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Plant-based Natural inhibitors of human liver carcinogenesis: A mechanistic overview, focusing on hepatitis B and hepatitis C viruses

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Abstract

Hepatitis B and C viruses can lead to serious complications such as hepatic fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC) and are therefore responsible for a significant portion of liver cancer cases worldwide, with over 1.3 million deaths annually. The mechanisms by which hepatitis viruses contribute to HCC include DNA integration into the host genome, metabolic reprogramming, induction of the cellular stress response pathway, and interference with tumour suppressors. HBV is a DNA virus from the *Hepadnaviridae* family, and HCV is an RNA virus from the *Flaviviridae* family. Both viruses are transmitted through contact with infected bodily fluids, such as blood or sexual fluids. It is important to get tested for hepatitis B and C and to seek treatment as early as possible to prevent the progression to liver cancer. While there is a vaccine available for Hepatitis B, there is currently no vaccine for Hepatitis C. But some natural medicines have demonstrated antiviral activity against the hepatitis B and C viruses. Therefore, it is important to explore natural alternatives for the treatment of this disease. This review aims to summarise the pathogenesis of hepatitis B and C and their link to hepatocellular carcinoma, as well as to highlight natural compounds with the potential to treat hepatitis through various mechanisms at different stages of infection. These natural compounds may offer an alternative to chemical-based medications in the treatment and control of hepatitis by inhibiting or disrupting the entry, activity, or replication of the virus within the host.

Keywords: Hepatitis B; Hepatitis C; Human Liver Cancer; Hepatitis B virus associated Hepatocellular carcinoma; Natural inhibitors

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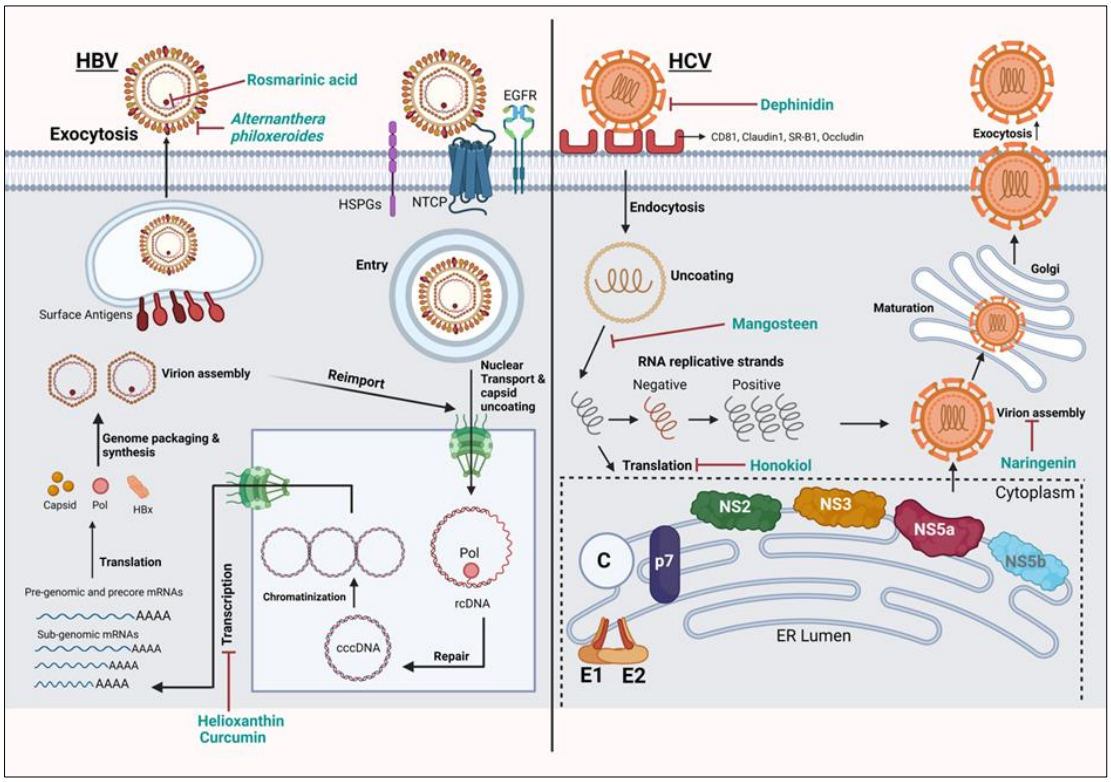
Graphical Abstract



Plant-based compounds and their mechanism of action against hepatitis B and C viruses.

1. Introduction

Infections with HBV and HCV are responsible for a significant portion of the liver disorders that are seen around the world. As a result of the fact that the two hepatotropic viruses share the same mode of transmission, co-infection with the two viruses is extremely common. This is especially true in areas of the world where HBV infection is more common, as well as in populations that are at a high risk for parenteral infection [1].

1.1. Hepatitis B Virus

Hepatitis B virus (HBV) is a member of the *Hepadnaviridae* family of viruses that infects exclusively the hepatocytes (liver cells) of humans and some non-human primates. It is found in several different forms in the blood, with the infectious form being known as the Dane particle. The Dane particle is a small, enveloped virus with a partially double-stranded DNA genome of approximately 3.2 kilobases in size and a diameter of 42nm, that is linked to a polymerase and surrounded by a nucleocapsid. It also contains three envelope proteins called the large (L), middle (M), and small (S) surface proteins, which contain domains essential for attachment to hepatocytes (Fig.1) [2]. The C-terminal S domain is common to all three envelope proteins, while the M protein also contains an extra N-terminal preS2 domain and the L protein contains a preS1 domain in addition to the preS2 and S domains [3]. In addition to the Dane particle, there are two other forms of HBV that are secreted in large amounts and known as subviral particles (SVPs) [4]. These SVPs contain only envelope proteins and are non-infectious. The SVPs can be either spherical or filamentous in shape, with the spherical SVPs being composed of S (90%) and M proteins (10%), and the filamentous SVPs containing S (80%), M, and L proteins (10% each) [5]. The lipid composition of SVPs has been determined to consist mainly of phosphatidylcholine, cholesteryl ester, cholesterol, and triglycerides, but the lipid composition of Dane particles has not yet been determined [6]. The relevance of SVPs in the life cycle of HBV is unclear, but it has been suggested that they may act as a decoy for the immune system, protecting the Dane particle from the neutralizing humoral response [7].

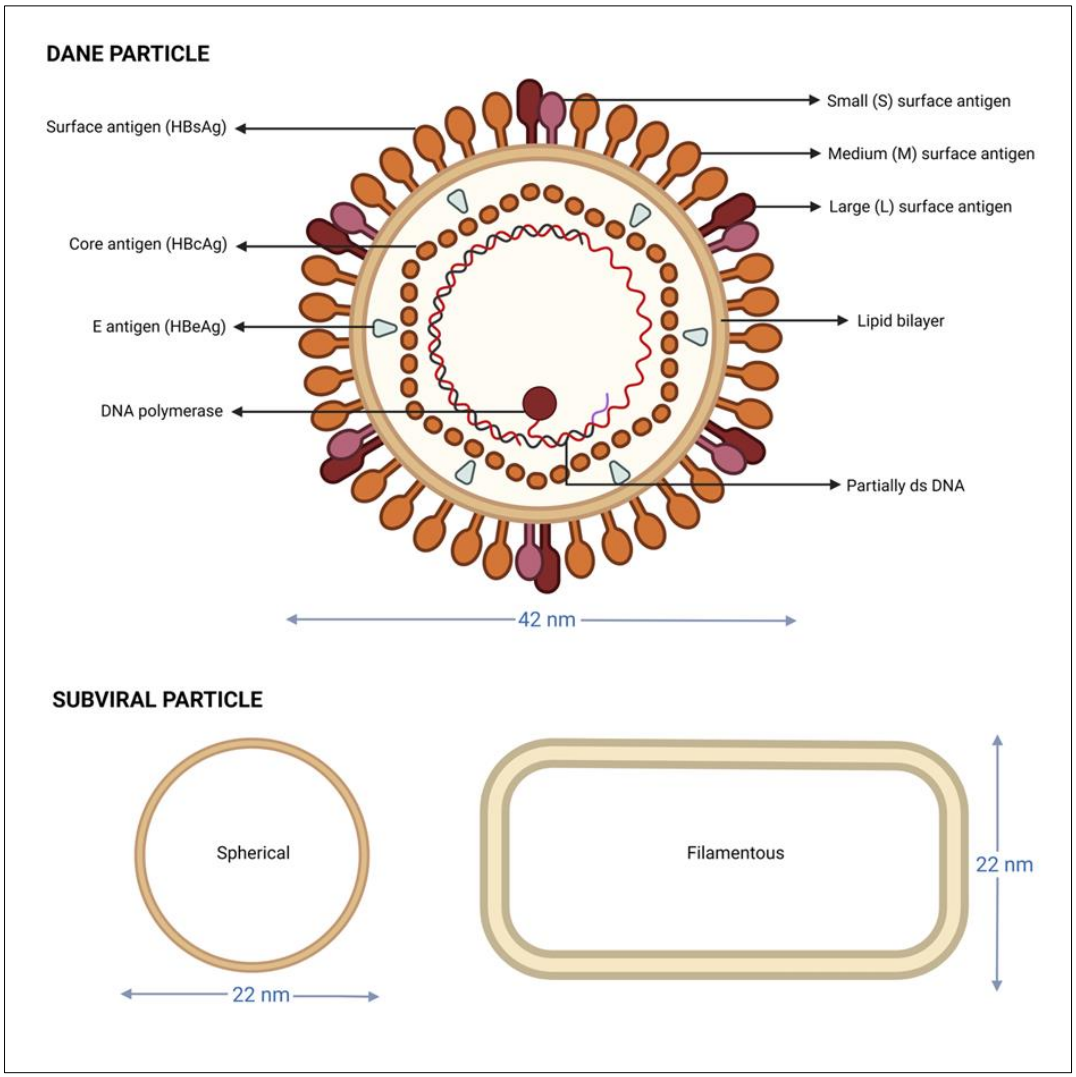


Figure 1 Schematic representation of Hepatitis B

1.1.1. Virus interaction with Hepatocytes

The hepatitis B virus (HBV) infects the liver and causes chronic liver disease by binding to liver cells via a protein called sodium taurocholate co-transporting polypeptide (NTCP) and then entering the cell to replicate **(Fig.2).** The process of HBV entering a cell may involve the interaction with multiple receptors and may be a complex, multi-step process that involves endocytosis, a process by which the cell takes in molecules from outside the cell. It is thought that additional host factors, or proteins produced by the host cell, are required for susceptibility to HBV infection[8]. Some host factors that may play a role in HBV infection include the epidermal growth factor receptor (EGFR) and the protein E-cadherin [9]. The mechanism by which HBV gains access to the cell once it has interacted with its receptor and coreceptor is not fully understood, but some studies have suggested that HBV enters cells through a process called Caveolin-1-mediated endocytosis, while others have found evidence for the involvement of Clathrin-mediated endocytosis (CME) [10]. Inhibition of CME has been shown to decrease HBV infection in some studies [11, 12, 13]. Further understanding of the mechanisms involved in HBV entry into cells could lead to the development of new inhibitors to eliminate the virus from infected liver cells.

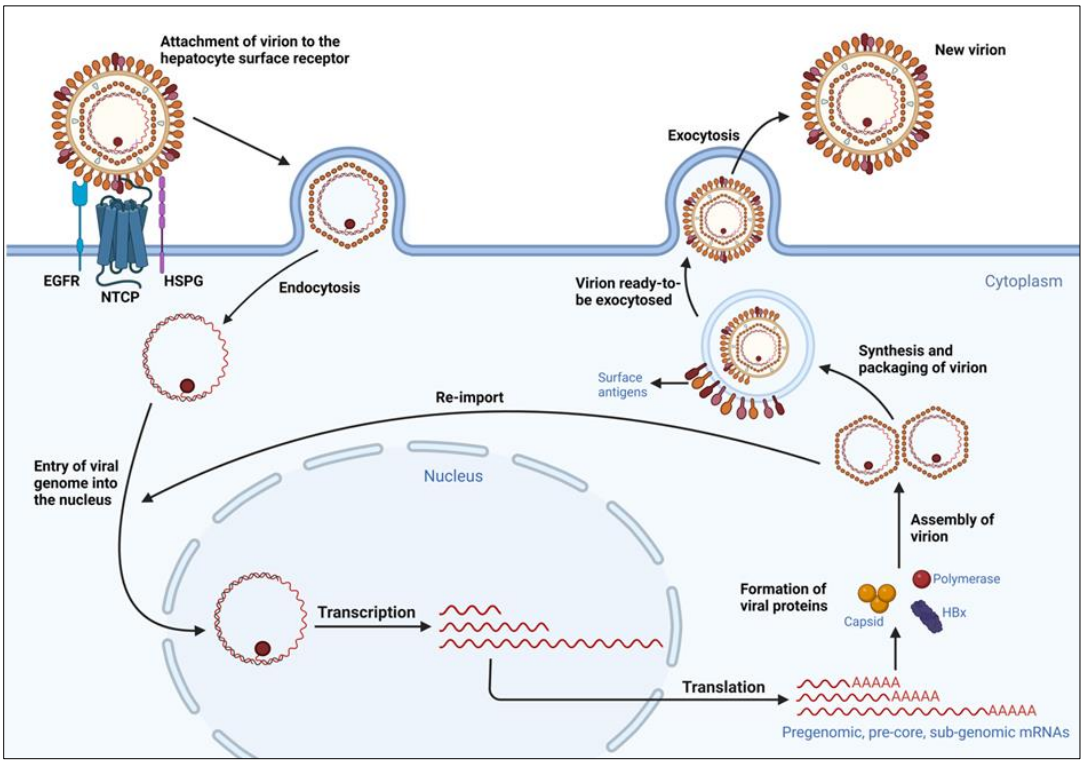


Figure 2 Schematic representation of Hepatitis B viral pathogenesis/ Hepatitis B Virus Pathogenesis: A Schematic Overview

1.1.2. Understanding the progression of Hepatocellular carcinoma by Hepatitis B virus

Hepatitis B virus (HBV) infects the liver and can range from being inactive to a more or less progressive form of hepatitis that can lead to cirrhosis and liver cancer (hepatocellular carcinoma, or HCC) [14, 15, 16]. There are two types of chronic hepatitis B: Hepatitis B envelope Antigen (HBeAg)-positive, which is due to wild-type HBV and represents the early phase of chronic HBV infection, and HBeAg-negative, which is caused by a naturally occurring HBV variant with mutations in certain regions of the genome and represents a late phase of the infection [17]. HBeAg-negative chronic hepatitis B is generally associated with more severe liver disease and a lower response rate to antiviral therapy [17, 18 ,19]. The risk of developing cirrhosis within 5 years of being diagnosed with chronic hepatitis B ranges from 8-20%, and the risk of developing liver failure, or HCC, is also significant [20]. HCC is one of the most common cancers worldwide, with about 75% of cases being related to chronic HBV infection [21]. The incidence of HCC varies geographically and is higher in people with advanced liver disease. The only way to improve survival after a diagnosis of HCC is through early detection and treatment such as surgical resection, liver transplantation, or percutaneous ablation. Universal vaccination and new therapeutic agents may help prevent the development of cirrhosis and HCC [22].

1.1.3. Current Treatment of Chronic Hepatitis B

There are currently seven drugs available for the treatment of chronic hepatitis B (CHB), including Interferon alpha, Lamivudine, Adefovir, pegylated interferon alpha-2a, Entecavir, Telbivudine, and Tenofovir [14, 15, 23]. These drugs have varying levels of effectiveness in suppressing the hepatitis B virus and improving clinical outcomes, and can be limited by factors such as poor tolerability, the development of resistance, and the presence of co-infections with other viruses (Table1).

Table 1 Overview of Antiviral drugs for treatment of Hepatitis B virus (HBV) infection

Drug	Description	Effectiveness	Resistance profile	Reference
Interferon-∝	Antiviral, antiproliferative, and immunomodulatory effects	Superior to placebo in undetectability of HBV DNA and HBeAg loss	Poor tolerability	[24, 25]
Lamivudine	Oral drug	Poor resistance profile		[26, 27]
Adefovir	Nucleotide analogue	Improved resistance profile compared to lamivudine but not more effective than lamivudine in viral suppression	Better resistance profile than lamivudine	[28]
Entecavir	Potent anti-HBV agent	High rates of undetectable HBV DNA and low HBeAg seroconversion rates	Low rate of resistance in naïve patients, high genetic barrier to resistance in lamivudine- resistance patients	[29, 30]
Telbivudine	Oral drug	High rates of undetachable HBV DNA and low HBeAg seroconversion rates	Low rate of resistance in naïve patients, moderate rate of resistance in lamivudine- resistance patients	[31,32]
Tenofovir	Nucleotide analogue	High rates of undetachable HBV DNA and low HBeAg seroconversion rates	Low rate of resistance in naïve patients, moderate rate of resistance in lamivudine- resistance patients	[33]

1.2. Hepatitis C virus

Table 2 Possible Multifunctional Roles of HCV Gene Products

Gene product	Function	Relevance to malignant formation	Reference
Core	May have immunosuppressive activities through interaction with pathways and C1qR.	Yes	[34] [39]
E2	Interferes with interferon actions; Interacts with cell surface marker CD81.	Yes	[40]
NS3	Viral protease; activates various signal transduction pathways	Yes	[35] [41]
NS5A	Implicated in diverse cellular functions including blocking interferon responses.	Yes	[42][43][44] [45][46][47] [48]
P7, NS2, NS4A, NS4B	Not fully defined	Unknown	[49] [50]

Note: The true biological relevance of these observations is not yet established, especially in regards to the development of hepatocellular carcinoma (HCC).

Hepatitis C virus (HCV) is a type of RNA virus that belongs to the *Flaviviridae* family and is classified in the genus *Hepacivirus*. Its genome is approximately 10 kilobases in length and encodes 10 viral gene products that are divided

into structural and non-structural genes (Fig.3). Some of the proposed functions of HCV gene products may be relevant to the development of cancer, such as the core gene product's interaction with pathways related to apoptosis, signal transduction, transcriptional activation, and transformation [34]. The non-structural proteins of HCV may also play a role in sustaining viral persistence and promoting carcinogenesis, such as the NS3 protein's activation of various signal transduction pathways and the NS5A protein's potential role in blocking interferon responses [35, 36, 37, 38]. However, the true biological relevance of these observations is not yet established, especially in regards to the development of hepatocellular carcinoma (HCC) (Table2).

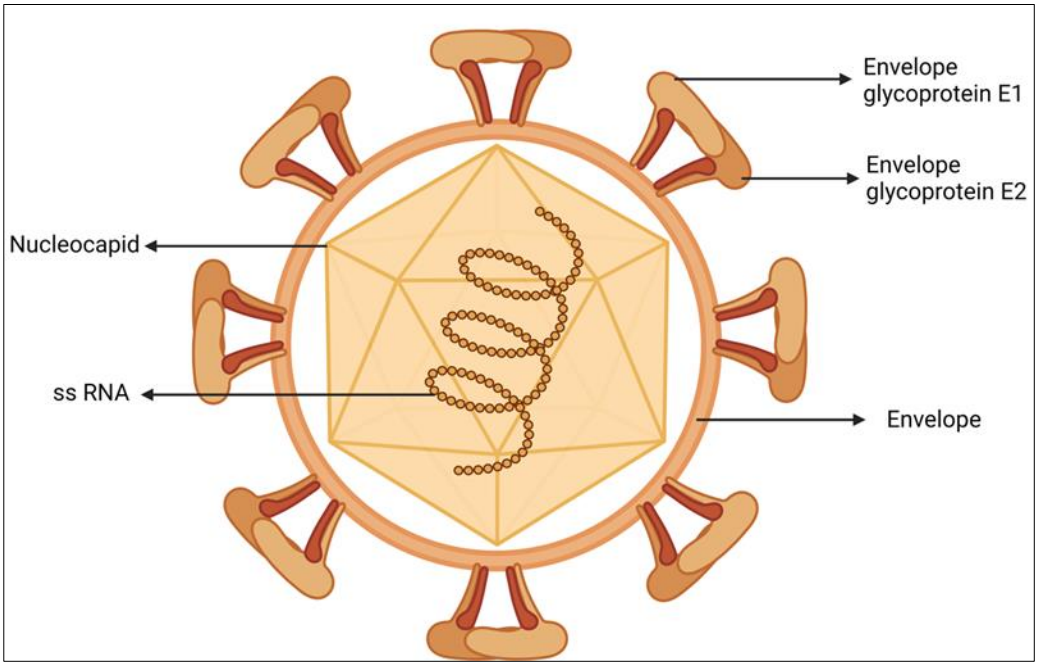


Figure 3 Schematic representation of Hepatitis C

1.2.1. Molecular Mechanisms of HCV pathogenesis

The molecular mechanisms of HCV pathogenesis involve a complex interplay between the virus and the host, with the virus exploiting various host pathways and mechanisms to establish infection and persist in the liver **(Fig.4.).** HCV has been linked to the production of reactive oxygen species (ROS), which can contribute to liver injury and oxidative stress in infected individuals. HCV gene products such as the core and NS5A proteins have been shown to induce ROS production through various mechanisms, including the release of cytochrome C by the core protein and the release of calcium by the NS5A protein [51, 43]. Chronic inflammation caused by HCV infection can also contribute to ROS production. The presence of ROS and the resulting oxidative stress can create a pro-carcinogenic environment that leads to chromosomal damage and increased mutation rates in infected cells. In particular, HCV infection has been associated with mutations in p53, beta-catenin, and other proto-oncogenes and tumour suppressor genes in HCC [52, 53]. A study also found that HCV infection can cause a "hypermutator" phenotype in lymphoma cells, with increased mutational frequencies in certain genes, double-stranded chromosomal breaks, and the activation of error-prone DNA polymerases and activation-induced cytidine deaminase. These findings suggest that hypermutational events may be a mechanism of carcinogenesis during HCV infection[54, 55, 56].

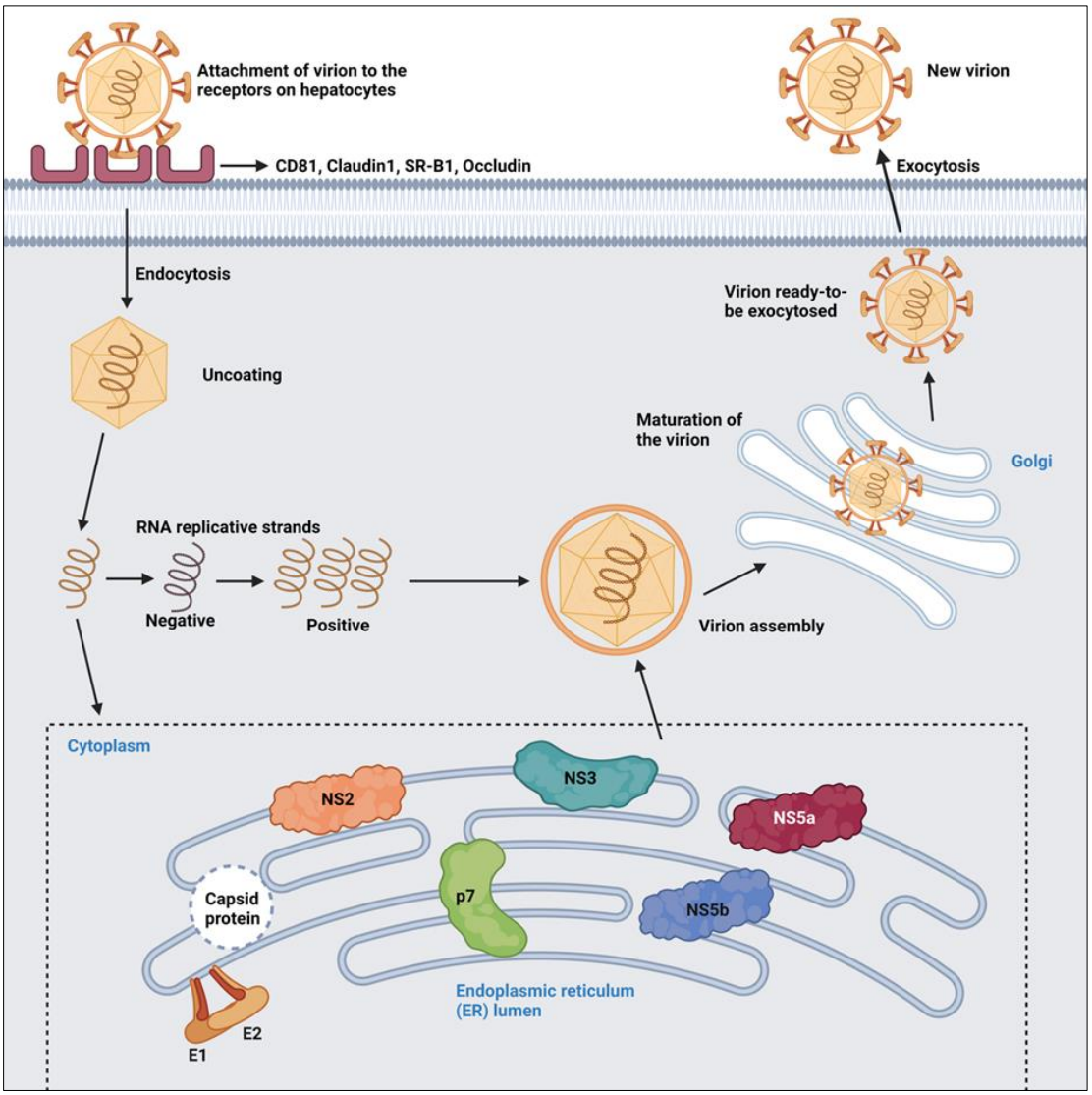


Figure 4 Schematic representation of Hepatitis C viral pathogenesis/ Hepatitis C Virus Pathogenesis: A Schematic Overview

1.2.2. Understanding the Progression of Chronic Hepatitis C Infection

Chronic hepatitis C (HCV) is a viral infection that can cause liver scarring (fibrosis). The progression of fibrosis is a key factor in determining the need for treatment and the overall outlook of the infection [57, 58, 59]. A number of factors, including inflammation and stellate cell activation, contribute to the development of fibrosis. Risk factors for fibrosis progression include age at infection, male gender, heavy alcohol use, and being immunocompromised. Obesity, diabetes, and hepatic steatosis (excess fat accumulation in the liver) may also affect the progression of fibrosis in HCV-infected individuals [60, 61] . There is currently no reliable test to predict the rate of fibrosis progression in a specific case. Normal ALT levels do not necessarily mean a patient is not at risk for fibrosis worsening, but elevated ALT levels are a risk factor[62]. The most accurate way to assess fibrosis progression is to repeat a liver biopsy 3-5 years after the initial biopsy. However, there is a need for improvement and validation in the use of fibrosis serum markers.

1.2.3. Current Treatment of Hepatitis C

Chronic hepatitis C (HCV) is currently treated with a combination of pegylated interferon and ribavirin, which has a success rate of around 50% in achieving sustained virologic response (SVR) [63,64,65,66,67]. However, this treatment has significant side effects and is not well tolerated by many patients. Researchers are currently developing enzyme inhibitors, such as protease and polymerase inhibitors, which show promise in combination with pegylated interferon and ribavirin[68]. It is likely that interferon-based therapy and ribavirin will remain the main treatment for HCV in the coming years, but the use of specifically targeted antiviral therapy drugs could improve SVR rates and eventually lead

to the use of interferon- and ribavirin-sparing regimens[69,70]. Many natural compounds derived from plants have been shown to have antiviral activity and have been studied as potential sources for new drugs. Approximately 200 antiviral agents have been developed in the past 50 years, with around 40% being vaccines and the rest being natural or semi-synthetic compounds inspired by nature. These compounds, including flavonoids, polyphenols, alkaloids, stilbenoids, and terpenes, have been shown to prevent the adhesion, penetration, duplication, or replication of viruses. Some of these compounds have previously been shown to be effective against viruses that are similar to HCV.

Natural products and synthetic therapeutic chemicals can work together to produce potent and effective medicines. This theory is supported by the existing research on the development of new drugs. This strategy has the potential to serve as the foundation for the development of therapeutic natural products that are designed for consumption by humans. Furthermore, the treatment of Hepatitis B and Hepatitis C virus infections, which can lead to liver carcinoma, should consider the use of such plant-based natural products exhibiting elevated antiviral activity. In this context, the current review discusses certain classes of natural compounds that have previously been reported to exhibit antiviral activity against similar viruses.

2. Search methodology

A thorough literature search was conducted for retrieving the studies published until September 2021 in the following databases: PubMed, Science Direct & Google Scholar. Inclusion and exclusion criteria were used to screen out 96 relevant papers. The papers included extensive data on several known and novel natural compounds studied for Hepatitis B & C. **Table 3** provides a concise summary of each of these papers, as well as the mechanism of action of the natural inhibitors that target viral proteins. The papers identified focused on bioactive plant-derived compounds derived from natural sources and their impact on various stages of viral replication, with a preference for compounds with low toxicity and the potential for multi-site inhibition.

3. Natural plant derived inhibitors targeting Hepatitis B and C viral proteins during their replication

In the section that follows, natural products with demonstrated anti-HBV (hepatitis B virus) and anti-HCV (Hepatitis C virus) activities will be discussed, with a particular focus on those that utilize mechanisms that differ from those of currently approved drugs. The aim is to explore the potential of these natural products as alternative or complementary treatments for HBV and HCV infections, and to highlight any unique or novel modes of action that they may possess. Given the significant burden of HBV and HCV on global health, the identification and characterization of these natural products may provide valuable insights for the development of new and more effective treatments for these viral infections.

Table 3 List of the natural compounds with anti-Hep B and anti-Hep C activities along with their mechanisms of action.

S.No	Compound	Plant source	Virus	Target protein	Mechanism of action	IC_{50}/EC_{50}	Reference
1.	Rosmarinic acid (phenolic compound)	*Lamiaceae*	HBV	ε-polymerase	Inhibits replication by binding to ε-polymerase	NR	[73]
2.	Methanolic extract	*Hybanthus enneaspermus*	HBV	Surface antigen	Entry, replication, and maturation of HBV particles are all inhibited.		[74,75]
3.	Methanolic extract	*Terminalia bellerica*	HBV	DNA polymerase			
4.	Methanolic extract	*Enicostemma axillare*	HBV	DNA polymerase			
5.	Crude extract	*Phyllanthus amarus*	HBV	DNA polymerase			
6.	Caudatin	*Cynanchum auriculatum*	HBV	anti-HBV action by interfering HBV enhancers and promoters	Inhibitory activity against HBsAg secretion and	142.67 μmol/L	[76, 77]

					HBV DNA replication		
7.	Helioxanthin	*Taiwania cryptomerioides*	HBV	HBV mRNAs transcription, or in a post-transcriptional manner	To inhibit the HBV RNA and the viral protein expression	0.1 ±0.2mM 32 ±1.4 mM	[78, 79]
8.	Curcumin	Rhizome of *Curcuma longa* L.	HBV	gluconeogenesis gene coactivator PGC-1α	Inhibits HBV replication in part by preventing the acetylation of histones bound to cccDNA.	NA	[80, 81, 82]
9.	Asiaticoside	*Hydrocotyle sibthorpioides*	HBV	core, S1, S2, and X gene promoter activities	Reduced viral DNA transcription and replication	23.5 µmol/L	[83]
10.	Phytoconstituents extract	*Gymnema sylvestre R.Br.*	HBV	Surface Antigen	Inhibits HBsAg and HBV DNA polymerase activity	NR	[84]
11.	LPRP-Et-97543	*Liriope platyphylla* roots	HBV	Entry of target protein	Controls gene expression and DNA replication	NR	[85]
12.	C-boivinopyranosyl flavones (luteolin-6-C-*ß*-d-boivinopyranosyl-*3'-O-ß*-D-glucopyranoside and chrysoeriol-6-C-*ß*-D-boivinopyranosyl-*4'-O-ß*-D-glucopyranoside) extracts	*Alternanthera philoxeroides*	HBV	Surface Antigen	Reduces the amount of HBsAg that is secreted by HepG2.15	NR	[86]
13.	Dephinidin	Anthocyanidin abundant in *Vaccinium corymbosum*	HCV	E1 and E2 glyco-proteins	Effects on virus-host interaction via action on E1 and E2 glycoproteins, which results in conformational changes in viral particles.	EC_{50} - 3.7 + 0.8 µM	[87]
14.	Mangosteen	*Garcinia mangostana L*	HCV	NS5b	Reduces HCV protein and RNA levels in 1b & 2a	EC_{50} – 1b- 5.1 µg/ml	[88]

					infectious replicon systems.	2a- 3.8 µg/ml	
15.	Epigallocatechin-3-gallate (EGCG)	*Camellia sinensis*	HCV	E1 and E2 glyco-proteins	EGCG's direct action on E1, E2 envelope glycoprotein alters the viral envelope structure without destroying it and blocks HCV cell binding. Blocks cell-to-cell communication	IC_{50}- 5-21 µM	[89, 90, 91, 92, 93]
16.	Gallic acid	*Limonium sinense*	HCV	NS's (NS 5A)	Inhibits entry and replication, inhibits HCV protease function, and downregulates HCV-RNA.		[94, 95]
17.	Apigenin	*Petroselinum crispum* OR flowers of chamomile *Eclipta alba*	HCV	NS5B	Has an inhibitory effect on the development and replication of HCV viral particles containing miR122 (microRNA 122). HCV replication can be suppressed by blocking the RNA-dependent RNA polymerase, NS5B, in vitro.	IC_{50} - 4.3-7.9 µM	[96, 97, 100, 101]
18.	Lucidone	*Lindera erythrocarpa*	HCV	NS3/4A	Increases IFN response and blocks NS3/4A protease by upregulating HO-1.	IC_{50} - 1.1 µM	[102]
19.	Vitisin B	*Vitis vinifera*	HCV	NS3	Inhibitor of HCV replication that targets NS3 helicase	IC_{50} - 0.006 µM or 6nM	[103]

20.	Saikosaponin B2	*Bupleurum kaoi*	HCV	E2	Blocks viral entry by neutralizing the viral particles	16.13 + 2.41 μM	[104]
21.	Honokiol	*Magnolia officinalis*	HCV	Components of replication complex, NS3, NS5A and NS5B, were downregulated	Have multiple effects on HCV infection, inhibiting entry, translation and replication	(LD50/ EC90 = 5.4	[105]
22.	Naringenin	Grapefruit flavonoid	HCV	Against NS2 protease	Inhibits HCV assembly, as it reduces the buildup of infectious particles within cells.	109 μM	[106]

3.1. Natural compounds targeting viral proteins associated with Hepatitis B for therapeutic intervention

As summarised in the Table.3. Rosmarinic acid is a natural compound that is found in abundance in various herbs belonging to the *Lamiaceae* family, such as spearmint, sage, peppermint, and perilla. It is commonly used as a dietary supplement and in Chinese herbal medicine. This compound has been shown to inhibit the binding of ε-Pol without affecting the binding of dsRNA-RIG-I, the helicase activity of RIG-I, or the binding of ε-ISG20 [71, 72]. Therefore, it is believed that rosmarinic acid does not interfere with the host's antiviral immune response. In vitro studies have also shown that rosmarinic acid treatment strongly abolishes ε-Pol binding, and it has been demonstrated to suppress HBV replication in cells [73].

Inhibitors of viral entry and fusion are receiving increasing attention for HBV treatment due to the highly selective tropism of the virus. Methanolic extracts of *Hybanthus enneaspermus* have been shown to inhibit HBs Ag binding, while methanolic extracts from seeds of *Terminalia bellerica* and leaves of *Enicostemma axillare* have been demonstrated to block HBV DNA polymerase. *Phyllanthus amarus* extracts have been found to downregulate hepatitis B virus mRNA transcription, suppress hepatitis B virus polymerase activity, and inhibit the release of the virus into Hep-G/2.2.15 cells [74]. The antiviral activity of these three plants was further investigated and it was found that the methanol extract of *Hybanthus enneaspermus* inhibited HBs Ag binding, while methanolic extracts of *Terminalia bellerica* and *Enicostemma axillare* inhibited HBV DNA polymerase. However, none of the three plants exhibited inhibition of both HBs Ag binding and HBV DNA polymerase, indicating that simply screening for antiviral activity using a single assay is not conclusive proof and further molecular studies are necessary. These studies also revealed the HBV receptor binding capability of all three plants. While there are no published antiviral studies on these three plants, there are numerous other plants that have been studied elsewhere and their results are cited for comparison. The methanol extract of *Hybanthus enneaspermus* was found to inhibit both HBs Ag binding and HBV DNA polymerase, while only the methanolic extracts of *Terminalia bellerica* and *Enicostemma axillare* inhibited HBV DNA polymerase [75].

Caudatin is a steroidal compound found in the plant *Cynanchum auriculatum*. It has been shown to have anti-cancer and antiangiogenic properties, meaning it can inhibit the growth of cancer cells and prevent the formation of new blood vessels. Caudatin has also been found to have inhibitory activity against the secretion of HBsAg (a protein produced by the hepatitis B virus) and the replication of HBV DNA. In particular, the compound 3-O-(3,4,5-trimethoxy) cinnamoyl caudatin has been shown to have a novel mechanism of anti-HBV action by interfering with HBV enhancers and promoters. In laboratory studies, caudatin has been shown to cause cell cycle arrest and induce apoptosis (a type of programmed cell death). The IC50 values for caudatin's inhibitory activity against HBsAg secretion and HBV DNA replication have been measured at 142.67 μmol/L (SI = 1.7) and 40.62 mmol/L (SI = 6.0), respectively [76, 77].

Helioxanthin and its derivative are small molecules that have been found to inhibit HBV RNA and viral protein expression. These compounds have unique structures compared to other anti-HBV compounds and may have unique modes of action. In laboratory studies, the treatment of HepG2.2.15 cells with these compounds resulted in the inhibition of HBV mRNA transcripts, including both 3.5 kb and 2.4/2.1 kb mRNAs. This led to a decrease in the HBV core

protein. The 3.5 kb mRNA plays a key role in the HBV life cycle as it encodes the HBV core protein and DNA polymerase, and serves as the template for minus strand DNA synthesis. These results suggest that helioxanthin and 5-4-2 target multiple steps of the viral life cycle and effectively inhibit HBV replication [78, 79].

Curcumin, a natural compound found in the spice turmeric, has been shown to have antiviral properties against HBV (hepatitis B virus) infection. It is believed to inhibit HBV by down-regulating the expression of certain genes, such as PGC-1α, and increasing the stability of the p53 protein [80, 81]. In a study, researchers examined the effects of curcumin on cccDNA (circular, covalently closed DNA), a form of the HBV genome found in infected liver cells. They found that curcumin was able to reduce the levels of cccDNA-bound histones and overall levels of cccDNA in HBV-infected cells, suggesting it may be a promising agent for the treatment of HBV. Further research is needed to fully understand how curcumin exerts its antiviral effects [82].

Asiaticoside, a compound isolated from the plant *Hydrocotyle sibthorpioides*, has been shown to effectively suppress the levels of HBsAg/HBeAg (proteins produced by the hepatitis B virus), extracellular HBV DNA, and intracellular cccDNA (a form of the HBV genome) in a dose-dependent manner. It also inhibits viral DNA transcription and replication by inhibiting the activity of certain gene promoters, and reduces replication of the hepatitis B virus (DHBV) without causing any obvious signs of toxicity. These findings suggest that asiaticoside may be a promising agent for the treatment of HBV infection [83].

Gymnema sylvestre R. Br. is a plant that has been shown to have antiviral activity. Its active components, known as phytoconstituents, have been shown to inhibit the binding of HBsAg (a protein produced by the hepatitis B virus) and the activity of HBV DNA polymerase, an enzyme involved in the replication of HBV DNA. These findings suggest that *Gymnema sylvestre* may be a useful agent for the treatment of HBV infection [84].

LPRP-Et-97543 is a compound that was isolated from the roots of the plant *Liriope platyphylla*. It has been shown to inhibit the mode of action of the hepatitis B virus (HBV) by controlling gene expression and DNA replication by viral proteins. This interference with the viral proteins disrupts the nuclear factor NF-κB pathway, which is a signaling pathway that plays a role in the regulation of immune and inflammatory responses. These findings suggest that LPRP-Et-97543 may be a promising agent for the treatment of HBV infection [85].

Two new compounds called luteolin-6-C-β-d-boivinopyranosyl-3′-O-β-D-glucopyranoside and chrysoeriol-6-C-β-D-boivinopyranosyl-4′-O-β-D-glucopyranoside have been identified in the plant *Alternanthera philoxeroides*. These compounds, known as C-boivinopyranosyl flavones, have been shown to have significant anti-HBV (hepatitis B virus) activity. Specifically, they have been found to reduce the secretion of HBsAg, a protein produced by HBV, in HepG2.15 cells, a type of liver cell line. These findings suggest that these C-boivinopyranosyl flavones may be useful agents for the treatment of HBV infection [86].

3.2. Natural compounds targeting viral proteins associated with Hepatitis C for therapeutic intervention

Delphinidin, a plant pigment found in anthocyanidins, has been shown to be a more effective HCV entry inhibitor. It has been observed to inhibit HCV attachment to the cell surface and is effective in primary hepatocytes. It combats the HCV entry through the use of HCV pseudo particle (HCVpp), which harbor E1E2 envelope glycoproteins of various genotypes, indicating that its inhibitory effects are not limited to a specific genotype. In addition to inhibiting HCVpp entry, delphinidin has also been shown to inhibit HCV cell culture (HCVcc) infections, suggesting that it may interfere with the function of the E1E2 envelope glycoprotein on the viral particle. Overall, delphinidin appears to be a promising new HCV entry inhibitor with potential for use in the treatment of HCV infection [87].

Garcinia mangostana, also known as Mangosteen, is a plant native to Indonesia, Malaysia, the Philippines, and Thailand that has been shown to have antioxidant and antiviral properties. Researchers have hypothesized that it may have therapeutic potential against HCV infection, and a study found that the ethanol extract of mangosteen (MG-EtOH) had the most potent anti-HCV replication activity. Further analysis identified two molecules, α- and γ-mangostins, as the major contributors to this antiviral effect. The study also found that MG-EtOH was able to restore normal levels of ROS production in HCV-infected cells, suggesting that its ROS-scavenging activity may be involved in its inhibitory effect on HCV replication [88].

Epigallocatechin-3-gallate (EGCG), a flavonoid found in green tea, has been shown to inhibit HCV entry. It has been tested in HCVcc and HCVpp systems, as well as in primary human hepatocytes, and has been shown to directly act on the viral particle to prevent attachment to the cell surface [89, 90, 91]. EGCG has also been observed to have a pan-genotypic effect against HCV, meaning it is effective against all genotypes of the virus. It is thought that EGCG may alter

the structure of the HCV envelope by acting on the E1E2 envelope glycoprotein, without disrupting it, leading to the blockade of HCV binding to cells. EGCG has also been suggested to inhibit the binding of the HCV envelope to cell surface heparan sulphate [92, 93]. Gallic acid is a type of phenolic acid that has been identified as an anti-HCV (hepatitis C virus) agent when isolated from grape seed extract. It has also been isolated from a plant called *Limonium sinense*, which belongs to the *Plumbaginaceae* family and is commonly used in traditional medicine. When a root water extract from *L. sinense* was tested, it was found to inhibit HCV infection at the entry step, which refers to the initial stage of the virus entering and infecting a host cell. This inhibition occurred more specifically during the attachment and fusion/endocytosis processes, which involve the virus attaching to and merging with the host cell membrane. Gallic acid was found to be the most active in inhibiting this process, with an inhibitory activity on viral entry with an IC50 value of 36.4 (μM) [94, 95]. Apigenin is a flavonoid found in certain plants that has been shown to inhibit the replication of hepatitis C virus (HCV) by blocking the maturation of microRNA (miRNA) called miR122 [96, 97]. miR122 is essential for the replication of HCV RNA in liver cells [98, 99]. Research has also shown that apigenin and another flavonoid called luteolin can inhibit HCV infection and replication in cells expressing HCV replicon [100]. These compounds were identified using a pharmacophore and structure-based study targeting NS5B, and the anti-NS5B polymerase activity of luteolin was confirmed in vitro. Apigenin and luteolin extracted from the plant *Eclipta alba*, which is used in Ayurvedic medicine, have also been shown to inhibit HCV replication by inhibiting NS5B RNA-dependent RNA polymerase in vitro and in cells expressing a sub genomic replicon [101]. Lucidone, a compound isolated from the fruit of the plant *Lindera erythrocarpa*, has been shown to specifically inhibit the replication of hepatitis C virus (HCV) RNA. *L. erythrocarpa*, a plant native to Asia, has traditionally been used in folk medicine and its fruit has a range of pharmacological properties. Research has shown that HCV RNA levels are suppressed by lucidone in a concentration-dependent manner, with an EC50 (concentration required to achieve 50% effectiveness) of 15 ± 0.5 μM in HCV replicon cells. The compound was also found to have a CC50 (concentration required to achieve 50% cytotoxicity) of 620 ± 5 μM, indicating that it is not cytotoxic at effective antiviral concentrations. An infectious assay confirmed the inhibitory effect of lucidone on viral RNA replication with an EC50 of 20 ± 1.1 μM, and a selectivity index (SI; CC50/EC50) of approximately 31, suggesting that it could be a promising lead compound for the development of new anti-HCV agents [102]. The resveratrol tetramer Vitisin B, which is found in the root of grapevines, has been shown to have the highest anti-hepatitis C virus (HCV) replication activity. Further analysis of several HCV variants resistant to vitisin B, as well as in vitro binding and helicase assays, suggests that the mode of action of vitisin B is the inhibition of the viral helicase NS3. Vitisin B was found to have the greatest activity against HCV replication, and it is thought that its direct binding to and inhibition of HCV NS3 helicase may be an important factor in its ability to effectively suppress HCV replication [103]. Inhibition of early hepatitis C virus (HCV) entry has been demonstrated for Saikosaponin Sb2. Virus particles are neutralised, attachment is prevented, and entry and fusion are blocked. It has been shown that SSb2 acts on the HCV E2 protein through analysis of soluble viral glycoproteins. Furthermore, SSb2 has been shown to prevent the binding of serum-derived HCV to hepatoma cells, as well as inhibit infection by multiple genotypic strains of HCV. Researchers have discovered that SSb2 can prevent HCV infection in primary human hepatocytes when used as a treatment [104]. Honokiol is a natural compound found in the *Magnolia officinalis* plant that has been shown to have several pharmacological effects, including anti-inflammatory and anti-cancer properties. In pre-clinical studies, honokiol has demonstrated effectiveness in inhibiting the replication of the hepatitis C virus (HCV) by reducing the expression of proteins that are necessary for HCV infection. This antiviral activity is observed at low concentrations of honokiol and does not appear to be toxic to cells. Honokiol may also inhibit HCV replication by modulating signaling pathways related to reactive oxygen species (ROS), PI3K/Akt, NFκB, and STAT3. When combined with a low dose of interferon-a, honokiol has been shown to have an even more potent inhibitory effect on HCV replication compared to the standard treatment with ribavirin [105]. Naringenin is a flavonoid that is found in grapefruit and is commonly used as a dietary supplement. It has been shown to have anti-oxidant, anti-inflammatory, and anti-carcinogenic properties both in laboratory and animal studies. Naringenin has been found to inhibit the secretion of ApoB and HCV particles in a dose-dependent manner, without affecting the levels of HCV RNA or protein within cells. This suggests that naringenin may prevent the accumulation of infectious particles by blocking the assembly of HCV [106].

4. Conclusion

HBV & HCV, are a major cause of liver cancer and pose a significant threat to global health. As there are only a few drugs against HBV & HCV and no vaccine for HCV, there is an urgent need for the discovery of new and natural agents, having lesser side effects compared to chemical drugs. In this context, several attempts are made to successfully identify the inhibitors of hepatotropic viruses, including peptides, vaccines, small molecule compounds, and even natural products exhibiting anti-viral activity. The present review is, therefore, an attempt to review the existing literature for potential natural inhibitors against hepatotropic viruses causing liver cancer to provide an overview that could assist in further investigations related to this topic of concern. These natural inhibitors are suitable for managing Liver cancer infections through the modulation of a wide range of molecular targets through effective mechanisms of action (Table.3) and minimum toxicity. For example, Rosmarinic acid can be used against HBV which hinders viral replication and

Epigallocatechin-3-gallate (EGCG) alters HCV envelop protein's structure hampering the viral entry in the host. Overall, the data collected from various sources indicated the availability of different classes of compounds with high favourable efficacy are included are flavonoids, flavanones, flavanols, alkaloids, polyphenols, and terpenes. As a future scope, it would be valuable to investigate the use of combinations of these compounds for checking the potential of improvement in overall therapeutic success. Considering the promising and powerful effects of these natural products, they should be further researched, developed and investigated as alternative therapies to current standard treatments.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest with respect to the current study.

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