Network pharmacology and experimental validation to reveal the anti-hepatitis B virus pharmacological mechanism of *Phyllanthus urinaria* L.

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**Author contributions**
Peng Q designed the experiment, analyzed and interpreted the experimental data, and wrote the first draft. Fu QJ conducted experiments, collected and summarized data, and wrote in English. Xiao M, Sang SG and Rong H planned, executed, managed and coordinated the work. All the authors participated in the final approval of the manuscript.

**Competing interests**
The authors declare no conflicts of interest.

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**Abbreviations**
HBV, hepatitis B virus; ETV, entecavir; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein protein interaction; R. urinaria, *Phyllanthus urinaria* L.; DMSO, dimethyl-sulfoxide.

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**Abstract**

**Background:** To explore the pharmacological mechanism of the anti-hepatitis B virus of *Phyllanthus urinaria* L. through network pharmacological analysis and experimental validation. **Method:** The active ingredient, target of action and target of action related to hepatitis B were clarified by searching the herb group identification, GeneCards and OMIM databases, and the protein interaction relationship was obtained by using the String database, and the protein interaction network map was constructed by using Cytoscape software. We also performed gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis of key targets of the anti-hepatitis B action of *Phyllanthus urinaria* L. and predicted the core targets and pathways of *Phyllanthus urinaria* L. anti-hepatitis B. The main targets predicted by network pharmacology were then validated by HepG2.2.15 cell experiments. **Results:** By searching active ingredient targets and hepatitis B disease targets, a total of 19 active ingredients and 64 related targets of action were retrieved from *Phyllanthus urinaria* L., and a total of 51 common targets were obtained by mapping the obtained hepatitis B disease targets and drug targets. protein protein interaction network analysis indicated that targets including TNF, JUN, AKT1, IL-10, IL-1B, CAT, HMOX1, NFE2L2, and CASP3 and other targets may be the core targets.gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis showed that the treatment of hepatitis B by *Phyllanthus urinaria* L. mainly included inflammation and oxidation-related processes, and the signaling pathways mainly included fluid shear stress and atherosclerosis, VEGF, and hepatocellular carcinoma. The results of the in vitro test showed that after the action of different concentrations of the extracts of the *Phyllanthus urinaria* L in the safe concentration range on cells HepG2.2.15, HBsAg, HBeAg and hepatitis B virus DNA levels were significantly inhibited, and NFE2L2 and HMOX1 were affecting hepatitis B virus transcription and replication by regulating the oxidative stress response. **Conclusion:** Using an integrated network pharmacology approach, this study revealed the active components and potential targets of *Phyllanthus urinaria* L. for the treatment of the hepatitis B virus, providing a theoretical basis for the research and clinical application of *Phyllanthus urinaria* L.

**Keywords:** *Phyllanthus urinaria* L.; hepatitis B; mechanism of action; network pharmacology
HIGHLIGHTS
This study mainly used network pharmacology to explore the action target of Phyllanthus urinaria L. and predict its possible molecular mechanism, analyzed the action mechanism of Phyllanthus urinaria L. on hepatitis B disease at multiple levels and verified the prediction results of network pharmacology, so as to provide reference for the subsequent research on the treatment of hepatitis B, and provide more comprehensive and powerful theoretical support for the clinical application of Phyllanthus urinaria L.

MEDICAL HISTORY OF OBJECTIVE
Phyllanthus urinaria L. is an herb belonging to the genus Phyllanthus of the Euphorbiaceae. This product was first listed in the “Compendium of Materia Medica” under the name of “true pearl grass”. In ancient times, the dried whole herb is used as medicine, slightly bitter, sweet and cool in nature, with the effects of clearing heat and toxin (using drugs to relieve inflammation or fever), inducing diuresis (reducing swelling) and improving eyesight (using drugs to make people’s eyes clear and bright without visual obstacles). In current studies, Phyllanthus urinaria L. has the functions of liver protection, bacteriostasis, antiviral, antioxidant and anti-tumor.

INTRODUCTION
Viral hepatitis B (“hepatitis B”) is a hepatitis caused by hepatitis B virus (HBV) infection. HBV is a DNA virus with hemophilic characteristics. HBV infection is the most common viral infection in the world and can be transmitted through contact with HBV-infected blood and semen, and HBV infection is a major and intractable public health problem in the world [1]. The progression of the disease course after HBV infection is complex and variable and can be influenced by age, viral replication and the immune status of the infected organism [2]. It often leads to chronic hepatitis B. The natural course of chronic hepatitis B often ranges from HBeAg positivity in the early stages to negativity in the late stages, and HBeAg-negative chronic hepatitis B is often associated with more severe liver disease, so HBV infection may even evolve into cirrhosis and hepatocellular carcinoma. After diagnosis of chronic hepatitis B, the 5-year incidence of cirrhosis is cumulatively 8% to 20%, and the 5-year survival rate for patients in the compensated stage of cirrhosis is approximately 80% to 86%, while the survival rate for patients in the decompensated stage of cirrhosis is poorer, at approximately 14% to 35%, and HBV-related end-stage liver disease or liver cancer causes at least 500,000 deaths per year [3]. According to the World Health Organization and other incomplete data, about 30% of the world’s population is or has been infected with HBV. Since 1981, the safe and effective hepatitis B vaccine has had good success in preventing hepatitis B virus infection, but many people are still infected with HBV and suffer from serious illnesses caused by the virus. Therefore, the treatment of hepatitis B remains a major challenge for mankind. Currently, the main drugs used to treat hepatitis B are interferons and nucleoside analogs, but both of these drugs have limitations, such as significant side effects or the need to take the drugs for a long time, resulting in viral resistance. Therefore, it is urgent to develop new drug treatments or adjuvant therapies.

Phyllanthus urinaria L. is an herb belonging to the genus Phyllanthus of the Euphorbiaceae. [4]. The main types of compounds reported to be contained in Phyllanthus urinaria L. include flavonoids, tannins, coumarins, lignans, phenolic acids, etc. Related experiments have shown that Phyllanthus urinaria L. has antiviral, antitumor, hepatoprotective, antioxidant, anti-inflammatory and antibacterial effects [5-8]. It has a variety of active ingredients and various pharmacological activities. Therefore, the mechanisms of action of Phyllanthus urinaria L. are also very diverse. Chinese medicine has been widely used in the treatment of hepatitis B in China, including the use of traditional Chinese medicine preparations alone or in combination with interferon or nucleoside analogs [9]. Cheng Yan’an et al. found that Phyllanthus urinaria L. was effective against HBV and had a high negative rate of hepatitis B-related indexes through clinical studies [10]. It can also be used in combination with thymidine α1 to treat patients with chronic hepatitis B. It can restore liver function and improve the rate of HBeAg and HBV DNA regression [11]. A number of studies have shown that Phyllanthus urinaria L. has a significant effect on hepatitis B, but the exact mechanism of action is not well understood.

Network pharmacology is a new discipline that studies disease mechanisms and drug action mechanisms in the context of larger biological networks. Network pharmacology is a popular tool for studying herbal medicines, which can efficiently screen for genes commonly associated with drugs and diseases, display reciprocal networks, and predict the target genes and possible molecular mechanisms of drugs acting on diseases. Therefore, in this study, a network pharmacology approach was used to collect the targets of the major active ingredients of Phyllanthus urinaria L. and the targets of the hepatitis B virus. A protein interaction network map was established, followed by enrichment analysis using gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) to predict the core targets and pathways of the anti-hepatitis B of Phyllanthus urinaria L. Finally, the proposed major targets were experimentally validated.

In this study, we used network pharmacology to explore the main components and predict the possible molecular mechanism of the anti-hepatitis B of Phyllanthus urinaria L. And provide a reference for the subsequent studies on the treatment of hepatitis B with Phyllanthus urinaria L. and more comprehensive and strong theoretical support for the clinical application of Phyllanthus urinaria L.

MATERIALS AND METHODS
SCREENING FOR ACTIVE COMPONENTS AND TARGETS
The active ingredients of Phyllanthus urinaria L. and targets were searched using the HERB (http://herb.ac.cn/) online platform, which has data from SymMap, TCMDID 2.0, TCMSD 2.3 and TCM-ID [12-16].

COLLECTION OF RELATED TARGETS
Using “Hepatitis B” as the search term, we searched in the databases OMIM (https://omim.org/), HERB and GeneCards (http://www.genecards.org/). The final data were displayed in a Venn diagram of all genes obtained i.e., duplicate genes were removed. The collected Phyllanthus urinaria L. action targets and target genes of hepatitis B disease were displayed in a Venn diagram, with the cross-section of the targets shared by the Phyllanthus urinaria L. and hepatitis B, i.e., the target genes of Phyllanthus urinaria L. acting on hepatitis B.

CONSTRUCTION OF PROTEIN INTERACTION NETWORK (PPIN)
The common genetic data of Phyllanthus urinaria L. and hepatitis B disease collected were introduced into a protein interaction platform String (https://www.string-db.org/), setting human as the species source, and extracting the interaction pairs file import the software Cytoscape 3.6.2, and filter the key targets according to the degree value to construct the protein interaction network diagram.

SIGNALLING PATHWAY AND FUNCTIONAL ENRICHMENT ANALYSIS
The previously obtained common genes of Phyllanthus urinaria L. and hepatitis B disease were imported into the DAVID (https://david.ncifcrf.gov/) database, restricting the species to H. sapiens, for enrichment analysis, which functional enrichment analysis includes a cellular component, biological process, molecular function, and signaling pathway enrichment analysis, i.e., gene ontology/KEGG enrichment analysis.

DRUG PREPARATION AND CELL CULTURE
We purchased the whole pearl of Phyllanthus urinaria L. at the First
Affiliated Hospital of Hainan Medical University. Weighing 100 g of *Phyllanthus urinaria* L. with a balance, put the herbal medicine into a decoction pot, add 1,000 mL of pure water, steep for 30 minutes, then heat, boil and decort for 1 hour, decort two times, then mix the decoction, filtering with filter paper and repeat four times, put the filtered liquid in a rotary evaporator for evaporation and concentration; then pour the liquid into a vessel and put it in a dryer at 65 °C, dry for 8 h and finally obtained 16.14 ± 0.15 g of infusion. Weigh the appropriate amount of extract and add dimethyl sulfoxide DMSO (Sigma, St. Louis, MO, USA) at a concentration of 50 mg/mL to prepare the masterbatch. Filtered through a 0.22 μm filter (Biosharp, Anhui, China) and stored in a centrifuge tube, sealed at −20 °C. HepG2.2.15 cell line was purchased from Shanghai Mingjin Biotechnology Co., Ltd. A 50 mL sterile centrifuge tube (Biosharp, Anhui, China) was taken, and the ratio of each reagent was 10% fetal bovine serum (Boehringer Ingelheim, Ingham, Germany ), 1% double antibody (Gibco, Grand Island, NE, USA), 0.4 mg/mL G418 (Sigma, St. Louis, MO, USA) and minimum essential medium (Gibco, Grand Island, NE, USA). Following the principle of ready-to-use, The Cryopreservation Medium was prepared with a ratio of fetal bovine serum: DMSO (Sigma, St. Louis, MO, USA) of 9:1. Then the cells were cultured by cell recovery, fluid exchange, passaging, and freezing.

**Anti-HBV toxicity test of Phyllanthus urinaria L.**

CCK8 (DOJINDO, Kumamoto Prefecture, Japan) was used to determine the range of drug concentrations that were not significantly toxic to cells: six experimental groups were set up, which were HepG2.2.15 + *P. urinaria* (31.25 μg/mL), HepG2.2.15 + *P. urinaria* (62.5 μg/mL), HepG2.2.15 + *P. urinaria* (125 μg/mL), HepG2.2.15 + *P. urinaria* (250 μg/mL), HepG2.2.15 + entecavir (ETV) (30 nmol/L), HepG2.2.15 + 0.25% DMSO; Control group: HepG2.2.15; Blank group: only medium.

ETV is an antiviral drug that inhibits the replication of hepatitis B virus. It is a guanine nucleoside analog and is mainly used to treat slow-onset hepatitis B in the presence of viral replication. ETV only detects one concentration of 30 nmol/L because it is the maximum concentration that does not damage cells and has the most effective effect.

**Anti-HBV efficacy of Phyllanthus urinaria L.**

The levels of HBsAg (Abbott, IL, USA), HBeAg (Abbott, IL, USA), and HBV DNA (Tianlong, Shaanxi, China) in the supernate were measured by the action of different concentrations of *Phyllanthus urinaria* L. extracts on cells HepG2.2.15 in the safe concentration range.

**Quantitative real-time PCR**

Eastep®/Super Total RNA Extraction Kit (YEASEN, Shanghai, China) was selected for the extraction of total cellular RNA, then remove the residual DNA from the RNA and add 2 μl of 25XgDNA digestase Buffer; 1 μl of gDNA digestase; and 1–7 μl of sample RNA, adjust according to RNA concentration; RNase free dH2O, make up the total volume to 10 μl, mix well; incubate at 42 °C for 2 min; add 10 μl of SuperMix plus, mix well; The program setting parameters were 25 °C for 5 min; 42 °C for 30 min; 85 °C for 5 min; after the program was completed, the obtained cDNA was stored at −80 °C for backup to complete RNA reverse transcription. The designed upstream and downstream primer sequences are shown in (Table 1).

**Western blot analysis**

The expression of NFE2L2 and HMOX1 proteins was detected using Western Blot (Bio-Rad, CA, USA) by adding a primary antibody after 1:1000 dilution and a secondary antibody after 1:2000 dilution.

**Statistical analysis**

Three replicate experiments were conducted. The obtained data were processed with GraphPadPrism 9.0.0 to make graphs and do statistical analysis. T-test was used to evaluate whether the difference between the experimental data of each group was statistically significant, and the difference was considered statistically significant if *P* < 0.05.

**Result**

**Active ingredients and related targets**

Firstly, through Network pharmacology, 9 chemical constituents that have been studied more frequently in *Phyllanthus urinaria* L. (Table 2) and 64 relevant targets (IL6, DIO1, IRF1, IL1B, IRAK4, NOSTRIN, SOD1, CYP1B1, GSTM2, VEGF1, EBAG9, GSTP1, AKR1C3, TP53C0R1, Ptges3-ps, NRI13, SLC2A4, Cppg1os, NFE2L2, HMOX1 ect.) were obtained from the Herb database. And then, the disease-related targets were obtained from OMIM, Herb and GeneCards databases with “Hepatitis B” as the keyword. A total of 12,672 targets related to hepatitis B disease were obtained after removing duplicate targets (Figure 1), and 51 common targets were obtained by mapping the obtained hepatitis B disease targets and drug targets through the Venn diagram (Figure 2).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFE2L2</td>
<td>TCCGTCGACAAACCAGTGAT</td>
<td>GAATGTCTGGCCGAAAAGCTG</td>
</tr>
<tr>
<td>HMOX1</td>
<td>AAGACTGCCTTCCTGCTCAAC</td>
<td>AAAGCCCTACAGCAGCTGTCG</td>
</tr>
<tr>
<td>ACTB</td>
<td>CCTGCGACCCCAAGCAAT</td>
<td>GGCGCGGACTCGTCAAT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>CAS number</th>
<th>Mol ID</th>
<th>OB(%)</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>149-91-7</td>
<td>MOL000513</td>
<td>31.69</td>
<td>0.04</td>
</tr>
<tr>
<td>Gallicacid-3-O-(6'-galloyl)-glucoside</td>
<td>/</td>
<td>MOL006789</td>
<td>2.81</td>
<td>0.67</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>520-18-3</td>
<td>MOL000422</td>
<td>2.81</td>
<td>0.24</td>
</tr>
<tr>
<td>Methyl brevifolin carboxylate</td>
<td>107646-82-2</td>
<td>MOL005073</td>
<td>30.86</td>
<td>0.33</td>
</tr>
<tr>
<td>Corilagin</td>
<td>23094-69-1</td>
<td>MOL005079</td>
<td>3.01</td>
<td>0.44</td>
</tr>
<tr>
<td>Quercetin</td>
<td>73123-10-1</td>
<td>MOL000098</td>
<td>46.43</td>
<td>0.28</td>
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<tr>
<td>Ruvoside</td>
<td>6859-20-7</td>
<td>MOL004349</td>
<td>18.13</td>
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<tr>
<td>Rutin</td>
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<td>MOL000415</td>
<td>3.20</td>
<td>0.68</td>
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<tr>
<td>Kaempferol-3-arabofuranoside</td>
<td>5041-67-8</td>
<td>MOL002377</td>
<td>2.73</td>
<td>0.65</td>
</tr>
</tbody>
</table>

CAS, Chemical Abstracts Service; ID, identity document; OB, oral bioavailability; DL, drug likeness.
Construction of protein protein interaction (PPI) network and core targets

The intersection targets were imported into the STRING database, and after removing the free nodes, extracting the interaction relationship pair file for saving, and the PPI network diagram was constructed by importing Cytoscape 3.8.2 software, with 50 nodes and 308 interactions, as shown in (Figure 3). The key target was filtered according to the degree value: the higher the degree value, the larger the node.

Signaling pathway and functional enrichment analysis

The common targets of *Phyllanthus urinaria* L. and hepatitis B were imported into the DAVID database and enrichment analysis was performed, with \( P < 0.05 \) as a significant response for the biological function of the protein. A total of 1,046 biological processes, including responses to reactive oxygen species, responses to lipopolysaccharides and responses to molecules of bacterial origin. A total of 22 signaling pathways were identified in cell composition, involving membrane regions, membrane microdomains, membrane rafts, etc.; 63 signaling pathways were identified in molecular functions, mainly involving nuclear receptor activity, glutathione binding, etc., as shown in (Figure 4). The relevant signaling pathways enriched by KEGG included fluid shear stress and atherosclerosis, hepatitis B, TNF signaling pathway, VEGF signaling pathway, hepatocellular carcinoma, and JAK-STAT signaling pathway, and the specific results are shown in (Figure 5).

Toxicity test of *Phyllanthus urinaria* L. against hepatitis B cells

The range of drug concentrations that were not significantly toxic to cells was determined using CCK8, as shown in (Figure 6), and the results showed that a drug concentration of 250 \( \mu \text{g/mL} \) had a significant effect on cell survival, and the difference was statistically significant \( (P < 0.05) \).

Anti-hepatitis B efficacy of *Phyllanthus urinaria* L.

The other drug concentrations had no significant effect on the survival of HepG2.2.15 cells. After the action of different concentrations of the extract of *Phyllanthus urinaria* L. on the cells HepG2.2.15 within the safe concentration range, the levels of HBsAg, HBeAg and HBV DNA in the supernatant were measured, and it was found that *Phyllanthus*
Figure 3 Protein network interaction diagram

Figure 4 GO functional enrichment analysis histogram. GO, gene ontology; BP, biological process; CC, cell component; MF, molecular function.
Figure 5 KEGG pathway enrichment bubble map. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 6 CCK8 assay cell survival rate. (a) Drug action 24 hours. (b) Drug action 48 hours (***P < 0.0001). CCK8, cell counting Kit-8.
**Regulation of the NFE2L2/HMOX1 signaling axis**

qPCR is used to compare the gene expression of samples by monitoring the fluorescence intensity of the PCR process. The expression of target genes NFE2L2, HMOX1 after drug action was detected by qPCR. The relative expression of target genes was expressed by $2^{-\Delta\Delta C_t}$. The expression of NFE2L2 and HMOX1 was significantly higher after drug action and the difference was significant at $P < 0.05$. As shown in (Figure 8); the expression of NFE2L2, HMOX1 proteins were detected using WB. The results showed that the expression levels of NFE2L2 and HMOX1 proteins were elevated in the cells after the action of *Phyllanthus urinaria* L. compared with the blank control group, and the differences were statistically significant ($P < 0.05$). The specific results are shown in (Figure 9).

**Discussion**

Hepatitis B is a very complex disease that involves the process of viral infection and the body’s reaction to the infection. Although much research has been done on hepatitis B, there is still a lot of information about hepatitis B that has not yet been discovered. Likewise, hepatitis B is a major problem plaguing human health, and there are no very safe and effective treatments with few side effects. Those factors have driven scholars to explore hepatitis B. With the booming development of Chinese medicine, people are focusing more and more attention on the research of Chinese medicine. To date, several herbal medicines have been clinically applied in the treatment of hepatitis B. However, the complexity of herbal medicines has led to many mechanisms of action that are still unclear. Numerous studies have shown that *Phyllanthus urinaria* L. has significant effects on hepatitis B, but the specific mechanism of action is not well understood. In this study, the target genes and molecular mechanisms of action of *Phyllanthus urinaria* L. were investigated based on network pharmacology analysis and experimental validation. Network pharmacology predicted that most of the core targets of *Phyllanthus urinaria* L. anti-Hepatitis B were enriched in oxidative stress and inflammation-related signaling pathways, and then the efficacy of *Phyllanthus urinaria* L. against Hepatitis B was determined by experimental validation, and it was found that *Phyllanthus urinaria* L. could elevate NFE2L2 and HMOX expression in HepG2.2.15.

Based on the search of the constituents of *Phyllanthus urinaria* L., querceitin, corilagin, kaempferol, rutinoside and gallic acid are the main active ingredients of *Phyllanthus urinaria* L. Many chemical components such as flavonoids and lignans have been isolated from...
the plant and have been studied for anti-HBV activity in vitro and in vivo, and they work by inhibiting antigen secretion or inhibiting DNA replication through different or synergistic mechanisms of action [17]. Among them, quercetin significantly inhibited the synthesis of hepatitis B-related antigens, with 60% inhibition of HBsAg and 62% inhibition of HBeAg [18]. Ge et al. experimentally found the anti-HBV effect of kaempferol and others in the Chinese patent medicine Liu Wei Wu Ling Tablets [19]. A study by Perwez Alam [20] et al. using RP- and NP-HPTLC methods affirmed the anti-HBV activity of quercetin, gallic acid, etc. Corilagin was shown to be dose-dependent through oxidative stress to achieve anti-HBV effects, thus having a protective effect on cells [21].

The results by PPI showed that the anti-hepatitis B effect of *Phyllanthus urinaria* L. is closely associated with genes such as TNF, JUN, AKT1, IL-10, IL-1β, CAT, HMOX1, NFE2L2 and CASP3, which may be the core targets of *Phyllanthus urinaria* L. action on hepatitis B. Most of these genes are in signaling pathways associated with inflammation, oxidative stress and cancer. The pathogenesis of hepatitis B is mediated by persistent intrahepatic immunopathology due to HBV infection, i.e., HBV itself does not damage hepatocytes, and it is the viral infection that causes the body to undergo an immune response that leads to disease development [22]. Combined with the network pharmacology prediction of the possible targets of the action of *Phyllanthus urinaria* L. on hepatitis B and the analysis of the possible mechanisms of action with the targets, it can be found that the action of *Phyllanthus urinaria* L. on hepatitis B is not only limited to antiviral, but also may slow down the progression of hepatitis B-related diseases through other pathways.

A study of the pharmacological effects of *Phyllanthus urinaria* L. on hepatitis B revealed a significant inhibitory effect on HBsAg, HBeAg and HBV DNA levels. HBsAg is a specific indicator of acute or chronic infection with HBV, and HBsAg is secreted by HBV-infected cells in the form of subviral particles, which may be a mechanism for evading the host immune response [23–25]. HBSAg may lead to impairment of innate and adaptive immunity and depletion of T- and B-cell responses; therefore, lowering HBsAg levels may promote recovery of the host’s immune system [26]. Loss of HBsAg reflects a functional cure of hepatitis B virus infection, and therefore HBsAg levels have been widely used in clinical practice [27]. HBeAg is a soluble protein, which is a nonsstructural protein that is not required for viral replication but is used as a marker of infectivity and is an immunomodulatory protein with tolerance and immunomodulatory activity that plays an important role in viral persistence [28]. In clinical practice, HBeAg is an indicator of viral replication, infectivity, inflammation, disease severity, and response to antiviral therapy. Seroconversion from HBeAg-positive to HBeAg-negative or anti-HBeAg-positive usually predicts the elimination of infection [29]. Seroconversion of HBeAg is considered to be the main short-term goal of antiviral therapy for chronic hepatitis B [30]. HBV is a small hemophilic DNA virus that replicates by reverse transcription [31]. HBV DNA is a double-stranded DNA containing the hepatitis B virus genome, after entering the host cell nucleus, it undergoes a series of reactions to form covalent closed circular DNA (cccDNA), which essential role is to serve as a template for all viral RNAs and the resulting generation of new viral particles [31, 32]. There is a good positive correlation between intrahepatic cccDNA levels and the levels of HBV DNA and HBsAg released into peripheral blood, and both serum HBV DNA and HBsAg can be influenced by viral replication activity [33–35]. So far, serum HBV DNA levels and HBsAg titers are the main and classical markers for monitoring viral replication and assessing the efficacy of antiviral therapy in patients with chronic HBV infection [36].

It was experimentally verified that *Phyllanthus urinaria* L. can increase the expression of NFE2L2 and HMOX1 in HepG2.2.15. Nuclear factor-E2-related factor 2 (NFE2L2/Nrf2) is a prevalent major transcription factor that regulates the expression of antioxidant enzymes through antioxidant response elements, such as antioxidants, oxidants and pro-oxidants that can activate Nrf2 expression [37]. Nrf2 plays an important role in mediating cellular antioxidantation, protecting the liver from various injuries and promoting liver regeneration [38]. The active ingredient of Corilagin can alleviate liver function damage in vitro and in vivo, and its mechanism of action may be to induce the expression of antioxidant enzymes and upregulate AMPK/GSK3β-Nrf2 signaling pathway, and it can also induce apoptosis and autophagy by decreasing the expression of Nrf2 [39, 40]. Combined with the experimental results of this study and other scholars’ studies, we suggest that *Phyllanthus urinaria* L. can against hepatitis B by regulating NFE2L2. Heme oxygenase (HO/HMOX) is the rate-limiting enzyme of heme metabolism that degrades heme to CO, free iron, and biliverdin and includes three isofoms: HO-1, HO-2, and HO-3, of which HO-1 is mainly expressed in the liver and is a marker of oxidative stress [41, 42]. As a protective enzyme, HO-1 has been shown to cause severe tissue damage in deficient conditions due to oxidative stress and multiple regulatory disorders [43]. NFE2L2 and HMOX1 are key genes that regulate oxidative stress, which plays an important role in HBV-related diseases. Many studies have shown that persistent HBV infection can promote oxidative responses in patients, leading to HBV gene alterations and abnormal expression, which in
turn may lead to disruption of the oxidative/antioxidant balance and induce cIRrhosis and hepatocellular carcinoma in some patients [44]. According to the experimental results, *Phyllanthus urinaria* L. showed good therapeutic effects on hepatitis B. Moreover, the mRNA and protein expression of NFE2L2 and HMox1 were significantly increased in HepG2.2.15 cells after the action of *Phyllanthus urinaria* L. in vitro experiments, therefore, *Phyllanthus urinaria* L. can regulate the NFE2L2/HMox1 signaling axis to exert antioxidant effects to influence the development of hepatitis B. This provides a theoretical basis for the clinical application of *Phyllanthus urinaria* L. and also provides a basis for further studies of *Phyllanthus urinaria* L.

**Conclusion**

In conclusion, a network pharmacology analysis and in vitro experimental validation were performed to investigate the pharmacological mechanism of *Phyllanthus urinaria* L. against the hepatitis B virus. The network pharmacology analysis predicted that *Phyllanthus urinaria* L. exerts its therapeutic effects against the hepatitis B virus by modulating multiple components, targets, and pathways. In vitro, experiments confirmed that the mechanism of Hepatitis B treatment by *Phyllanthus urinaria* L. is through the regulation of the NFE2L2/HMox1 signaling axis to exert antioxidant effects to affect the development of Hepatitis B. This provides a theoretical basis for the clinical application of *Phyllanthus urinaria* L., and it also provides a basis for further research.

**References**


