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Anti-hepatitis B virus activities of natural products and their antiviral mechanisms

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[ABSTRACT] Chronic hepatitis B (CHB) infections caused by the hepatitis B virus (HBV) continue to pose a significant global public health challenge. Currently, the approved treatments for CHB are limited to interferon and nucleos(t)ide analogs, both of which have their limitations, and achieving a complete cure remains an elusive goal. Therefore, the identification of new therapeutic targets and the development of novel antiviral strategies are of utmost importance. Natural products (NPs) constitute a class of substances known for their diverse chemical structures, wide-ranging biological activities, and low toxicity profiles. They have shown promise as potential candidates for combating various diseases, with a substantial number demonstrating anti-HBV properties. This comprehensive review focuses on the current applications of NPs in the fight against HBV and provides a summary of their antiviral mechanisms, considering their impact on the viral life cycle and host hepatocytes. By offering insights into the world of anti-HBV NPs, this review aims to furnish valuable information to support the future development of antiviral drugs.

[KEY WORDS] Hepatitis B virus; Natural products; Antiviral stategy; Mechanisms; Host hepatocyte; Life cycle
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Introduction

Hepatitis B virus (HBV), a primary cause of liver disease and hepatocellular carcinoma (HCC), remains a pressing global health concern. In 2019, the World Health Organization (WHO) reported an estimated 296 million chronic HBV infections, with 1.5 million new cases annually and approximately 820 000 resultant deaths [1]. Current treatment strategies for chronic hepatitis B (CHB) are centered around nucleos(t)ide analog (NA) and pegylated interferon (IFN) therapies [2]. However, their limitations, including NA's propensity for drug resistance and IFN's side effects, coupled with their high costs and limited accessibility, make the eradication of the virus a significant challenge. This landscape underscores the urgency of developing innovative, affordable, safe, and more effective anti-HBV drugs.

HBV is a small double-stranded DNA virus classified as a member of the hepadnaviridae family. It enters hepatocytes *via* the sodium taurocholate cotransporting polypeptide (NTCP) receptor. After endocytosis, the viral nucleocapsid is released into the cytoplasm, after which the 3.2 kb viral relaxed circular DNA (rcDNA) is transferred to the nucleus, where it becomes covalently closed circular DNA (cccDNA) [3]. This cccDNA serves as a transcriptional template for the production of 3.5 kb pregenomic RNA (pgRNA) and four other viral mRNAs. Among these, the 3.5 kb precore mRNA contains all open reading frames for viral proteins but exclusively translates HBV e antigen (HBeAg). Furthermore, the 2.4 kb and 2.1 kb mRNA, and 0.7 kb mRNA under the control of their own promoters. In addition to encoding the viral polymerase and the core protein, the pgRNA serves as

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the template of the HBV genome. The 2.4 kb RNA translates into HBV large surface antigen (L-HBsAg, abbreviated as L-HBs, including preS1 and preS2 extensions). Meanwhile, the 2.1 kb RNA synthesizes the other two surface antigens, HBV middle (M-HBs, which includes preS2 region) and small surface antigen (S-HBs). The 0.7 kb RNA is responsible for the synthesis of the HBV X protein (HBx). After the viral polymerase binds to pgRNA, the complex is packaged into nucleocapsids, wherein the pgRNA is converted into rcDNA by the polymerase [4]. The ensuing processes entail the nucleocapsid undergoing either envelopment and secretion as a virion or redirecting the rcDNA back into the nucleus to replenish the cccDNA pool. This intricate viral life cycle offers a plethora of opportunities for drug targeting—either by directly targeting the viral mechanisms or by indirectly influencing host factors (Fig. 1).

Natural products (NPs), sourced from plants, animals, fungi, and microorganisms and their analogs [5], emerge as promising candidates in the quest for effective anti-HBV agents due to their diverse structures, broad bioactivity, and minimal toxicity. While these compounds can be classified into terpenes, flavonoids, alkaloids, phenolic acids, lignans, anthraquinones, and glycosides based on their chemical properties [6], a holistic understanding of their anti-HBV mechanisms is yet to be established [7-9]. This review delineates natural anti-HBV products into two primary categories: direct antiviral agents that interfere with the HBV life cycle, including viral proteins, viral transcription, cccDNA, viral assembly, and viral secretion, and indirect antiviral agents that modulate host hepatocytes, including membrane proteins, the immune system, the autophagy, and other signaling pathways, potentially impacting HBV replication (Fig. 1).

Directly Targeting the HBV Life Cycle

Targeting viral proteins

Surface protein: HBV infection commences with the virus's attachment to hepatocytes, a process mediated by HBsAgs that constitute the viral envelope. Clinically, the loss of HBsAgs is currently regarded as one of two benchmarks for achieving a functional HBV cure [10]. Consequently, therapeutic agents designed to target HBsAgs could impede viral particle interactions, thus obstructing viral entry, and bear significant therapeutic implications. In this context, proanthocyanidin (a plant-derived oligomeric flavonoid) [11] and amentoflavone (a plant-derived polyphenolic bioflavonoid) [12], have demonstrated dose-dependent antiviral efficacies in both HBV-susceptible hepatoma cells and primary human hepatocytes. It has been discerned that these compounds hinder the

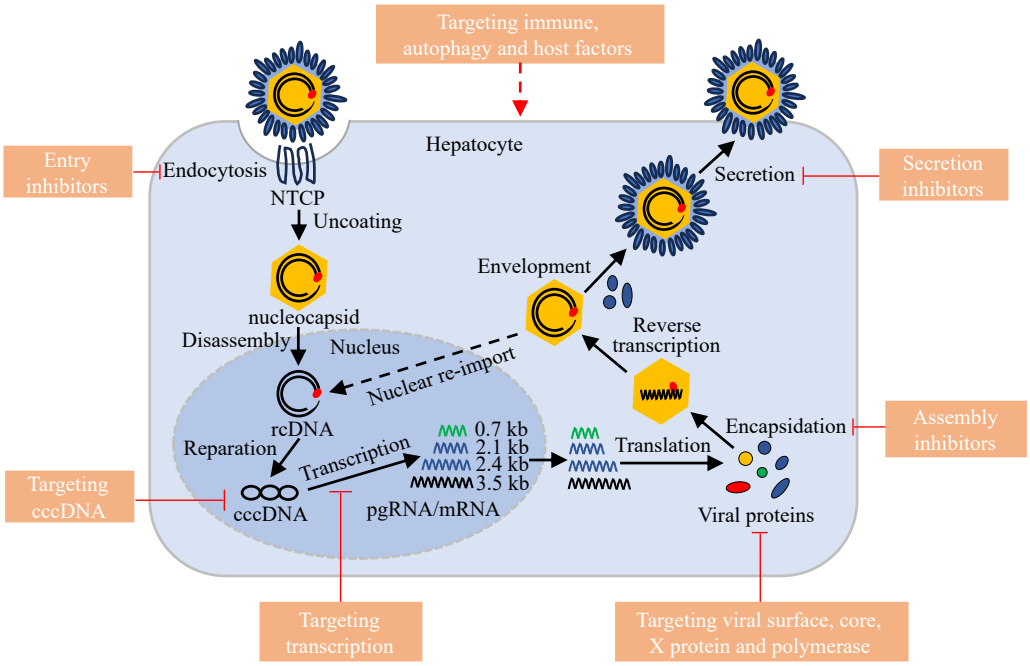

Fig. 1 A schematic representation of the HBV life cycle and antiviral targets. Following NTCP-mediated endocytosis, viral nucleocapsids are transported to the nucleus, where rcDNA is converted to cccDNA. cccDNA acts as the template to transcribe pgRNA and four viral mRNA, which are then transferred into the cytoplasm and translated into three viral envelope proteins, precore/core proteins, X proteins, and polymerase. The core protein acts as a packaging unit for polymerase and pgRNA. In this capsid, pgRNA reversely transcribes into viral DNA under the control of polymerase. The nucleocapsid is subsequently enveloped with viral surface proteins, either being exported from the hepatocyte to instigate new infections or re-enter the nucleus to replenish the cccDNA pool. Potential drugs may exert their antiviral effects *via* inhibiting viral entry and transcription, targeting four viral proteins and cccDNA, suppressing viral assembly and secretion, and modulating immune responses, autophagy, and other host factors to regulate viral replication. The visualization differentiates envelope, core, X proteins, and polymerase in blue, yellow, green, and red hues, respectively.

binding of HBV particles and the HBV preS1 peptide to the NTCP by directly interacting with the essential receptor-binding domain in the preS1 region of the LHBs protein. Notably, this intervention leaves the NTCP's bile acid transport functions unaltered. These results indicate that these compounds have pan-genotypic anti-HBV activity and can be particularly potent against HBV variants that display resistance to nucleoside analogs.

Core protein: The HBV core protein, pivotal in the viral life cycle, constitutes the structural framework for the icosahedral shell of capsids, which is indispensable for the maintenance and transcriptional regulation of cccDNA [13]. This process commences with two core protein monomers forming a homodimer intermediate through an intradimer interface. Subsequently, numerous homodimers assemble to form an intact capsid *via* a dimer-dimer interface, a crucial step that supports pgRNA encapsidation and capsid assembly [14]. Additionally, the core protein has been identified in the nucleus of hepatocytes, where it interacts with host factors responsible for transcriptional regulation [15]. Consequently, targeting the core protein presents a promising strategy for the development of new anti-HBV drugs. For example, myricetin-3-O-rhamnoside and quercetin, both isolated from *Guiera senegalensis* leaves, have demonstrated their ability to downregulate HBV replication by hydrogen bonding and hydrophobic interactions with the viral core protein [16]. Additionally, two flavonoids, sakuranetin and velutin, extracted from the dichloromethane fraction of *Rhus retinorrhoea* aerial parts, have exhibited dose- and time-dependent inhibitory activities against HBsAg and HBeAg in cultured HepG2.2.15 cells. Subsequent studies have further revealed that these two flavonoids display anti-HBV potential through interactions with the viral core protein, subsequently inhibiting its activity [17].

Polymerase: The HBV polymerase, the largest viral protein, is a multifaceted reverse transcriptase with four domains, each performing critical functions for the virus. Among these domains, the reverse transcriptase (RT domain) is particularly noteworthy. It embodies both reverse transcriptase and DNA polymerase activities, enabling the reverse transcription of HBV pgRNA into viral negative-stranded DNA. This DNA then serves as a template for synthesizing positive-stranded DNA [18]. Moreover, for effective function, the HBV polymerase must first bind to a short RNA structure termed epsilon at the 5' end of pgRNA [19]. This binding is not only essential for protein-primed DNA synthesis by the polymerase but also for the packaging of pgRNA into viral nucleocapsids [20, 21]. Therefore, the polymerase emerges as an ideal target for inhibiting viral genome amplification. Several natural compounds have demonstrated potential in inhibiting the polymerase. For example, rosmarinic acid, abundant in plants like *Salvia officinalis*, displays its anti-HBV activity by inhibiting the interaction between HBV epsilon RNA and the viral polymerase [22]. Furthermore, Parvez *et al.* identified multiple substances with anti-HBV effects targeting the polymerase. These substances include anthraquinones (aloe-emodin, chrysophanol, and aloin B) derived from *Aloe vera*, catechin and epicatechin from *Rhus tripartite*, four flavonoids (myricetin-3-*O*-rhamnoside, quercetin, sakuranetin, and velutin) isolated from *Guiera senegalensis* leaves and *Rhus retinorrhoea* aerial parts, and solanopubamine, a rare alkaloid from *Solanum schimperianum*. Each of these compounds interacts with the active-site residues of the HBV polymerase, forming stable complexes that inhibit its activity [16, 17, 23-25].

X protein: HBx is a multifunctional regulatory protein that promotes viral production through various mechanisms, notably by guiding the epigenetic control of cccDNA transcription and ensuring the initiation and maintenance of viral replication [26]. Given its significant role in the viral life cycle, the HBx protein presents an appealing target for strategies aimed at inhibiting HBV replication. Two NPs, dicoumarol and sphondin, have demonstrated potential in this area. Dicoumarol, a natural hydroxycoumarin derived from *Melilotus officinalis* (L.) Pall fungi [27] and sphondin, a furanocoumarin isolated from *Heracleum laciniatum* [28], have shown inhibitory effects on HBV RNA and DNA formation, as well as on viral surface and core protein production in HBV-infected cells and humanized liver mouse models. Mechanistic studies have indicated that the two NPs inhibit the recruitment of HBx to cccDNA [28, 29]. This action, in turn, suppresses cccDNA transcription and the subsequent expression of viral proteins.

Targeting cccDNA

The persistence of cccDNA in liver cells is a primary obstacle in eradicating HBV. This persistence is responsible for the reactivation of HBV replication in individuals who have previously recovered but are immunosuppressed. Even though NA therapies can diminish viremia, they often fall short in directing destablizing cccDNA in numerous cases, necessitating prolonged treatments. Consequently, directly targeting cccDNA might be the key to eradicating HBV [30]. Several NPs have shown promise in this regard. Hydrolyzable tannins (punicalagin, punicalin, and geraniin), extracted from Chinese herbal remedies, inhibit HBV cccDNA production by suppressing the formation of cccDNA and promoting its degradation [31]. Chrysin, also known as 5, 7-dihydroxyflavone, found abundantly in honey, propolis, and various plant extracts, has long been celebrated for its hepatoprotective qualities in traditional Chinese medicine. Bhat *et al.* revealed that chrysin significantly reduces HBV cccDNA levels in a dose-dependent manner. In addition, increasing doses of chrysin gradually inhibit the formation of intracellular cccDNA in the cell culture system, pointing to its potential in suppressing the buildup of the cccDNA pool [32].

Targeting viral DNA and transcriptional cis-elements

In the HBV life cycle, the transcription of viral DNA is a pivotal step, and it is well-established that a high antigen load contributes to the persistence of chronic HBV infection. Therefore, developing strategies to selectively target viral

RNA synthesis and prevent viral antigen expression holds promise for novel antiviral drug development. One such strategy involves regulating gene expression through methylation, as alterations in HBV DNA methylation can disrupt the aberrant transcription of viral genes [33]. For example, sophoridine, a matrine-type alkaloid derived from *Sophora flavescens*, exerts its anti-HBV effects by increasing the methylation of HBV DNA, resulting in the downregulation of HBV viral protein expression and secretion, and also by suppressing pgRNA and HBV DNA [34].

HBV genome transcription is tightly controlled by two enhancers (enhancer I and II) and four promoters, including core, preS1, preS2, and X promoters [35]. Consequently, drugs targeting the activities of these viral *cis*-elements can profoundly impact viral protein expression and interfere with viral replication. For instance, (-)-lariciresinol ((-)-LRSL), isolated from water-soluble chemical constituents in the roots of *Isatis indigotica* Fortune ex Lindl., has been demonstrated to inhibit HBV DNA replication of both wild-type and NA-resistant strains *in vitro*. Delving into its mechanism of action, (-)-LRSL can block viral transcription by inhibiting the activities of HBV preS1, core promoter, and enhancer II [36]. Similarly, psoralen, a principal bioactive component in the dried fruits of *Cullen corylifolium* (L.) Medik (*syn. Psoralea corylifolia* L), suppresses HBV RNA transcription by inhibiting HBV pre-core/core promoter enhancer II activities, resulting in a reduction in the core protein expression [37]. Another notable compound, calcitriol (1α, 25-dihydroxyvitamin D3), the metabolically activated form of vitamin D, selectively inhibits the HBV core promoter in multiple HBV cell culture models without affecting the preS1, preS2, or X promoters [38]. Such observations provide a molecular basis for the clinically noted correlation between vitamin D levels and HBV activity.

Targeting assembly and secretion

Viral capsid assembly and progeny virion secretion are two pivotal phases in the HBV life cycle. Therefore, in addition to targeting viral proteins or DNA, antiviral therapies that target these processes have proven effective. For example, isothiafludine (NZ-4), a derivative of bis-heterocycle tandem pairs from the NP leucamide A, which is extracted from the Australian marine sponge *Leucetta microraphis* [39], suppresses the replication of intracellular wild-type HBV and various drug-resistant HBV mutants in HepG2.2.15 cells *via* interfering with the interaction between pgRNA and viral core protein, resulting in the assembly of replication-deficient capsids [40]. Furthermore, the matrine-type alkaloid sophoridine from *Sophora flavescens* plays a dual role. It regulates the HBV life cycle by increasing HBV DNA methylation, and interestingly, it also impacts viral secretion. There is a noted reduction in extracellular HBV DNA and HBsAg levels after its administration. This effect is paired with a dose-dependent spike in intracellular HBV DNA levels after a short 24-hour exposure, hinting at sophoridine's involvement in HBV envelopment or virion release processes [34].

Indirectly Targeting Host Hepatocytes

Targeting NTCP and endocytosis

HBV exhibits a strong affinity for hepatocytes, primarily driven by its interaction with specific receptors or co-receptors within these liver cells. A breakthrough in understanding this specificity came in 2012 with the identification of NTCP, a hepatocyte-expressed transmembrane protein. NTCP was recognized as the key receptor facilitating the specific interaction between the receptor-binding region of pre-S1 and HBV, thereby governing the virus's cellular entry [41]. This pivotal discovery paved the way for the development of NPs aimed at inhibiting HBV entry by targeting NTCP. Examples of such products include vanitaracin A [42] and exophillic acid [43], both of which are fungus-derived secondary metabolites. These compounds demonstrate an ability to inhibit the infection of all HBV genotypes tested (genotypes A to D) and even clinically relevant NA-resistant HBV strains by directly interacting with the HBV receptor NTCP. Further, Myricetin-3-*O*-rhamnoside and quercetin showcase inhibitory effects on the HBV life cycle through their strong binding affinities towards NTCP [16]. Another compound, ergosterol peroxide, extracted from mushrooms, interferes with the early phase of HBV entry into dimethylsulfoxide-differentiated immortalized human primary hepatocytes overexpressing NTCP. Specifically, it directly hinders the NTCP-LHB interaction without affecting HBV genome replication or virion secretion [44].

HBV enters hepatocytes *via* the clathrin-mediated endocytosis pathway after binding with NTCP [45]. Epigallocatechin-3-gallate, a polyphenol extracted from green tea, inhibited the secretion of HBsAgs, HBeAgs, and extracellular HBV DNA from HepG2 2.2.15 cells in a dose- and time-dependent manner, with an inhibitory effect even more potent than lamivudine [46]. Further study demonstrated that its anti-HBV capability manifested in an early step of viral entry by functionally inhibiting clathrin-mediated endocytosis, while HBV replication, assembly, or release remains unaffected [47]. Silibinin, a constituent of milk thistle extract, inhibited the entry of HBV into hepatocytes dose-dependently by inhibiting clathrin-mediated endocytosis [48].

Targeting immune responses

HBV is a non-cytopathic virus capable of precipitating both acute and chronic infections. While individuals with acute infections often manifest robust T-cell-mediated responses facilitating viral elimination, those diagnosed with CHB typically exhibit diminished innate and adaptive immune responses to HBV, culminating in persistent viral infections with potential progression to cirrhosis and HCC [49]. To ameliorate these outcomes, strategic modulation of the host's immune system —either by augmenting innate immune defenses or eliciting HBV-specific adaptive immunity —may prove efficacious. A seminal study by Wang *et al.* elucidated that tannic acid, a polyphenol ubiquitously present in various botanical sources [50], and dihydromyricetin, a flavonoid isol-

ated from *Ampelopsis grossedentata* [51], exert profound inhibitory effects on HBV RNA transcription, in tandem with the suppression of HBsAg and HBeAg secretions. This inhibitory cascade is attributed to the upregulation of specific inflammatory cytokines, as well as the IFN-*α*-mediated antiviral immune effector pathway and the nuclear factor-kappa B/mitogen-activated protein kinase pathway, ultimately culminating in the abrogation of HBV replication [50, 51]. Concurrently, several kinds of water-soluble polysaccharides, extracted from diverse organisms, including *Flammulina velutipes* [52], *Thais clavigera* (Küster 1860) [53], flaxseed hull [54], and Radix Isatidis (*Isatis indigotica* Fortune) [55], display pronounced anti-HBV activity *via* the activation of the IFN-dependent signaling pathway and the induction of immune activity.

Toll-like receptors (TLRs) are pivotal pattern recognition receptors instrumental in the host cell's recognition of and responses to microbial pathogens. Their significance is underscored in the innate immune system, where they discern viral and bacterial pathogen-associated molecular patterns, facilitating the transition to adaptive immunity [56]. Ginsenoside Rg3, derived from *Genus Panax*, exhibits anti-HBV potential in patients with HBV-induced hepatic ailments by inhibiting viral proteins, viral particle formation, and viral replication. Further studies have demonstrated that Rg3 also mitigates the TLR-myeloid differentiation primary response gene 88-dependent pathway by downregulating the tumor necrosis factor receptor-associated factor 6/transforming growth factor activated kinase-1 signaling and suppressing c-Jun N-terminal kinase/AP-1 signaling [57]. Our investigations have identified that polysaccharides sourced from sea urchins and *Eupolyphaga sinensis Walker* can reduce the secretion of HBsAg and HBeAg, as well as the replication and transcription of HBV, both *in vitro* and *in vivo*. This effect is attributed to the activation of the TLR-4-mediated immune pathway, and the compounds have demonstrated broad-spectrum antiviral activity spanning numerous HBV genotypes [58, 59].

Targeting autophagy

Autophagy, a cellular homeostatic process, orchestrates the degradation of persistent proteins and damaged organelles by sequestering them into specialized double-membraned vesicles known as autophagosomes. These vesicles subsequently merge with lysosomes to enable the degradation of their contents [60]. Intriguingly, HBV has demonstrated an ability to exploit the autophagic pathway. It selectively activates the initial stages while inhibiting the terminal stages of autophagy by hindering lysosomal acidification [61, 62]. Given this nuanced interaction between HBV and autophagy, therapeutic modulation of this process presents a compelling avenue for anti-HBV strategies. Notably, phytocompounds such as tannic acid and dihydromyricetin have exhibited potent anti-HBV activity. Their efficacy is twofold: modulation of immune-related cytokines and stimulation of autophagy in hepatocytes [50, 51]. Epigallocatechin-3-gallate (EGCG), the most abundant polyphenol in green tea, effectively suppresses the secretion of HBV DNA in hepatoma cells. This inhibitory effect is attributed to EGCG's facilitation of a complete autophagic cycle, particularly by enhancing lysosomal acidification [63]. Similarly, lithospermic acid (LA), a polyphenol derived from *Salvia miltiorrhiza,* inhibits HBV protein expression and HBV DNA replication in dose- and time-dependent manners both *in vitro* and *in vivo*. This suppression is mechanistically linked to LA's promotion of light chain 3-II protein expression and increased autolysosome formation, ultimately inducing a robust autophagic response [64].

Targeting other pathways in hepatocytes

HBV relies heavily on host cell factors and metabolic processes for its survival and replication. Intriguingly, the recruitment of host cellular transcription factors and coactivators to facilitate HBV genome transcription is related to the nutritional state within host cells. Moreover, the expression of HBV genes intricately intertwines with hepatic metabolic pathways [65]. As such, the development of novel antiviral therapeutics is predicated on the ability to target these host cellular processes within hepatocytes. Curcumin, a natural phenolic compound, has been documented to exhibit profound inhibitory effects on HBV gene expression and replication. Its mechanism of action involves the downregulation of peroxisome proliferator-activated receptor γ coactivator-1α, a protein induced under conditions of nutritional deprivation that plays a pivotal role in initiating the gluconeogenesis cascade and, notably, coactivates HBV transcription [66]. Additionally, oxymatrine, derived from *Sophora japonica* and cepharanthine hydrochloride, a natural alkaloid-derived compound extracted from *Stephania cepharantha Hayata*, have exhibited antiviral activity against both lamivudine-resistant and clinically wild-type HBV. Investigations have indicated that these two NPs can inhibit the stabilization of heat-stress cognate 70 (Hsc70) [67] or inhibit Hsc70 mRNA expression [68], consequently downregulating the expression of Hsc70, which is a host ATP-binding protein required for HBV replication and participate in the viral reverse transcription process [69].

In addition, numerous natural extracts, such as geranyl phenyl ethers from *Illicium micranthum* [70], quercetin and kaempferol derivatives from *Euphorbia schimperi* [71], and swertisin isolated from *Iris tectorum* Maxim [72], have exhibited notable inhibitory effects on HBV replication, although the precise underlying mechanisms remain enigmatic. Notably, two chalcone derivatives, namely 2,4'-dihydroxy-4-methoxydihydrochalcone and 2,4'-dihydroxy-4-methoxyhydrochalcone, extracted from the endemic *Socotraen Dracaena cinnabari*, have shown a remarkable capacity to inhibit HBsAg production in a dose- and time-dependent manner [73]. Additionally, esculetin derived from Microsorium fortune has demonstrated the ability to suppress the expressions of HBV antigens and HBV DNA *in vitro* and *in vivo* [74]. Furthermore, several matrine-type alkaloids and analogs isolated from the roots of *Sophora flavescens* have exhibited anti-

HBV ability comparable to that of matrine [75]. While these NPs undoubtedly possess anti-HBV activities, further investigations are warranted to unveil the precise mechanistic underpinnings of their action.

Conclusion

A plethora of NPs, distributed globally, have been documented to exhibit antiviral properties through a spectrum of mechanisms. Based on their modulatory effects on the viral life cycle, the anti-HBV mechanisms of NPs can be categorized into two primary classifications: direct and indirect actions. Direct action implies that certain NPs target the virus itself, intervening with the viral surface protein, core protein, polymerase, X protein, cccDNA functionality, or the transcription of viral DNA. Conversely, indirect action suggests that NPs exert their antiviral prowess by modulating hepatocytic membrane proteins, immune responses, autophagy, and other host proteins and pathways that regulate viral replication (Table 1).

Prospects For Advancements In Anti-HBV Therapeutics

CHB remains a global health concern, necessitating the pursuit of innovative therapeutic approaches. The paramount goal in CHB treatment is the achievement of a functional cure, defined as the sustained absence of HBsAg and undetectable HBV DNA for at least six months post-treatment cessation [76]. Currently, the predominant treatment modalities, IFN and NA therapies, primarily center on regulating host immune responses and inhibiting viral polymerase activ-

Table 1 The direct and indirect actions of NPs on the regulation of HBV

Mechanisms of antiviral action		Natural products	Sources	Ref.
Direct anti-HBV action based on the viral life cycle	Targeting viral surface protein	Proanthocyanidin, amentoflavone	Plants	[11, 12]
	Targeting viral core protein	Myricetin-3-*O*-rhamnoside, quercetin, sakuranetin, velutin	*Guiera senegalensis*, *Rhus retinorrhoea*	[16, 17]
	Targeting viral polymerase	Rosmarinic acid, anthraquinones, catechin, epicatechin, myricetin-3-*O*-rhamnoside, quercetin, sakuranetin, velutin, solanopubamine	*Salvia officinalis*, *Aloe vera*, *Rhus tripartite*, *Guiera senegalensis*, *Rhus retinorrhoea*, *Solanum schimperianum*	[16, 17, 22-25]
	Targeting viral X protein	Dicoumarol, furanocoumarin derivative	*Melilotus officinalis* (L.) Pall fungi, *Heracleum laciniatum*	[27-29]
	Targeting cccDNA	Punicalagin, punicalin, geraniin, chrysin	Honey, propolis, plant	[31, 32]
	Targeting viral DNA	Sophoridine, (−)-lariciresinol, Psoralen, calcitriol	*Sophora flavescens*, *Isatis indigotica*, *Cullen corylifolium* (L.)	[34, 36-38]
	Targeting viral assembly	Isothiafludine	*Leucetta microraphis*	[39]
	Targeting virion secretion	Sophoridine	*Sophora flavescens*	[34]
Indirect anti-HBV action based on host cells	Targeting NTCP	Vanitaracin A, exophillic acid, myricetin-3-*O*-rhamnoside, quercetin, ergosterol peroxide	Fungus, *Guiera senegalensis*, mushroom	[16, 42-44]
	Targeting endocytosis	Epigallocatechin-3-gallate, silibinin	Green tea, milk	[46, 48]
	Targeting immune responses	Tannic acid, dihydromyricetin, polysaccharides	Foods, fruits, plants, *Ampelopsis grossedentata*, *Flammulina velutipes*, *Thais clavigera*, flaxseed hull, *Isatis indigotica*, *Genus Panax*, sea urchins, *Eupolyphaga sinensis Walker*	[51-55, 57-59]
	Targeting autophagy	Tannic acid, dihydromyricetin, epigallocatechin-3-gallate, lithospermic acid	Foods, fruits, plants, *Ampelopsis grossedentata*, green tea, *Salvia miltiorrhiza*	[50, 51, 63, 64]
	Targeting other pathways	Curcumin, oxymatrine, cepharanthine hydrochloride, geranyl phenyl ethers, quercetin and kaempferol derivatives, swertisin, 2,4′-dihydroxy-4-methoxydihydrochalcone, 2,4′-dihydroxy-4-methoxyhydrochalcone, esculetin, alkaloids	*Sophora japonica*, *Stephania cepharantha Hayata*, *Illicium micranthum*, *Euphorbia schimperi*, *Iris tectorum*, *Socotraen Dracaena cinnabari*, microsorium, *Sophora flavescens*	[66-68, 70-75]

ity. While these treatments effectively reduce HBV DNA levels and mitigate adverse outcomes, such as liver cirrhosis and HCC, they harbor limitations. Even with prolonged therapy, they yield relatively low rates of functional cure [77]. Therefore, the search for novel anti-HBV medications to achieve functional cure is an imperative endeavor. NPs have garnered substantial attention as a source of potential therapeutic agents. NPs have exhibited diverse antiviral mechanisms, with many demonstrating the capacity to significantly reduce HBsAg levels—a pivotal surrogate marker of HBV functional cure [10]. Consequently, the exploration of naturally sourced anti-HBV medications has gained momentum. However, despite the abundance of NPs showcasing promising antiviral ability in cell culture and animal models, most of them confront substantial translational barriers. A central issue revolves around the uncertainty of whether HBsAg loss achieved through NP interventions aligns with the clinical outcomes seen in spontaneous seroclearance or IFN/NA therapies. Therefore, substantiating sustainable off-treatment HBsAg seroclearance becomes an essential research endeavor.

Given the multifaceted nature of HBV replication, an optimal strategy for achieving a durable functional cure in CHB may entail combination therapies targeting various facets of the HBV life cycle and host factors, particularly immunomodulatory pathways. For example, the combination of IFNs with matrine has been explored in CHB patients, revealing enhanced clinical efficacy and reduced adverse effects relative to IFN monotherapy [78]. Similarly, concurrent administration of ma-huang-tang (maoto), an agent that induces host gene tropomyosin 2 expression to suppress HBV production, and lamivudine has exhibited superior efficacy in restraining HBV replication compared with individual regimens [79]. The established safety profile of ma-huang-tang positions it as a promising candidate for clinical application in the realm of anti-HBV therapeutics. Furthermore, several traditional Chinese medicines, characterized by their rich herbal composition, have become integral adjuncts in antiviral therapies. For example, Xiao Chai Hu Tang, a traditional Chinese herbal medicine formula comprising seven medicinal herbs (*Bupleurum falcatum*, *Pinellia ternata*, *Zizyphus jujuba*, *Panax ginseng*, *zingiber officinale*, *Scutellaria baicalensis*, *Glycyrrhiza glabra)*, has shown efficacy in ameliorating CHB clinical manifestations and viral load, especially in populations where conventional antiviral therapies are contraindicated due to adverse event profiles or financial constraints [80]. *Dichondra repens* J.R.Forst. and G.Forst. (Convolvulaceae, termed 'Matijin' in Chinese), a perennial creeping herb and an ethnomedicine mainly used by Dai and Miao nationalities in Southwest China. Its chief phytochemical constituents, phenylalanine dipeptides, termed 'Matijin-Su', have showcased anti-HBV properties. Significantly, a phase I anti-HBV clinical trial of a candidate compound, bentysrepinine (Y101 or 'Tifentai'), has culminated [81]. Typically, these traditional Chinese medicinal formulations are perceived as favorable alternatives to IFNs and NAs, predominantly due to their cost-efficiency and favorable safety spectra.

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