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. 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.
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](https://example.com/figure1.png)

**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 protein 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
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.

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**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.

**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).
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).

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.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Target protein</th>
<th>PDB-ID</th>
<th>Vina score</th>
<th>Cavity size</th>
<th>Center</th>
<th>Size</th>
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</thead>
<tbody>
<tr>
<td>Ellagic Acid</td>
<td>AKT1</td>
<td>1unq</td>
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<td>14 6 17</td>
<td>19 19 19</td>
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<td>19 19 19</td>
</tr>
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<td>VEGFA</td>
<td>1vpf</td>
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<td>37 11 17</td>
<td>19 19 19</td>
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<tr>
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<td>29 9 50</td>
<td>30 31 30</td>
</tr>
<tr>
<td></td>
<td>CCND1</td>
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<td>15 33 30</td>
</tr>
<tr>
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<td>19 19 19</td>
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<td>CREB1</td>
<td>5zko</td>
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<td>28 49 143</td>
<td>19 19 28</td>
</tr>
<tr>
<td></td>
<td>MMP2</td>
<td>1qib</td>
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<td>19 19 19</td>
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<td>JUN</td>
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<tr>
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<td>CREB1</td>
<td>5zko</td>
<td>-6.3</td>
<td>297</td>
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<td>21 21 28</td>
</tr>
<tr>
<td></td>
<td>MMP2</td>
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<td>682</td>
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<td>21 21 21</td>
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<td>2,304</td>
<td>-7 53 11</td>
<td>30 21 21</td>
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</table>

[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.]

[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-REL. (n) Quercetin-REL. (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.

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

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

ACKNOWLEDGMENTS

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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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Ramdani ED, Yanuar A, Tjandrawinata RR. Comparison of dopamine D2 receptor (homology model and X-ray structure) and virtual screening protocol validation for the antagonism mechanism. J App Pharm Sci, 2019; 9:17–22.


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