al*., 2022; Wadhawan *et al*., 2021). The bioactive components were selected from all components with oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 in the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP, https://tcmsp-e.com/tcmsp.php) or components that passed Lipinski's rule of five for DL of OB in SwissADME (http://www.swissadme.ch/index.php) using each canonical simplified molecular input line entry system (canonical SMILES) obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

A high probability of being of being an oral drug (or the DL) is indicated by molecular weight <500 Da, MLOGP ≤4.15, number of H-bond acceptors ≤10, and number of H-bond donors ≤5 (Daina *et al*., 2017; Jia *et al*., 2021; Lipinski *et al*., 2001; Ranjith and Ravikumar, 2019; Zhang *et al*., 2019; Zhou *et al*., 2022).

Phyllanthus niruri-related target proteins collection and screening

Phyllanthus niruri-related target proteins were collected from Similarity Ensemble Approach (https://sea.bkslab.org/) and the SwissTargetPrediction platform (http://www.swisstargetprediction.ch/) by input canonical SMILES, which were obtained from PubChem for each bioactive component of *P. niruri* (Wadhawan *et al*., 2021). Target protein collection was limited in *Homo sapiens* (human) and Tanimoto coefficient (TC) or probability of drug similarity ≥0.5 (Rahman *et al*., 2022). A similarity threshold for TC is different in several studies. However, the common range applied is 0.5–0.85. As a note, the higher the threshold, the fewer predicted target proteins (Gottlieb *et al*., 2012; Rahman *et al*., 2022). Target proteins collected from both databases were combined and removed

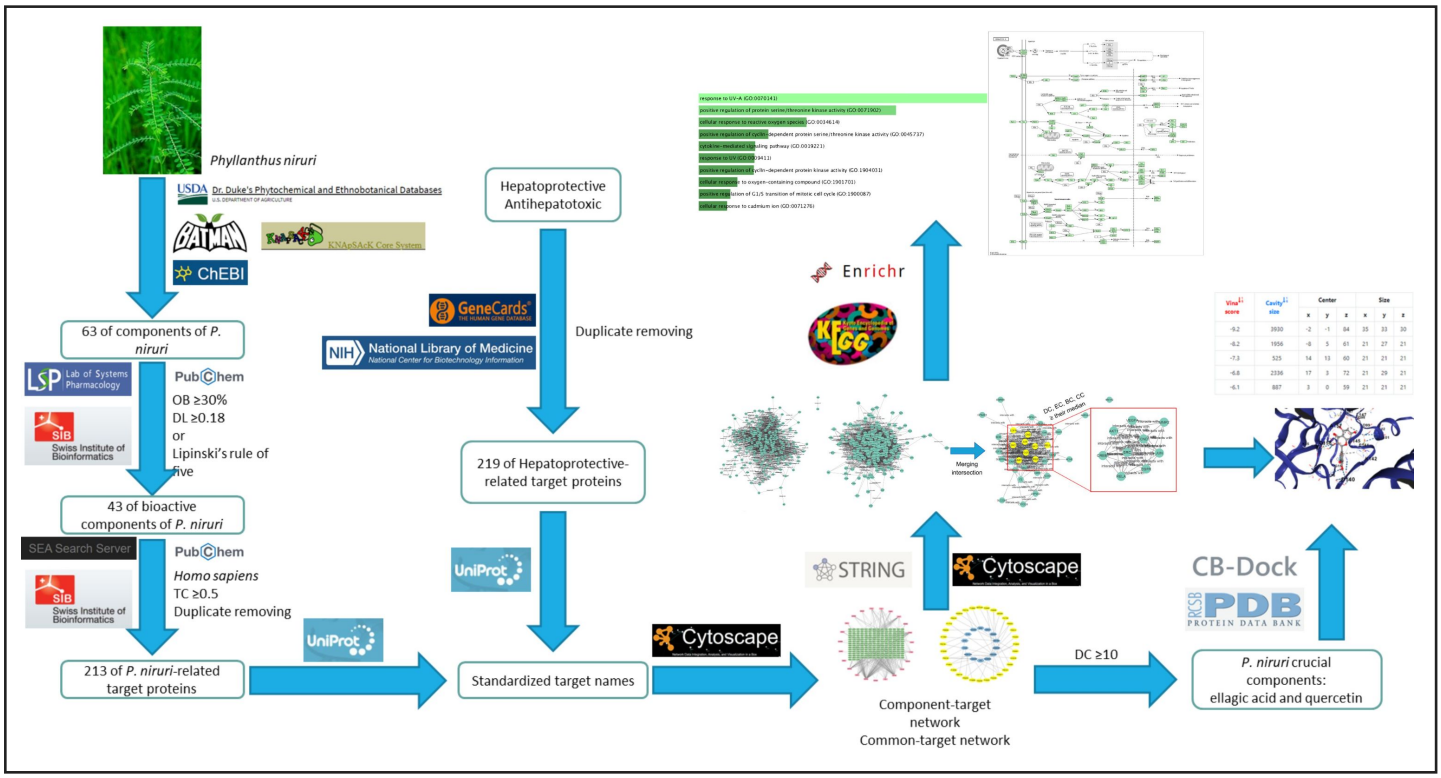

Figure 1. The workflow diagram of network pharmacology study and molecular docking validation of *P. niruri* on hepatoprotective activities.

for duplicate targets (Jia *et al*., 2021; Zhou *et al*., 2022). Those target protein names should be standardized using the UniProt database (https://beta.uniprot.org/) (Wadhawan *et al*., 2021).

Hepatoprotective-related target proteins collection and screening

Hepatoprotective-related target proteins were obtained from the GeneCards database (https://www.genecards.org) and the National Center for Biotechnology Information Gene (NCBI Gene, https://www.ncbi.nlm.nih.gov/gene/) (Li *et al*., 2019; Zhou *et al*., 2022) using keywords of "hepatoprotective" and "antihepatotoxic". Target proteins collected from both databases were combined and removed for duplicate targets, then those target protein names should be standardized using the UniProt database (Wadhawan *et al*., 2021).

Component-target network and common-target network construction

Phyllanthus niruri-related target proteins and the bioactive components of *P. niruri* were collected to build a component-target network using Cytoscape v3.9.1 (https://cytoscape.org/). *Phyllanthus niruri*-related target proteins and the bioactive components of *P. niruri* were represented as nodes, while the interactions between them were represented as edges (Wadhawan *et al*., 2021).

The intersection of *P. niruri*-related target proteins and hepatoprotective-related target proteins was used to construct a common-target network using Cytoscape v3.9.1. This common-target network might be analyzed to determine the important proteins, i.e., the nodes with degree ≥ median degree. The greater number of important proteins were the target of a component; the component could be considered crucial (Li *et al*., 2019).

Protein-protein interaction (PPI) network

Phyllanthus niruri-related target proteins and hepatoprotective-related target proteins were used to build PPI networks using the STRING database (https://www.string-db.org/). *Homo sapiens* (human) organisms with medium confidence of 0.400 were selected as a limitation (Zhang *et al*., 2019). PPI network from the STRING database was downloaded in tab-separated value format and visualized into Cytoscape v3.9.1 (Jia *et al*., 2021; Zhou *et al*., 2022). Both PPI networks were merged in Cytoscape to obtain the intersection. Then, the merging intersection was analyzed using CytoNCA, a plug-in of Cytoscape, resulting in the important proteins. Target proteins should be eliminated when they do not meet the screening criteria of "degree centrality (DC), eigenvector centrality (EC), betweenness centrality (BC), and closeness centrality (CC) are greater than or equal to their median." The remaining target proteins were determined as the important proteins (Li *et al*., 2019).

Enrichment analysis

Phyllanthus niruri-and hepatoprotective-related important proteins were further analyzed to result in information on biological processes (BPs), molecular functions (MFs), cellular components (CCs), and signaling pathways regarding potential hepatoprotective activities using Enrichr (https://amp.pharm.mssm.edu/Enrichr/) and Kyoto encyclopedia of genes and genomes (KEGG) PATHWAY database (https://www.genome.jp/kegg/pathway.html) with p-value ≤0.05 (Jia *et al*., 2021; Li *et al*., 2019; Shahid *et al*., 2021; Zhang *et al*., 2019).

Molecular docking validation

This approach aimed to confirm the network pharmacology study. The crucial components obtained from network pharmacology were used as small molecular ligands to perform molecular docking with potential target proteins. We used the PubChem database to download the 2D structures of ligands in structure-data file format. The 3D structures of receptor proteins were searched in the UniProt database linked directly to Research Collaboratory for Structural Bioinformatics Protein Data Bank (https://www.rcsb.org/) (Rahardjo *et al*., 2020; Ramdani *et al*., 2019) to be downloaded in PDB format. After removing the original ligands and water molecules by University of California, San Francisco Chimera v.1.16 (https://www.cgl.ucsf.edu/chimera/download.html), we obtained receptor protein structure.

To predict the binding regions of a target protein and to calculate the centers and sizes to obtain the best pose with the smallest binding energy, we employed the server of CB-Dock (http://clab.labshare.cn/cb-dock/php/). CB-Dock ranked the binding modes according to Vina score and showed an interactive 3D visualization of the binding modes (Liu *et al*., 2020). Based on the principle of molecular docking, the most negative value of energy (shown as the Vina score) indicates the most stable ligand structure (Abbas *et al*., 2018). If the minimum binding energy is less than −5.0, it implies that ligand-receptor binding activity is high (Lin *et al*., 2021; Zhou *et al*., 2022).

RESULTS

Data collection and screening

In total, 63 components of *P. niruri* were collected from the previous phytochemical studies and 4 natural product databases, including Dr. Duke's phytochemical and ethnobotanical databases, BATMAN-TCM, KNApSAcK core system, and ChEBI (Supplementary Table S1). A total of 43 bioactive components were selected from 63 components according to 2 types of criteria (Supplementary Table S2). First, the criteria from the TCMSP database, including OB ≥30% and DL ≥0.18. Second, the compounds unavailable in the TCMSP database were screened by the criteria of Lipinski's rule of five from SwissADME.

Network construction

From 43 bioactive components of *P. niruri*, we collected 380 target proteins. After removing duplicates, we obtained 213 target proteins (Supplementary Table S3a and b). Subsequently, we constructed a *P. niruri* component-target network containing 236 nodes and 380 edges using Cytoscape v3.9.1 (Fig. 2).

After deleting duplicates, this study collected 219 hepatoprotective-related target proteins from 2 human genomic databases (Supplementary Table S4). The amount of these target proteins in GeneCards and NCBI Gene was 224 and 15, respectively. A total of 32 from 219 target proteins were related to 14 bioactive components of *P. niruri* (Supplementary Table S5a and b, as well as Fig. 3).

Furthermore, we analyzed the common-target network using the criteria of DC. Degree centrality reflects

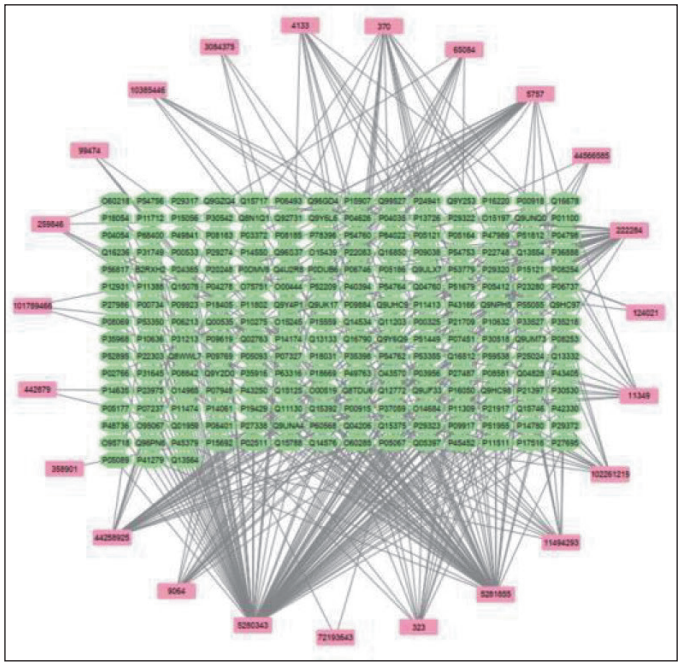

Figure 2. *Phyllanthus niruri* component-target network, containing 236 nodes and 380 edges; pink nodes and green nodes stand for bioactive components of *P. niruri* and target proteins, respectively.

the importance of nodes. The greater DC indicates the more connections a molecule has and the more important it is. With criteria of DC ≥10, we selected ellagic acid (PubChem ID: 5281855) and quercetin (PubChem ID: 5280343) as crucial components of *P. niruri*.

The PPI network of *P. niruri*- and hepatoprotective-related target proteins were constructed using the STRING database and visualized in Cytoscape v3.9.1. We merged and obtained the intersection of both PPI networks. We analyzed the merging intersection of the PPI network by using CytoNCA, a plug-in of Cytoscape. The screening criteria we used were "DC, EC, BC, and CC greater than or equal to their median." Subsequently, we obtained nine potential target proteins (Supplementary Table S6 and Fig. 4).

Enrichment analysis

We input nine potential target proteins into Enrichr for enrichment analysis, resulting in 568 BPs, 63 MFs, 19 CCs, and 139 KEGG pathways, as shown in Supplementary Tables S7–S10, respectively. The top 10 BPs, MFs, CCs, and signaling pathways are shown in Figure 5.

Molecular docking validation

Molecular docking results are shown in Table 1 and Figure 6.

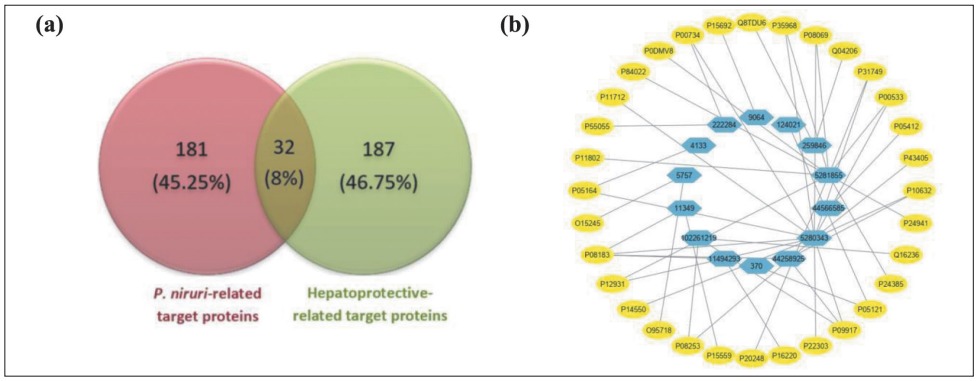

Figure 3. Common-target network. (a) Venn diagram, including 32 target proteins that are related to *P. niruri* and hepatoprotective. (b) Visual common-target network, containing 46 nodes and 47 edges; blue and yellow nodes stand for the bioactive components of *P. niruri* and target proteins, respectively.

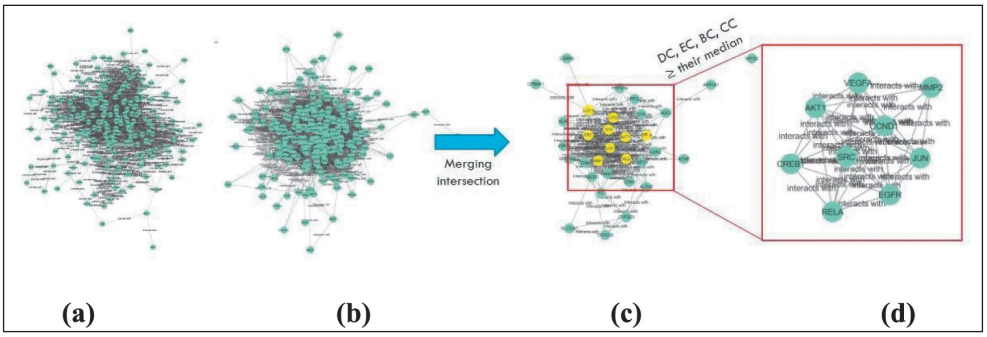

Figure 4. PPI network of target proteins related to *P. niruri* and hepatoprotective. (a) PPI network of *P. niruri*-related target proteins (208 nodes and 1,699 edges). (b) PPI network of hepatoprotective-related target proteins (215 nodes and 4,094 edges). (c) PPI network of common target (32 nodes and 157 edges). (d) PPI network by the screening criteria of DC ≥10, EC ≥0.16039226, BC ≥6.74254615, and CC ≥0.3625855 (9 nodes and 34 edges).

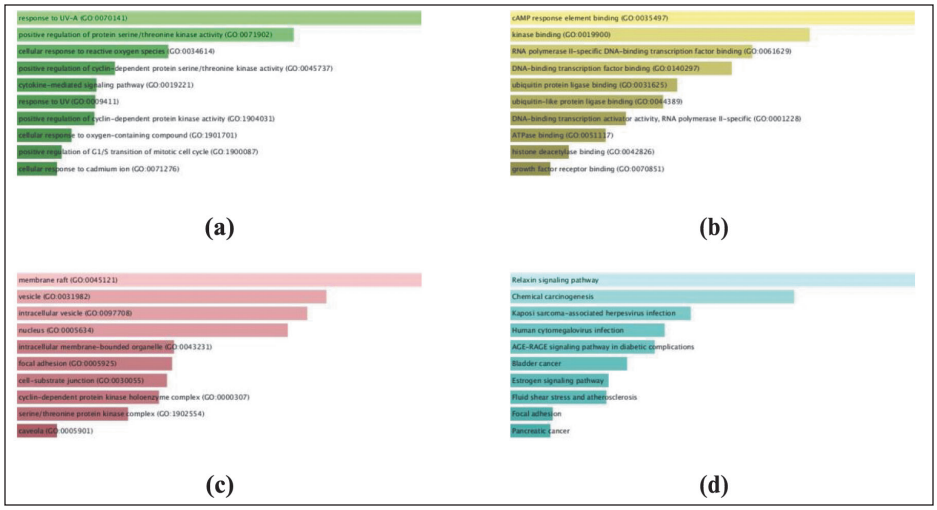

Figure 5. Bar graph of top 10 enriched BPs, MFs, CCs, and signaling pathways (sorted by *p*-value ranking). (a) Bar graph of top ten enriched BPs. (b) Bar graph of top 10 enriched MFs. (c) Bar graph of top 10 enriched CCs. (d) Bar graph of top 10 enriched signaling pathways.

Table 1. Vina score and cavity information of the docking simulation pose for each potential target protein and crucial component of *P. niruri* by using CB-Dock.

Ligand	Target protein	PDB-ID	Vina score	Cavity size	Center			Size		
					x	*y*	*z*	*x*	*y*	*z*
Ellagic Acid	AKT1	1unq	−6.3	116	14	6	17	19	19	19
	JUN	5fv8	−6.5	70	28	2	9	19	19	19
	VEGFA	1vpf	−6.7	322	37	11	17	19	19	19
	EGFR	1m14	−8	3,283	29	9	50	32	35	19
	CCND1	2w96	−8.4	3,930	−2	−1	84	35	33	30
	SRC	1a07	−7.8	1,209	42	12	28	19	19	19
	CREB1	5zko	−6.6	297	28	−49	143	19	19	28
	MMP2	1qib	−7.5	682	69	24	27	19	19	19
	RELA	1nfi	−8.4	3,683	−3	79	106	32	25	19
Quercetin	AKT1	1unq	−6	105	16	16	−2	21	21	21
	JUN	5fv8	−6.4	70	28	2	9	21	21	21
	VEGFA	1vpf	−7.4	416	4	12	6	21	21	21
	EGFR	1m14	−7.9	3,283	29	9	50	32	35	21
	CCND1	2w96	−9.2	3,930	−2	−1	84	35	33	30
	SRC	1a07	−8.1	1,209	42	12	28	21	21	21
	CREB1	5zko	−6.3	297	28	-49	143	21	21	28
	MMP2	1qib	−9.2	682	69	24	27	21	21	21
	RELA	1nfi	−9.2	2,304	−7	53	11	30	21	21

DISCUSSION

The two crucial components of *P. niruri*, namely, ellagic acid and quercetin, and the nine potential target proteins of AKT1, JUN, VEGFA, EGFR, CCND1, SRC, CREB1, MMP2, and RELA, were successfully identified in this network pharmacology study. The DC, EC, BC, and CC of those nine target proteins were greater than or equal to their median. The higher all those topological parameters indicate the more important the target proteins (nodes) (Li *et al*., 2019; Wadhawan *et al*., 2021).

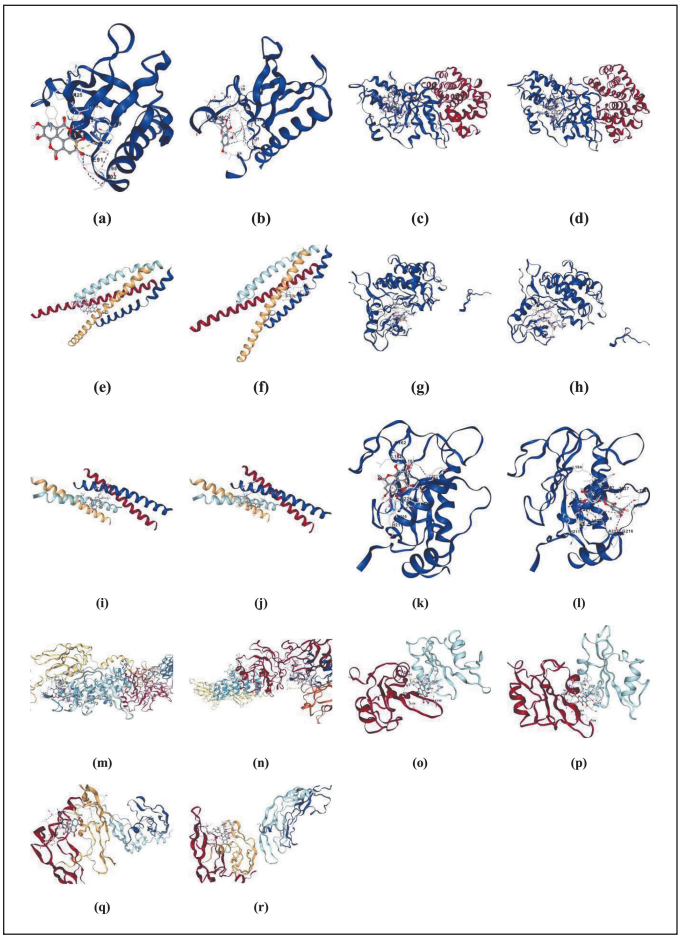

Figure 6. Docking model diagram for each potential target protein and crucial component of *P. niruri* by using CB-Dock. (a) Ellagic acid-AKT1. (b) Quercetin-AKT1. (c) Ellagic acid-CCND1. (d) Quercetin-CCND1. (e) Ellagic acid-CREB1. (f) Quercetin-CREB1. (g) Ellagic acid-EGFR. (h) Quercetin-EGFR. (i) Ellagic acid-JUN. (j) Quercetin-JUN. (k) Ellagic acid-MMP2. (l) Quercetin-MMP2. (m) Ellagic acid-RELA. (n) Quercetin-RELA. (o) Ellagic acid-SRC. (p) Quercetin-SRC. (q) Ellagic acid-VEGFA. (r) Quercetin-VEGFA.

Table 2. Functions of nine potential target proteins of *P. niruri* based on GO and KEGG pathway analyses through Enrichr.

Classification	ID	Term	Genes
Antioxidant	GO:0034599	Cellular response to oxidative stress	JUN, AKT1, EGFR
	GO:0034614	Cellular response to reactive oxygen species	JUN, AKT1, EGFR, RELA
	GO:2001022	Positive regulation of response to DNA damage stimulus	EGFR
	GO:0044773	Mitotic DNA damage checkpoint signaling	CCND1
	GO:0046322	Negative regulation of fatty acid oxidation	AKT1
	KEGG:05208	Chemical carcinogenesis	JUN, CREB1, CCND1, SRC, AKT1, EGFR, RELA, VEGFA
	KEGG:05225	HCC	CCND1, AKT1, EGFR
Anti-inflammation	GO:0019221	Cytokine-mediated signaling pathway	CCND1, SRC, MMP2, AKT1, RELA, VEGFA
	GO:1901222	Regulation of NIK/NF- kappaB signaling	EGFR, RELA
	GO:0034612	Response to TNF	AKT1, RELA
	GO:0042981	Regulation of apoptotic process	JUN, SRC, AKT1, EGFR, RELA, VEGFA
	GO:0050728	Negative regulation of inflammatory response	CREB1, SRC
	KEGG:04926	Relaxin signaling pathway	JUN, CREB1, SRC, MMP2, AKT1, EGFR, RELA, VEGFA
	KEGG:05161	Hepatitis B	JUN, CREB1, SRC, AKT1, RELA
	KEGG:05160	Hepatitis C	CCND1, AKT1, EGFR, RELA
	KEGG:04210	Apoptosis	JUN, AKT1, RELA
	KEGG:04932	Non-alcoholic fatty liver disease	JUN, AKT1, RELA

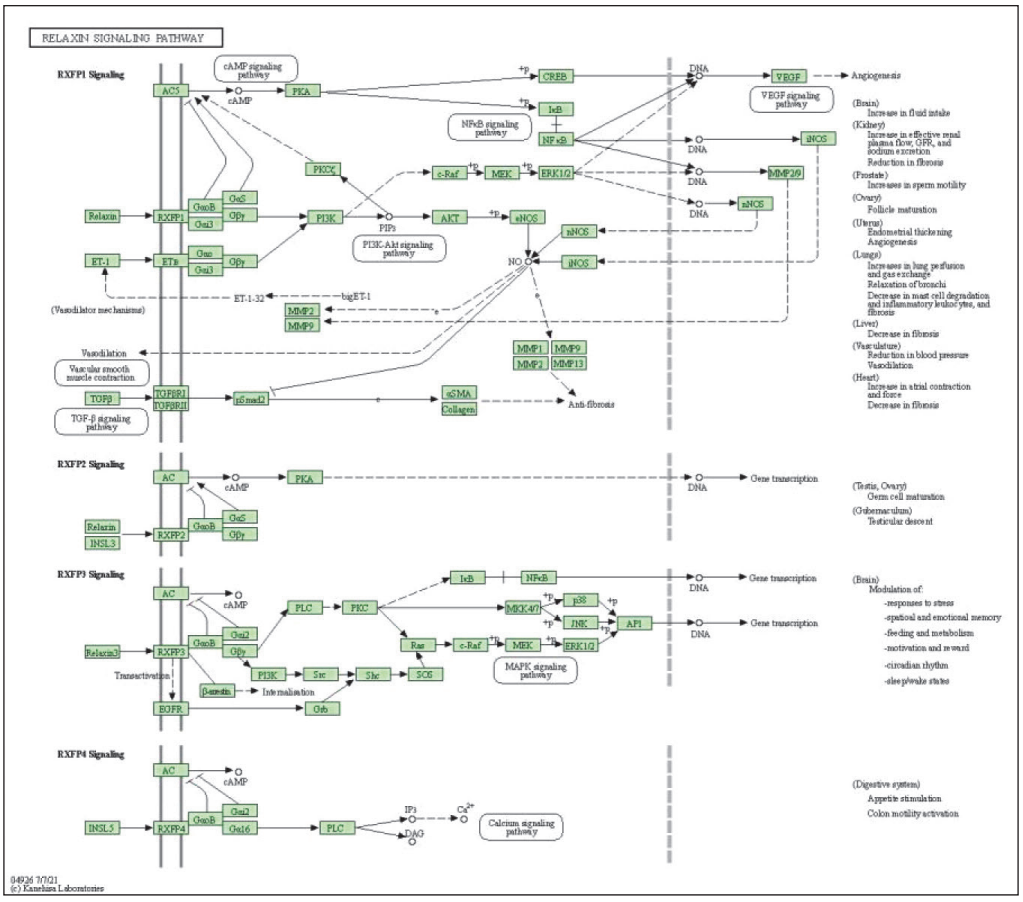

Figure 7. Relaxin signaling pathway (KEGG:04926).

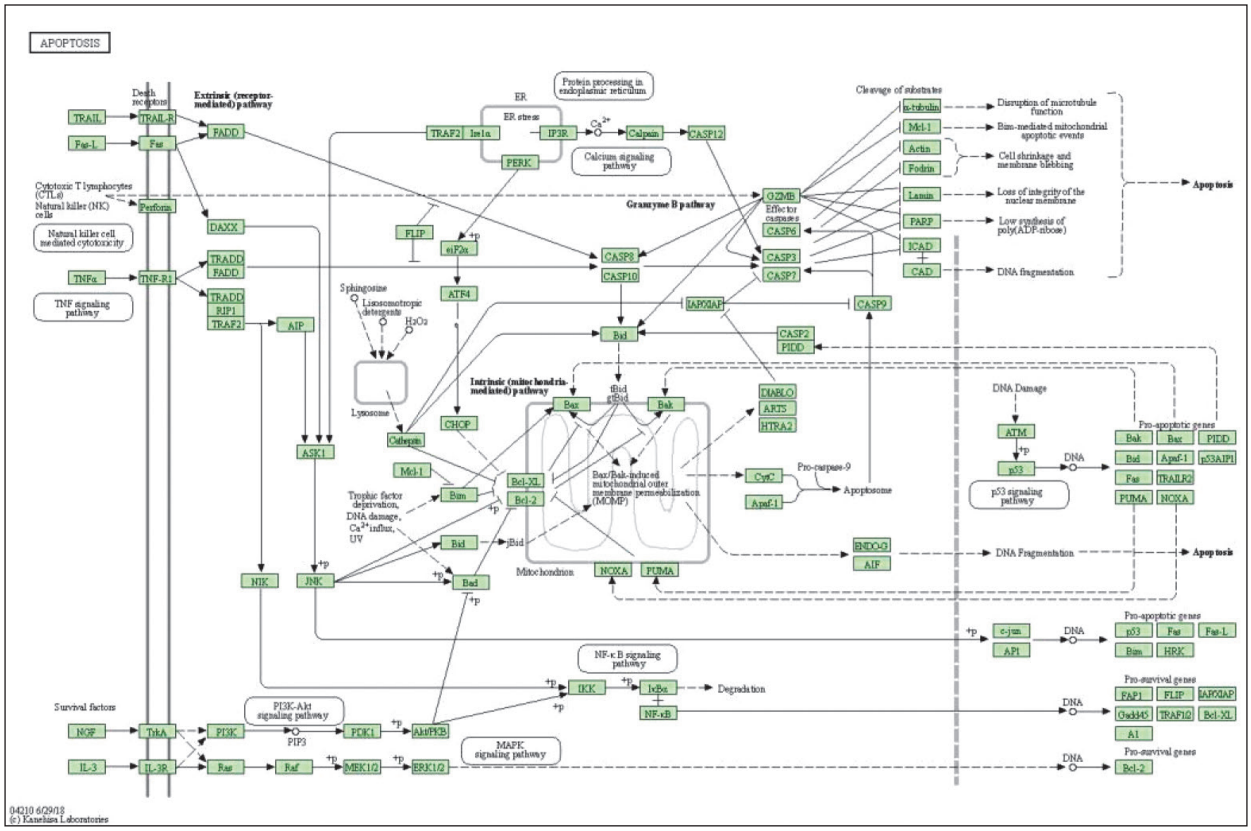

Figure 8. Apoptosis pathway (KEGG:04210).

Based on the gene ontology (GO) and KEGG pathway analyses, the nine potential target proteins' BPs and signaling pathways could be categorized into two primary pharmacological activities, i.e., antioxidant and anti-inflammation, as shown in Table 2.

As a typical wound-healing response to tissue injury, all hepatocellular pathways are commonly activated in chronic liver injuries. This is known as fibrogenesis, and it occurs when fibrogenic extracellular matrix (ECM) components are secreted to enclose and isolate the injured area of the tissue for repair (Acharya *et al*., 2021). Hepatic stellate cells transform into myofibroblasts after sustained liver injury, express alpha-smooth muscle actin, move to tissue healing sites, and release substantial amounts of ECM (Rachmawati *et al*., 2017; Sundari *et al*., 2018). According to this study, *P. niruri* possessed target proteins related to the prohibition of fibrogenesis response-mediating proteins. The relaxin signaling pathway (KEGG:04926) involved several target genes such as JUN, CREB1, SRC, MMP2, AKT1, EGFR, RELA, and VEGFA, which could explain this process, as shown in Figure 7.

Elevated reactive oxygen species (ROS) are the primary cause of liver fibrosis (Rachmawati *et al*., 2017). As demonstrated in Figure 8, ROS-induced hepatocyte apoptosis (KEGG:04210) might be the protective mechanism, resulting in the release of damaging mediators (e.g., TGF-β, TNF-α) (Sundari *et al*., 2018; Wardhani *et al*., 2020). Proapoptotic proteins, such as p53, CASP9, Fas, Fas-L, and Bax, are upregulated in regulating the apoptotic process (GO:0042981). Antiapoptotic proteins like Bcl2, on the other hand, are downregulated (Dhar *et al*., 2020; Tandrasasmita *et al*., 2010). Free radical activity has also been observed to be scavenged by ellagic acid (Aishwarya *et al*., 2021). This mechanism of action could be elicited by the BPs of cellular response to oxidative stress (GO:0034599) and cellular response to ROS (GO:0034614) that involved target genes of JUN, AKT1, EGFR, and RELA. HCV (KEGG:05160) and HBV (KEGG:05161) infections, as well as non-alcoholic fatty liver disease (GO:0046322, and KEGG:04932), also contributed to liver fibrosis (Dhar *et al*., 2020; Sundari *et al*., 2018).

As previously mentioned, ROS influenced the regulation of inflammatory response (GO:0050728). Overexpression of inflammatory mediators was closely associated with the inflammatory disease; therefore, NF-κB-Inducing Kinase (NIK)/NF-κB (GO:1901222), cytokine-mediated signaling (GO:0019221), and response to tumor necrosis factor (TNF) (GO:0034612) should be modulated (Pflug and Sitcheran, 2020; Yuliana *et al*., 2022). The previous study reported that the presence of fibrosis affected the development of HCC (Dhar *et al*., 2020; Wardhani *et al*., 2020). This study found that *P. niruri* also has target genes involved in the HCC pathway (KEGG:05225), including CCND1, AKT1, and EGFR. HCC is the most common type of liver cancer (Dhar *et al*., 2020), which is generally caused by DNA damage. Intriguingly, ellagic acid can prevent the binding of carcinogens to DNA (Bagalkotkar *et al*., 2010). This property has a close relationship with inhibiting DNA damage activities through various BPs and signaling pathways, including any

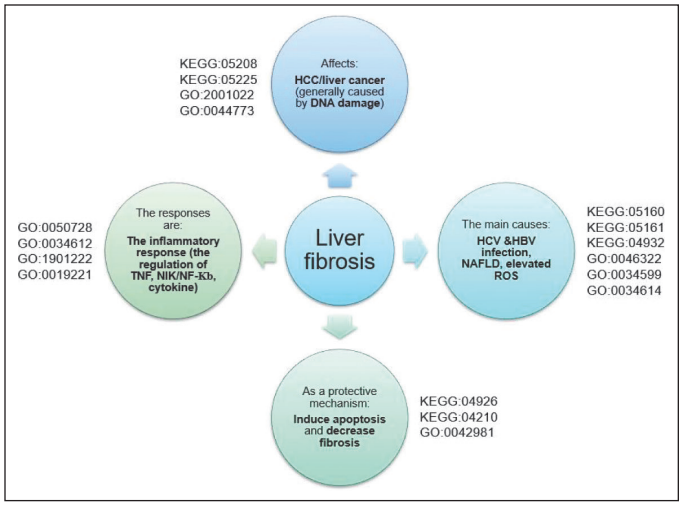

Figure 9. The summary of BPs and signaling pathways of *P. niruri* target proteins in hepatoprotective activities.

process that activates or increases the frequency, rate, or extent of the response to DNA damage stimulus (GO:2001022); signal transduction process involved in mitotic DNA damage checkpoint (GO:0044773); and ROS-induced chemical carcinogenesis pathway (KEGG:05208).

Ellagic acid and quercetin, which were identified as crucial components, were predicted to have a substantial role in *P. niruri* of antioxidant and anti-inflammatory effects, based on previous studies (Aishwarya *et al*., 2021; Bagalkotkar *et al*., 2010). Ellagic acid and quercetin have a promising role in preventing liver disease through various mechanisms of action (Aishwarya *et al*., 2021; Bagalkotkar *et al*., 2010; Tewari *et al*., 2017). According to the findings of this network pharmacology investigation, the modes of action are similar to those of *C. longa* and *C. xanthorrhiza* in protecting liver tissues (Devaraj *et al*., 2014; Ibrahim *et al*., 2020; Karamalakova *et al*., 2019; Oon *et al*., 2015; Rivera-Espinoza and Muriel, 2009; Salama *et al*., 2013). The molecular docking study confirmed this result. Our study found that CCND1 and RELA are the key targets of *P. niruri* in protecting liver tissues and have high binding activities with ellagic acid and quercetin.

This network pharmacology and molecular docking study revealed the BPs, target proteins, and signaling pathways to more comprehensively elucidate the ellagic acid and quercetin modes of action from upstream to downstream. We can develop a new therapeutic intervention that may be advantageous in treating liver fibrosis by better understanding the signaling pathways and target proteins of *P. niruri* in hepatoprotective activities and the interactions among their target proteins (Fig. 9). Eventually, it leads to promising hepatoprotective agent development.

CONCLUSION

In conclusion, our study found that CCND1 and RELA are the key targets of *P. niruri* in protecting liver tissues. They are both involved in many BPs and signaling pathways related to hepatic disease. The molecular mechanisms elicited by GO and KEGG analyses revealed similar mechanisms of action as *C. longa* and *C. xanthorrhiza* in hepatoprotective activities. In this molecular docking study, we discovered that ellagic acid and quercetin of *P. niruri* exhibited a high binding affinity to CCND1 and RELA. We can conclude that *P. niruri*, well-known for its ability to modulate the immune system, can also be a potential hepatoprotective agent like *C. longa* and *C. xanthorrhiza*. Concisely, the finding of this study can provide insight into developing a new promising hepatoprotective agent in the future. Therefore, studies of *P. niruri in vitro* and *in vivo*, especially those examining the effects of ellagic acid and quercetin on target proteins CCND1 and RELA, are suggested to be carried out for further confirmation.

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AUTHORS' CONTRIBUTIONS

S.T. searched the databases and references, collected and analyzed the data, developed the method, created the illustrations, and drafted the manuscript. A.Y. suggested the research topic and provided technical support. R.R.T was responsible for the funding and supervising. All authors reviewed the manuscript and provided insightful revision recommendations.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

This study does not involve any animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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APPENDIXES

All data generated or analyzed during this study are included in this published article and its Supplementary Tables.