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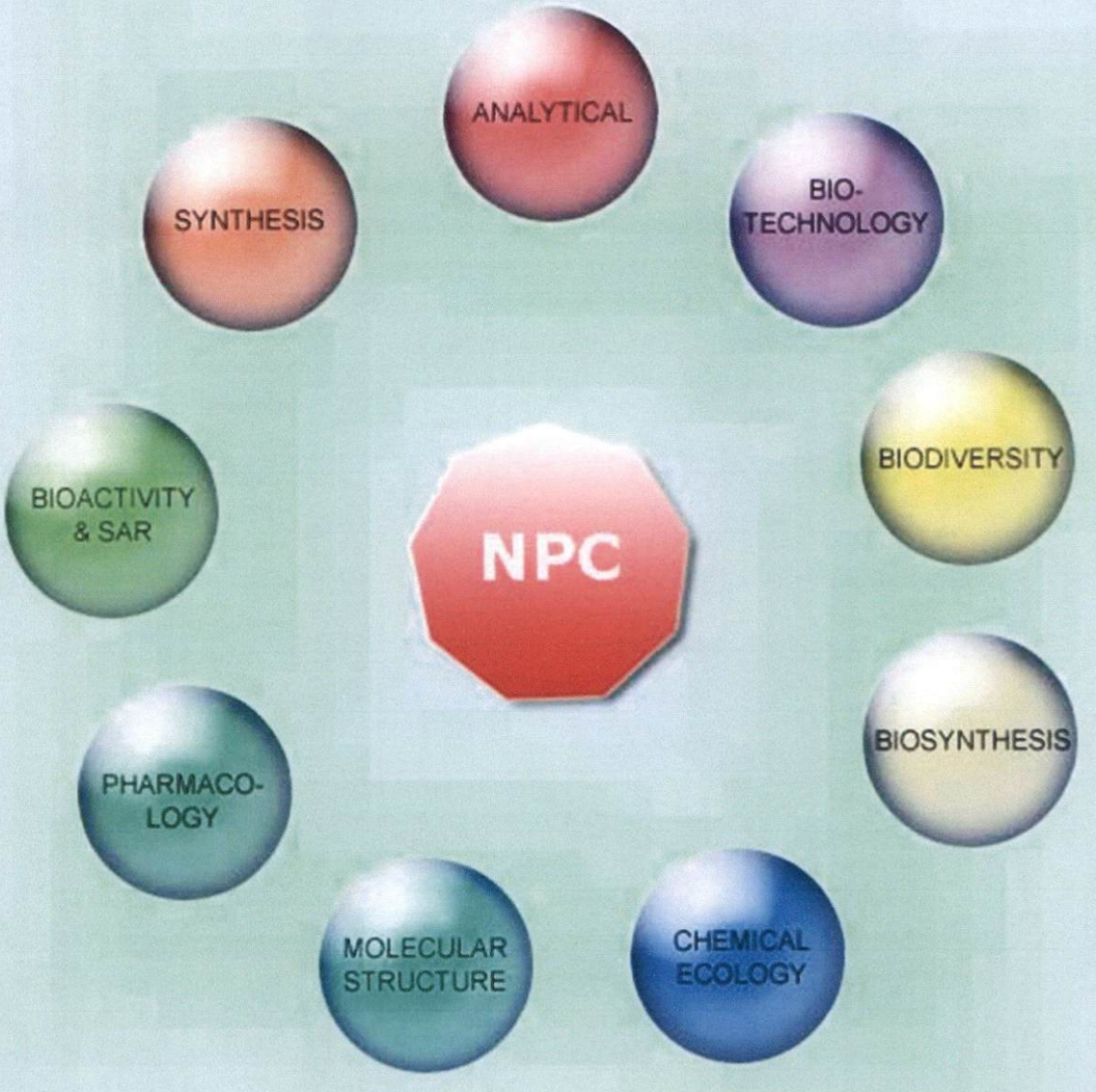

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On the Occasion of his 75th Birthday

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Promising Anti-Hepatitis C Virus Compounds from Natural Resources

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Hepatitis C virus (HCV) infection is a major worldwide problem, which involves approximately 170 million people. High morbidity of patients is caused by chronic infection, which leads to liver cirrhosis, hepatocellular carcinoma and other HCV-related diseases. The sustained virological response (SVR) has been markedly improved to be >90% by the current standard interferon (IFN)-free treatment regimens with a combination of direct-acting antiviral agents (DAAs) targeting the viral NS3 protease, NS5A multi-function protein and NS5B RNA-dependent RNA polymerase, compared with 50–70% of SVR rates achieved by the previous standard IFN-based treatment regimens with or without an NS3 protease inhibitor. However, the emergence of DAA-resistant HCV strains and the limited access to the DAAs due to their high cost could be major concerns. Also, the long-term prognosis of patients treated with DAAs, such as the possible development of hepatocellular carcinoma, still needs to be further evaluated. Natural resources are considered to be good candidates to develop anti-HCV agents. Here, we summarize anti-HCV compounds obtained from natural resources, including medicinal plant extracts, their isolated compounds and some of their derivatives that possess high antiviral potency against HCV.

Keywords: Hepatitis C virus, Medicinal plants, Extracts, Isolated compounds.

Hepatitis C virus (HCV) is a major cause of liver diseases. Persistent HCV infection will progress to chronic liver diseases including cirrhosis and hepatocellular carcinoma. It has been reported that HCV infects 170 million people in the world [1, 2]. More than 120-130 million people are at risk to develop cirrhosis and/or hepatocellular carcinoma, and importantly, around 350,000 patients die from HCV-related diseases every year. It is estimated that about four million people are chronically infected with HCV in the United States, 5-10 million in Europe, 12 million in India and 1.2 million in Japan. Approximately 80% of the acute cases progress to chronic infection and 20% of them progress to cirrhosis [3]. The HCV genome exhibits a sequence of heterogeneity, based on which HCV is currently classified into seven genotypes (1 to 7) with more than 70 subtypes (1a, 1b, 2a, 2b, etc.) [4]. The prevalence of each genotype varies with different geographic areas. The distribution of HCV genotypes 1, 2, and 3 are widely spread among global areas including the United States, South America, Europe, Australia and Eastern Asia. Genotype 4 is primarily found in Egypt, the Middle East, and Central Africa and genotype 5 in Southern Africa. Genotype 6 is mostly found in Southeast Asia [5, 6]. The distribution of HCV genotypes has an important clinical implication that influences the efficacy of therapies. HCV genotype 1 is most common, representing 46% of all HCV infections, with 22% being subtype 1b. Genotype 3 represents about 22% of all HCV infections, with genotypes 2 and 4 being 13% each, whereas genotypes 6 and 5 represent 2% and 1%, respectively [5].

A triple combination therapy with interferon (IFN)-α, ribavirin and the first generation of HCV NS3 protease inhibitors (telaprevir or boceprevir) has been used since 2011, which provides a higher sustained virological response (SVR) rate of ca. 70% for patients infected with HCV genotype 1 compared with ~50% achieved by a double combination therapy with IFN-α and ribavirin. More recently, novel direct-acting antiviral agents (DAAs) have been developed and IFN-free oral treatment regimens using a combination of the DAAs have become the standard for HCV treatment, with SVR rates being >90%. Those DAAs include NS5A inhibitors such as daclatasvir and ledipasvir, and NS5B RNA-dependent RNA polymerase (RdRp) inhibitors such as sofosbuvir, in addition to the second generation of NS3 protease inhibitors such as simeprevir, asunaprevir and vaniprevir. The IFN-free oral DAAs regimens are applicable to almost all HCV genotypes and have improved the SVR rate, with reduction in treatment duration and side effects. However, the long-term prognosis of patients treated with DAAs, such as the possible development of hepatocellular carcinoma, still needs to be further evaluated. Moreover, the high cost of the new DAA regimens is also an important issue; not all patients can have access to the therapy, particularly in countries with limited resources [1, 7-10]. Therefore, it would be necessary to find new compounds that not only have good efficacy for all HCV genotypes and drug-resistant strains but also which are available at much lower cost. Several compounds from natural resources and their derivatives have been reported to possess anti-HCV activities that have promise for development into anti-HCV agents. Here, we summarize medicinal plant extracts, isolated compounds and their derivative components that possess anti-HCV activities with diverse mechanisms.

HCV is a member of the genus *Hepacivirus* that belongs to the *Flaviviridae* family. The HCV genome consists of positive-sense single-stranded RNA of 9.6 kb with highly structured 5'- and 3'-untranslated regions (UTRs) (Figure 1). The viral genome encodes a polyprotein of about 3,000 amino acid residues, which is cleaved by

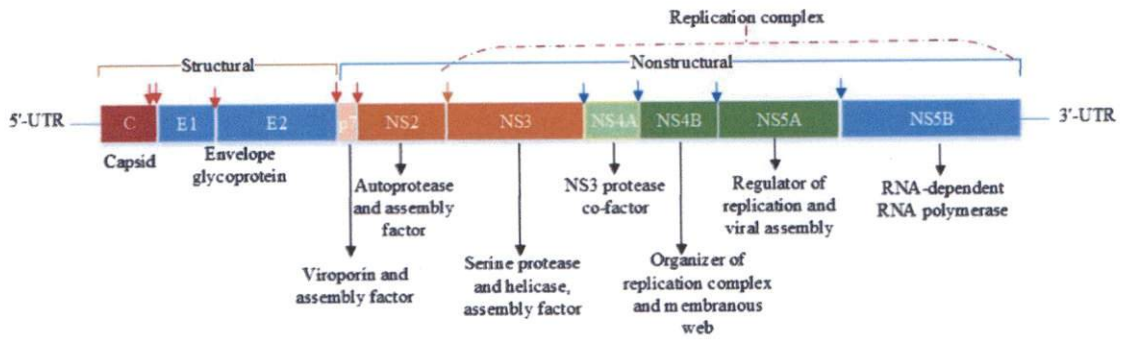

Figure 1: HCV genome organization.

The HCV genome contains a single open reading flame (ORF) of RNA that is flanked with 5'- and 3'-untranslated regions (UTRs). The 5'-UTR contains an internal ribosome entry site (IRES). The IRES-mediated translation of ORF generates a polyprotein, which is cleaved into ten viral proteins, structural proteins (Core, E1 and E2) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). Red arrows, cleavage by signal peptidase and the signal peptide peptidase; orange arrow, autocatalytic cleavage by NS2-NS3 metalloprotease; blue arrows, cleavage by NS3-NS4A serine protease. The functions of the individual protein are explained in the text.

the host and viral proteases to generate structural proteins (core, E1 and E2), a putative ion channel (p7), and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [11-13]. Each of these proteins has a significant role in HCV entry, replication, and particle formation, and therefore, can be a potential antiviral target(s). The E1 and E2 glycoproteins are involved in HCV entry steps, such as viral attachment and fusion. They are responsible for binding to a number of different virus receptor molecules on the cell surface, such as claudin 1 (CLDN1), scavenger receptor class B type I (SR-B1), CD81 and occludin (OCLN). On the other hand, nonstructural proteins play crucial roles in virus replication. They serve to coordinate the intracellular events of HCV replication, including HCV RNA synthesis, protein synthesis, virus assembly, and modulation of host defense mechanisms. The NS2 metalloprotease mediates cleavage between NS2 and NS3. NS3 exerts a serine protease activity that is responsible for the cleavage at the remaining cleavage sites of the polyprotein. NS3 also possesses a helicase activity that can be regulated by the interaction between the serine protease and helicase domains of NS3, and it is required for replication of the virus. NS4A stabilizes NS3 by forming a complex with it and also acts as an inducer of membrane alterations. NS4B is a hydrophobic protein and is involved in the membranous web formation, a characteristic feature of HCV-infected cells. NS5A is a phosphoprotein that is capable of interacting with the 3'-UTR of the HCV genome and is involved in viral RNA replication and particle assembly. The nonstructural proteins, NS3/4A, NS4B, NS5A and NS5B, form replication complexes that generate *de novo* viral genomic RNA [11, 13-17].

The HCV life cycle can be divided into several steps, i.e., viral attachment/entry, uncoating, viral translation, viral replication, viral assembly and release of the new virion (Figure 2). In the attachment/entry step, the HCV lipoviral particles attach to the cell surface and interact with glycosaminoglycans (GAG), low-density lipoprotein receptor (LDLR), SR-B1 and CD81. Then, the viral interaction with CLDN1 results in internalization of the virus via clathrin-mediated endocytosis. Following acidification of the endosome and subsequent fusion of viral and endosomal membranes, the viral genome is released into the cytoplasm. On the other hand, the lateral movement of HCV-CD81 causes the transmission of virus by cell-to-cell contact [17, 18]. The incoming viral genome is translated through an internal ribosome entry site (IRES) that is located in the 5-UTR of the viral genome. The 5'- and 3'-UTRs contain highly structured elements that are critical for genome translation, replication and encapsidation. The IRES initiates translation of the HCV genome into a single polyprotein. Following translation and cleavage of the HCV polyprotein, the nonstructural viral proteins, NS3/4A, NS4B, NS5A and NS5B, form replication complexes, which generate new viral genome RNA molecules. The HCV nonstructural proteins, together with cellular factors, mediate the formation of a membranous web, where the HCV RNA replication takes place. The NS3/4A, NS4B, NS5A, and NS5B are the viral proteins of the replication machinery, which replicates the positive sense RNA genome though the negative strand. The NS5B viral RdRp is the primarily essential enzyme for RNA synthesis. The RNA genome is translated to produce viral proteins, and also serves as the RNA template for further RNA replication. The newly replicated viral genomes are transferred to the assembly sites, where the HCV virion morphogenesis is tightly linked to the metabolism of VLDL assembly. The viral core protein interacts with genomic RNA to form the nucleocapsid, which is then covered in the viral envelope through the viral budding into the endoplasmic reticulum (ER) lumen on the site of VLDL production. After assembly and budding into the ER, HCV particles are released from the cells through the secretory pathway [13, 14, 18, 19].

The HCV proteins, HCV-specific RNA structures as well as host factors, are important targets of anti-HCV drugs. The new generations of DAAs have been developed as anti-HCV drugs for treatment of chronic HCV infection. Telaprevir, boceprevir, simeprevir, faldaprevir, vaniprevir, asunaprevir, paritaprevir, sovaprevir and grazoprevir are the first and second generations of NS3 protease inhibitors. On the other hand, daclatasvir, ledipasvir, ombitasvir, elbasvir and velpatasvir are known as NS5A inhibitors, and sofosbuvir, beclabuvir and dasabuvir as NS5B RdRp inhibitors [10, 20]. These DAAs have been approved by the United States Food and Drug Administration (FDA) for HCV treatment in combination with pegylated IFN and ribavirin or as IFN-free regimens. The current regimens with IFN-free DAAs have increased the SVR rates and are applicable for almost all patients except for those infected with drug-resistant strains. Sofosbuvir, a nucleotide NS5B RdRp inhibitor, is approved for treatment of patients with HCV genotypes 1, 2, 3 and 4 in combination with other DAAs. Daclatasvir, an NS5A inhibitor, has been approved by FDA for HCV genotypes 1, 3 and 4. Also, a triple combination of NS3, NS5A and NS5B inhibitors (asunaprevir, daclatasvir and beclabuvir, respectively) has been shown to be effective for HCV genotypes 1 through 5 and some strains of genotype 6. Another triple combination of ritonavir-boosted paritaprevir, ombitasvir and dasabuvir was used for HCV genotype 1 [7-10, 15, 16].

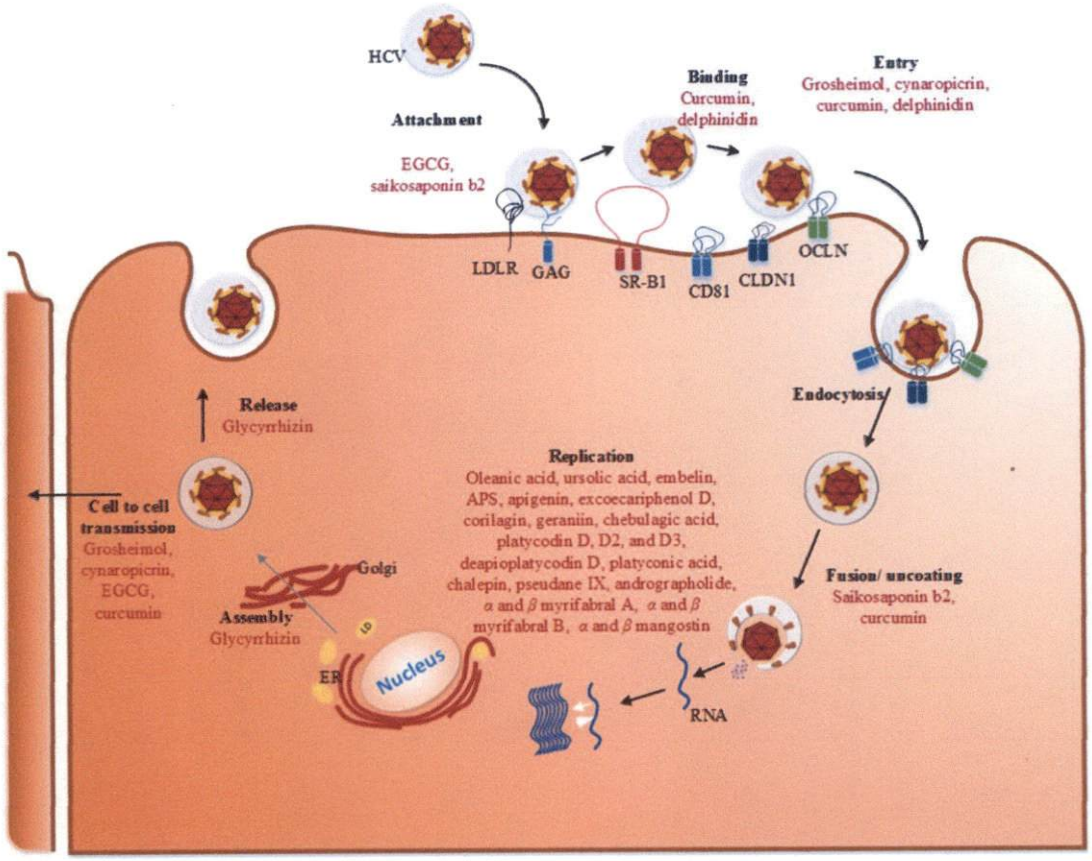

Figure 2: HCV life cycle and targets of natural compounds.

HCV infects hepatocytes by an attachment step at the cell surface and interacts with receptors on the host cells; first, interacts with glycosaminoglycans (GAG), associated with low density lipoprotein-receptor (LDL-R) and binds with entry factors, CD81, SRB1, CLDN1, and OCLN. Virus particles enter the cells via clathrin-mediated endocytosis and the low pH of the endosomal compartment induces HCV fusion. The viral genome is released into cytosol and continues for translating and polyprotein processing for RNA replication and towards virus assembly. Virus particles are assembled by recruiting E1 and E2 complexes, followed by budding into the ER, and HCV particles are released from the cells to infect new host cells.

In addition, several compounds that inhibit HCV replication by targeting host factors, such as alisporivir, a cyclophylin inhibitor, and miravirsen, a miR-122 antagonist, have also been reported [15]. Although the IFN-free DAA regimens have dramatically improved the outcome of HCV treatment, not all patients can access to the new therapy due to the high cost.

Developing anti-HCV agents from medicinal plants has currently become a significant issue. Products from medicinal plants are generally cost effective and easily available in many parts of the world. Medicinal plants contain many metabolites, both the secondary metabolites such as flavonoids, alkaloids, coumarins and polyphenol compounds, and primary metabolites such as peptides, which have been reported to possess antiviral effects including anti-HCV activities. We have summarized the medicinal plant extracts (Table 1), isolated compounds (Table 2) and their derivatives (Table 3) that inhibit HCV infection. Table 1 shows medicinal plant extracts that exert anti-HCV activities with IC_{50} values of less than 50 μg/mL. Medicinal plant extracts from Indonesia: *Toona sureni, Melicope latifolia, Melanolepis multiglandulosa, Ficus fistula* and *Ruta angustifolia* possess anti-HCV activities with IC_{50} values of 1.6 -17.1 μg/mL [21, 22]. *T. sureni* and *M. latifolia* inhibit HCV both at the entry and the post-entry steps, and decrease HCV RNA levels while *M. multiglandulosa* and *F. fistula* show significant HCV inhibition at the entry step. Moreover, these plant extracts mediated strong inhibition against almost all HCV genotypes [21]. As for the plant extracts derived from Cameroon, methanol extracts of *Trichilia dregeana, Detarium microcarpum* and *Phragmanthera capitata* were shown to possess anti-HCV activities by inhibiting the entry step, but showed no inhibition effect on viral replication and virion release [23].

Methanol and water extracts of Sudanese medicinal plants, *Boswellia carterii, Acacia nilotica, Embelia schimperi, Quercus infectoria* and *Syzygium aromaticum,* inhibited HCV protease activity by >90% at the concentration of 100 μg/mL [31]. Some traditional Chinese medicines, *Ligustrum lucidum* fruit and *Glycyrrhiza uralensis,* were also reported to have anti-HCV activities [24]. The ethyl acetate fractions of *L. lucidum* fruit mediated anti-HCV inhibition by acting on NS5B RdRp and thus blocking HCV RNA replication [25]. *G. uralensis*, commonly known as licorice, has been widely used in Chinese medicine. It contains about 20 triterpenoids and 300 flavonoid compounds. This plant was shown to decrease HCV particle release and possess an additive effect in combination with IFN-α [35, 36]. A methanol extract of *Embelia ribes* and a water extract of *Limonium sinense* root inhibit HCV infection at the entry step while extracts of *Phyllanthus amarus, Platycodon grandiflorum, Garcinia mangostana* and other medicinal plants inhibited HCV replication [26-30, 32-34]. The antiviral activities of these plant extracts might

Table 1: Plant extracts that possess anti-HCV activities.

Plant name	Plant part	Extract/ Fraction	IC_{50}	Viral target/ mechanism	Ref.
Trichilia dregeana (*Meliaceae*)	root	methanol	16.2 µg/mL	Inhibit HCV entry	[23]
Detarium microcarpum (*Caesalpinaceae*)	stem	methanol	1.4 µg/mL		
Pragmanthera capitata (*Loranthaceae*)	leaves	methanol	13.2 µg/mL		
Ruta angustifolia (*Rutaceae*)	leaves	ethanol, hexane, dichloromethane, and methanol	1.6 – 15.6 µg/mL	Inhibit HCV J6JFH1	[22]
Glycyrrhiza uralensis (*Fabaceae*)	roots	methanol	20 µg/mL	Inhibit HCV J6/JFH1	[24]
		chloroform	8.0 µg/mL		
Toona sureni (*Meliaceae*)	leaves	ethanol	2 – 13.9 µg/mL	Inhibit HCV J6/JFH1 and all HCV genotypes	[21]
Melicope latifolia (*Rutaceae*)	leaves	ethanol	2.1 – 3.5 µg/mL		
Melanolepis multiglandilosa (*Euphorbiaceae*)	stem	ethanol	6.2 – 17.1 µg/mL		
Ficus fistula (*Moraceae*)	leaves	ethanol	5.7 – 15.0 µg/mL		
Ligustrum lucidum (*Oleaceae*)	fruit	water,	10 µg/mL	Inhibit HCV NS5B RdRp and HCV replication	[25]
		ethyl acetate	11.9 – 51 µg/mL		
Platycodon grandiflorum (*Campanulaceae*)	root	water	35 µg/mL	Inhibit RNA replication against Con-1 and JFH1, and decrease NS5B level.	[26]
Embelia ribes (*Primulaceae*)	root	methanol	-	Inhibit HCV entry	[27]
Phyllanthus amarus (*Euphorbiaceae*)	root leaves	methanol	5 – 10 µg/mL	Inhibit HCV NS5B	[28]
Garcinia mangostana L (*Clusiaceae*)	fruit peels	ethanol	5.5 µg/mL	Inhibit HCV replication, decrease NS5A and ROS levels in HCV	[29]
Pinus massoniana (*Pinaceae*)	bark	-	9.6 µg/mL	Inhibit HCV replication, inhibit HCV NS3	[30]
Acacia nilotica (*Mimocaceae*), *Boswellia carterii* (*Burceraceae*), *Embelia schimperi* (*Myrsinaceae*), *Quercus infectoria* (*Fagaceae*), *Syzygium aromaticum* (*Myrtaceae*), *Piper cubeba* (*Piperaceae*)	-	methanol and water	1 – 40.5 µg/mL	Inhibit HCV protease	[31]
Limonium sinense (*Plumbaginaceae*)	root	water	9.71 µg/mL	Inhibit viral entry, attachment and fusion. Inactivate cell-free virion Inhibit virus binding to the host cell receptor.	[32]
Morinda citrifolia (*Rubiaceae*)	leaves	methanol	20.6 µg/mL	Inhibit HCV J6/JFH1	[33]
Dimocarpus longan (*Sapindaceae*)	leaves		19.4 µg/mL	Inhibit HCV primarily through a direct virucidal effect	[34]

be a sum of additive, synergistic or antagonistic effects of the mixed components of the extracts. The potential plant extracts are promising candidates as the drug of choice for alternative or complementary medicine for the treatment of HCV infection.

Further purification to obtain compounds responsible for anti-HCV activities is needed. Moreover, to increase the anti-HCV potency of the isolated compounds, structure modification to produce the semi-synthetic/derivative compounds has been made. Table 2 shows the isolated compounds from plants that exhibit anti-HCV activities, while Table 3 shows the semi-synthetic compounds. Benzoquinone compounds, embelin and 5-*O*-methylembelin, isolated from *E. schimperi*, were found to inhibit HCV protease at the concentrations of 21 and 46 µM, respectively [31]. Purification from *Maytrenus ilicifolia* and *Peperomia blanda*, Brazilian plants, yielded an alkaloid component, APS (IC_{50} 2.3 µM) and lignan compounds (IC_{50} 4.0 - 38.9 µM), that inhibited replication of HCV, including daclatasvir-resistant mutant subgenomic replicon [37]. Chalepin and pseudane IX, a coumarin and an alkaloid, both of which were isolated from *R. angustifolia*, inhibited HCV at the post-entry step and decreased the levels of HCV RNA replication and viral protein synthesis [22]. Interestingly, another alkaloid, caffeine, which is abundantly found in coffee, was shown to inhibit HCV with an IC_{50} of 0.72 mM. Caffeine acts by delaying fibrosis, and improving the function of liver cellular pathways, in addition to inhibiting HCV replication [38]. Potent alkaloids isolated from *Myrioneuron faberi*, which possess novel cyclohexane-fused oxtahydroquinolizine skeletons, inhibit HCV replication [39].

Terpenoid saponins from *P. grandiflorum*: platycodin D, D2, and D3, deapioplatycodin D, and D2 and platyconic acid A, were identified as active compounds for anti-HCV activities, which are shown to exert directly on NS5B RdRp, but did not show any inhibitory effect on NS3 protease. Animal experiments using a saponin mixture from *P. grandiflorum* revealed its half-life of 6.57 ± 0.7 h in rat and showed higher absorption from the duodenum and ileum than from the oral cavity. This might suggest that the compound could be absorbed better when used as an enteric-coated product. A clinical study using a coated product demonstrated decreased HCV titers by >2 logs after 8-weeks treatment in chronic HCV patients [26]. Other anti-HCV terpenoids that were isolated from fruits of *L. lucidum* are oleanic acid and ursolic acid. These compounds inhibit HCV replication and NS5B RdRp activity. A combination of oleanic acid and ursolic acid with IFN-γ significantly reduced HCV NS5A protein expression [25]. Andrographolide, a diterpenoid lactone from *Andrographis paniculata*, was identified to inhibit HCV replication by targeting host factors. This compound interferes with HCV replication by activating p38 MAPK phosphorylation, which stimulated nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated heme oxygenase-1 gene expression. A combination of andrographolide with IFN-α, telaprevir or PSI-7977 (NS5B inhibitor) revealed a synergistic effect [40]. On the other hand, flavonoid components also offer a bright part of the anti-HCV agent. Several flavonoids have been identified to inhibit HCV infection. Epigallocatechin-3-gallate (EGCG), which is present in green tea (*Camellia sinensis*), was reported to inhibit the HCV entry step in cell-to-cell transmission [41,66]. The structure-activity relationship demonstrated that the gallate ester hydroxyl groups are not essential for anti-HCV activity. However, the hydroxyl groups from the B-ring of EGCG play an important role in binding to HCV during the attachment/entry step [42]. A recent report described EGCG significantly enhancing HCV dsRNA-induced expression of IFN-λ1, Toll-like receptor 3 (TLR3), a retinoic acid-inducible gene I (RIG-I) and antiviral IFN-stimulated genes (ISGs), the important host factor(s) for intracellular innate immunity in hepatocytes [43]. A clinical trial study of healthy volunteers with an oral dose of 800 mg of EGCG per day over four weeks, which equals 8-16 cups of green tea, showed no toxic effects, however, this dose was not enough to eliminate HCV completely due probably to its poor *in vivo* bioavailability [41, 44]. Another natural compound, delphinidin, which has a similar molecular structure to EGCG, was identified as an anti-HCV entry inhibitor. Delphinidin acts directly on the virus particle and impairs viral attachment to the cell surface. This compound inhibits HCV

entry more efficiently than EGCG at the concentration of 50 μM. The IC_{50} value of delphinidin was 3.7 ± 0.8 μM, while that of EGCG was 10.6 ± 2.9 μM. Other polyphenol compounds, tricetinidin chloride, cyanidin chloride and myricetin, showed only moderate inhibition against the JFH1 strain of HCV. It was considered that trihydroxyphenyl and hydroxyl moieties in the benzene ring of flavonoids have an important contribution for their anti-HCV activities. The combination between delphinidin (5 and 10 μM) with IFN-α and boceprevir significantly potentiated the activity of IFN-α and boceprevir. The addition of delphinidin at the concentration of 5 μM potentiated the anti-HCV activity of boceprevir up to 5 fold (IC_{50} values from 0.15 μM to 0.03 μM) and that of IFN-α by10 fold (IC_{50} values from 6.30 IU/mL to 0.59 IU/mL). It was also observed that delphinidin and EGCG altered the morphology of HCV pseudoparticles (HCVpp) due possibly to a direct effect on the surface of HCVpp, including the E1/E2 glycoproteins [45]. Other isolated compounds that were reported to inhibit the entry step are grosheimol and cynaropicrin, isolated from wild Egyptian artichoke, which possess IC_{50} values between 0.4 and 4 μM. These compounds act directly on the viral particle and may prevent the virus-receptor interaction [46]. Currently, some of the isolated compounds from medicinal plants with anti-HCV activities are under clinical studies, such as naringenin, which is reported to be in phase 1 clinical study, and silymarin/silibinin, which is in phase 2/3 [18]. Both of them are bioflavonoid compounds that exert inhibition against HCV. Naringenin blocked NS5A-driven IRES-mediated translation of the viral genome [47]. Silymarin is a seed extract of *Silybum marianum*. This extract consists of eight flavonolignans, silybin A (16%), silybin B (24%), isosilibin A (6%), isosilybin B (4%), silydianin (16%), silychristin (12%), isosilychristin (2%), and taxifolin (2%). Monotherapy with oral administration of silymarin mediates little effect on viral enzymes and viral loads. Therefore, silymarin is used as a botanical medicine for complementary or alternative treatment and the impact of oral silymarin in combination with IFN-α, ribavirin or DAAs should be investigated [48, 49]. A randomized clinical trial of silymarin for patients with chronic HCV infection found that oral silymarin administration did not exert any significant effect on alanine amino transferase (ALT) and HCV RNA levels compared with the placebo/control group [50]. The impact of the anti-HCV activities of both silymarin and EGCG was relatively weak compared with the other DAAs in the *in vitro* culture cells [51]. Studies to improve the activity and bioavailability of these compounds are still ongoing. Another study of intravenous administration of silibinin (purified compound of silymarin) during the peri-transplant period evidenced antiviral properties that decreased viral load [52]. Ladanein, quercetin, and apigenin are other flavonoid compounds possessing anti-HCV activities [53]. Apigenin inhibits HCV replication and decreases the expression level of miR-122 [54].

Some polyphenols, such as curcumin, resveratrol, exoecariphenol D and corilagin, were found to have potential as anti-HCV substances [57,65]. Curcumin is a diarylheptanoid that possesses two phenol moieties. Curcumin was reported to act as an anti-inflammatory by suppressing pro-inflammatory cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and CXCL8.

It also activates antioxidant responses by activation of transcription factor, nuclear factor erythroid-derived 2-like 2 (Nrf2), which induces the expression of heme oxygenase 1, glutathione S-transferase (GST), and NAD(P)H-quinone oxidoreductase 1. These effects were modulated by other transcription factors, including NF-κB, β-catenin, and signal transducer and activator of transcription 3 (STAT3) [55, 56]. Curcumin inhibits the entry step of all HCV genotypes. However, it has no effect on HCV replication and assembly. Curcumin influences the fluidity of the HCV envelope and impairs viral binding and fusion. The membrane fluidity is controlled by anisotropic rotation of the phospholipid acyl chain and flip-flop movement of membrane molecules that affects membrane rigidity. Curcumin penetrates into the membrane and changes its rigidity. Curcumin also interferes with cell-to-cell transmission of HCV. Two of the curcumin derivatives, desmethoxycurcumin and bis-desmethoxycurcumin, also exert anti-HCV activity. However, the main metabolite of curcumin, tetrahydrocurcumin, did not show any significant inhibitory effect against HCV, indicating that the different structures on α- and β- unsaturated ketone groups play an importance role in anti-HCV activities [57]. Curcumin is an attractive antiviral candidate with a safety profile in humans and low cost. Phase 1 clinical study demonstrated no treatment-related toxicity. Animal experiments showed an improved bioavailability of curcumin when administered as nanoparticle and nanocrystal formulations [58]. Other polyphenols that inhibit HCV replication are α-mangostin and γ-mangostin, isolated compounds from *Garcinia mangostana*. Both of them reduced HCV RNA and NS3/NS5A protein levels. An ethanol extract of mangosteen was shown to decrease the reactive oxygen species (ROS) level in HCV-infected cells, while not inhibiting NS5B RdRp and IRES-dependent translation [29]. Novel derivatives of quercetin, 7-*O*-arylmethylquercetin and quercetin-3-*O*-benzoic acid ester, exhibited anti-HCV activities. Compound 3i, which has a 3-chlorobenzyl substitution in 7-*O*-arylmethylquercetin, showed stronger activity (IC_{50}: 3.8 μM) than that with substitution of carboxyl groups at quercetin-3-*O* (IC_{50}: 9.0 μM). This quercetin analog exerted potent inhibition of HCV NS5B RdRp activity through chelation of magnesium ions at the active site [59]. Other derivatives such as imidazo derivatives were also potent inhibitors of HCV [60, 61]. Compound 5o, an imidazo[1,2-α][1,8]naphthyridine derivative, revealed promising pharmacokinetics in rat at the concentration of 100 mg/kg [60]. A safe pharmacokinetic profile was also observed with MK-8831, which showed a bioavailability of 31% in rat and 17% in dog [62, 63]. A series of phenylalanine-based macrocyclic inhibitors, boronate-based inhibitors, phenylglycine-based inhibitors, α-ketoamide-based inhibitors, sulfonamide-capped inhibitors, indole derivatives, acridone derivatives and oleanolic acid derivatives have been designed as HCV NS3 protease inhibitors. Those NS3 protease inhibitors are considered as peptidomimetics, which mimic cleavage products (peptides) and inhibit the enzyme activity. Also, macrocyclic α-ketoamides were found to be potent HCV NS3 protease inhibitors [64]. Other semi-synthetic compounds possessing Ant-HCV activities are listed in Table 3.

Clemizole, anguizole and their structurally related compounds, *N*-(4-indol-2-yl)phenyl) sulfonamides, 6-(indol-2-yl)pyridine-3-sulfonamides and piperazine derivatives were developed as NS4B inhibitors. These compounds inhibit HCV replication by preventing either NS4B RNA binding activity or membranous web formation [74].

In conclusion, diverse natural resources, including medicinal plant extracts and their isolated compounds, interfere with HCV replication at different steps of the HCV life cycle. Many of the natural resources, especially herbal medicines, have been used for traditional medicine, with relatively less side effects and lower cost. The compounds described in this review article could be promising candidates for anti-HCV drugs. Increasing the antiviral potency by modification of the molecular structures would be an important strategy for developing clinically useful anti-HCV drugs. Also, *in vivo* study is necessary for drug development. Further studies on mechanisms of action, efficacy, pharmacokinetics and safety, both *in vitro* and *in vivo*, are greatly needed.

Table 2: Anti-HCV compounds isolated from medicinal plants.

Compound	Plant name (plant part)	IC_{50}	Viral target	Ref.
Embelin 5-*O*-Methyl embelin	*Embelia schimperi* (fruit)	21 μM 46 μM	Inhibit HCV NS3 protease	[31]
Chalepin Pseudane IX.	*Ruta angustifolia* (leaves)	1.7 μg/mL 1.4 μg/mL	Inhibit HCV replication and decrease the NS3 protein level	[22]
Oleanolic acid Ursolic acid	*Ligustrum lucidum* (fruit)	0.8 - 3.5 μg/mL 3.1 - 19.2 μg/mL	Inhibit HCV replication, HCV NS5B RdRp	[25]
APS (alkaloid)	*Maytrenus ilicifolia* (root bark)	2.3 μM	Inhibit HCV replication and decrease the NS5A level	[37]
Compound 3*43, 3*20 and 5*362	*Peperomia blanda* (aerial)	4.0, 8.2, 38.9 μM respectively.		
Glycycoumarin Glycerin, Glycerol	*Glycyrrhiza uralensis* (roots)	4.6 - 8.8 μg/mL	Inhibit post-entry step, Inhibit HCV NS3 protease	[24]
Excoecariphenols D Corilagin, Geraniin and Chebulagic acid	*Excoecaria agallocha* L.	3.4 - 9.0 μM	Inhibit HCV NS3-4A protease and RNA replication	[65]
Platycodin D, D2, and D3; Deapioplatycodin D, and D2; Platyconic acid A	*Platycodon grandiflorum* (roots)	0.35 - 2.45 μg/mL	Inhibit RNA replication against Con-1 and JFH1 Inhibit NS5B but not NS3 protease level	[26]
Grosheimol Cynaropicrin	*Cynara cardunculus* (leaves)	0.4 - 1.4 μM	Inhibit HCV pan-genotypes Inhibits entry step by cell to cell transmission. Directly act on the virus particle and prevent virus-receptor interaction.	[46]
Saikosaponin b2	*Bupleurum kooi/ Embelia ribes* (roots)	16.1 μM	Inhibit HCV entry, neutralization of virus particle, attachment, and fusion. Bind to E2 and disrupt E2-CD81interaction.	[27]
β-Myrifabral A α-Myrifabral A β-Myrifabral B α -Myrifabral B and their derivatives	*Myrioneuron faberi* (aerial)	0.9 - 4.7 μM	Inhibit HCV replication	[39]
α-Mangostin γ-Mangostin	*Garcinia mangostana* (fruit peels)	6.3 μM 2.7 μM	Inhibit HCV replication and decrease the NS3 and NS5A levels	[29]

Table 3: Semi-synthetic compounds and other anti-HCV agents from existing medicines.

Compound (plant origin)	IC_{50}	Viral target/ mechanism	Ref.
Glycyrrhizin (*Glycyrrhiza uralensis*; root)	16.5 μM	Inhibit HCV assembly and release by inhibiting phospholipase A2 (PLA2) and HCV NS3 protease	[24, 35, 36]
Andrographolide (*Andrographis paniculata*; aerial)	5.1 – 6 μM	Inhibit HCV replication by up-regulating the heme oxygenase-1 gene via the p38 MAPK/Nrf2 pathway	[40]
EGCG (*Camellia sinensis*; leaves)	5 – 21 μM	Inhibit HCV entry steps (attachment) Interfere with E1/E2 glycoprotein Enhance intracellular innate immunity against HCV	[42, 43, 45, 66]
Delphinidin (anthocyanidin in plant pigment)	3.7 μM	Inhibit HCV in the early step of entry (E1E2 glycoprotein)	[45]
Curcumin, Desmethoxycurcumin, Bis-desmethoxycurcumin (*Curcuma* species; rhizome)	8.46 μM	Inhibit HCV entry by affecting membrane fluidity Inhibit cell-to-cell transmission	[55-57]
Apigenin	50 μM	Inhibit HCV virus replication by decreasing miR22 expression level	[54]
Caffeine (*Coffea* species)	0.7 mM	Inhibit genotype 2a HCV replication	[38]
Licochalcone-A (*Glycyrrhiza* species; root and rhizome)	2.5 μg/mL	Inhibit HCV, dominantly acts in the post entry step.	[24, 36]
Glabridin (*Glycyrrhiza* species; root and rhizome)	6.3 μg/mL		
Isoliquiritigenin (*Glycyrrhiza* species; root and rhizome)	16.4 μg/mL		
5-Carba-pterocarpens derivatives	1.5 –5.5 μM	Inhibit HCV replicon and decrease HCV NS3 protease	[67]
7-*O*-Arylmethylquercetin derivate (compound 3i)	3.8 μM	Inhibit HCV RdRp	[59]
Quercetin-3-*O*-benzoic acid ester derivative (compound 4f)	9.0 μM		
Imidazo [2,1-b]thiazole derivative (compound 26f and 28g)	16 nM and 31 nM, respectively	Inhibit HCV NS4B (inhibit the second amphipathic α-helix of NS4B(4BAH2)	[61]
Imidazo[1,2-α][1,8]naphthyridine (compound RO81991) derivative (compound 5o)	0.017 – 0.159 μM	Inhibit HCV entry step	[60]
2-(4-sulfonamidophenyl)-indole 3 carboxamides	7 nM (GT-1a) and 2 nM (GT-1b)	Inhibit HCV NS4B	[68]
MK-8831	0.004 – 3.4 nM	Inhibit HCV-NS3/4a protease	[63]
MK-4882	0.001 – 0.4 nM	Inhibit HCV genotype 1a, 1b and 2a, potent NS5A inhibitor	[62]
Flunarizine	0.38 μM	Inhibit HCV entry by inhibiting membrane fusion (E1 and/or E2)	[69-71]
Fluphenazine, trifluopenazine and pinozide	0.5 – 1.0 μM	Inhibit entry step (E1 and/or E2)	[69]
Chloroquine (*Cinchona succirubra*; bark)	3.93 μM	Inhibit HCV entry by impairing endosome-mediated virus entry	[72, 73]
Ferroquine	0.26 – 0.85 μM	Inhibit all HCV genotype; inhibit entry step/fusion (E1)	[71, 73]

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