

Promising alkaloids and flavonoids compounds as anti-hepatitis c virus agents: a review

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Abstract

Background: Virus infections are presently seen as a major public health problem. Hepatitis C Virus (HCV) is recognized as a “silent killer” because the acute infection has no symptoms, and it develops as a chronic infection that causes hepatocellular carcinoma and liver damage. The World Health Organization (WHO) predicts that between 130-170 million people are estimated to have chronic Hepatitis C. Plants have various phytochemical compounds such as alkaloids and flavonoids that have prominent

antiviral effects especially anti-HCV. The current HCV treatment still has limitations related to side effects and can lead to viral resistance. Therefore, it is necessary for the discovery and development of novel anti-HCV drugs for alternative and complementary medicine.

Objective: This review intends to evaluate the alkaloids and flavonoids that have the potential to be used against HCV by looking at their classification and their mechanism of action.

Methods: Twenty-one articles from 2010 to 2022 obtained from PUBMED database using keywords such as isolated compounds, alkaloids, flavonoids, hepatitis C virus.

Results: 21 alkaloids and 37 flavonoids reported active against HCV. Alkaloids include quinoline, quinolizidine and isoquinoline. In addition, flavanone, flavonol, flavone, flavan-3-ol, flavonolignan, anthocyanidin and proanthocyanidin comprise flavonoids. The berberine alkaloids and eriodictyol 7-O-(6"-caffeoyl)-β-D-glucopyranoside flavonoids had the lowest IC₅₀ with values of 0.49 mM and 0.041 nM.

Conclusions: Alkaloids and flavonoids compound had good activity against HCV with various mechanisms. Our results provide information of alkaloids and flavonoids to the researcher for the development of alternative and complementary medicine of hepatitis C.

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Introduction

Virus infections are presently seen as a major public health problem. Hepatitis C (HCV) is the best-known of viral disease.¹ HCV is known as the “silent killer” because the acute infection has no symptoms and it develops as a chronic infection that causes hepatocellular carcinoma and liver damage before it's detected.² World Health Organization (WHO) predicts that between 130-170 million people are estimated to have chronic HCV.³

HCV is a single-strand RNA virus from Hepacivirus and Flaviviridae with a single strand with a 50-80 nm diameter⁴ and consists around a 9.6 kb positive-stranded RNA genome located between 5'NCR and 3'NCR, a lengthy active reading frame containing around 3000 amino acids that encodes 10 viral proteins consisting of structural proteins (Core, E1, E2) and non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B).⁵

Therapeutic agents for anti-HCV has been used Direct-Acting Antivirals (DAAs), but still has limitations related to side effects and can lead to viral resistance. Therefore, it is necessary for the discovery and development of novel anti-HCV drugs for alternative and complementary medicine.

Materials and Methods

The current study identified alkaloids and flavonoids as alternative and complementary treatments for hepatitis C *in vitro*, the articles used were obtained from the PUBMED database. The inclusion criteria used for extracting review were: i) the articles was published from 2010 to 2022 in English language; ii) isolated compounds, alkaloids, flavonoids, hepatitis C virus, were used as important keyword. The exclusion criteria were: i) the articles published prior to 2010; ii) articles published in languages other than English. A total of 21 articles were retrieved and analyze in this study.

Potential target on hepatitis C treatment

HCV genome provide structural and non-structural protein which play attractive target for therapeutic agents. The viral proteins, NS3, NS4A, NS4B, NS5A and NS5B proteins are necessary for HCV replication and is an NS3/4A plays a crucial role in producing proteins for viral maturity and infectivity by cleaving the viral polyprotein at four sites.⁶ NS3/NS4A inhibitors prevent HCV polyprotein cleavage by inhibiting the NS3/NS4A interaction or NS3 catalytic site.⁷

NS5A protein is essential for replication and assembly. NS5A consists of three domains, domain I assumed to be solely responsible for genome replication. In contrast, domains II and III are needed to form infectious virus particles.⁸ The interaction between inhibitors and NS5A could lead to conformational changes in NS5A and the creation of non-functional replication complexes.⁹

NS5B, known as RNA dependent RNA polymerases (RdRPs), are the catalytic components of the RNA replication and transcription transcribes viral RNA to facilitate protein translation and the formation of progeny genomes.¹⁰ NS5B inhibitors bind to catalytic sites known as nucleoside polymerase inhibitors (NPIs) as well as non-nucleoside analogs known as non-nucleoside polymerase inhibitors (NNPIs).¹¹

Current anti-hepatitis C drugs

Therapeutic agents for anti-HCV have been using DAAs. It has received more attention because its mechanism of action is to directly inhibit HCV non-structural proteins leading to increase Sustained Virological Response (SVR). DAAs are divided in 3 groups that inhibit non-structural proteins of HCV, including protease inhibitors, replication complex inhibitors and polymerase inhibitors, moreover non-nucleotide inhibitor.¹²

Natural sources as anti-hepatitis C agents

More than 50,000 plant species are utilized in pharmaceuticals and health products, which is more than one-tenth of all plant species that have been identified.¹³ 50% of FDA-approved medicines are from natural sources, the structures and biological activity of secondary metabolites derived from natural sources are various.^{14,15}

Natural sources have potential in the drug discovery of infectious diseases.^{16,17} Numerous studies have been conducted to determine the antiviral efficacy of natural sources. Historically alkaloids represent the largest antiviral category with a broad spectrum of antiviral properties¹⁸ and flavonoids represent the largest groups of secondary metabolites, since first report in 1938 were described as a broad spectrum that have antiviral activity.¹⁹ Based on clinical trial, alkaloid oxymatrine inhibit replication of HBV patients and flavonoid silymarin effective as an adjunct therapy for HCV patients.^{20,21}

Alkaloids compounds as anti-hepatitis C agents

Alkaloids are nitrogen-containing compounds and the most abundant types of natural products. The name alkaloid is derived from its fundamental properties (alkali-like), caused by the presence of one or more nitrogen atoms often in heterocycles.²²

We reported some alkaloid compounds with their anti-HCV activities (Table 1 and Figure 1). Based on the data obtained, it's showed that quinoline and quinolizidine alkaloids inhibit the replication of HCV. Quinoline alkaloids have the potential to aid in the creation of safe and efficient anti-HCV agents. Moreover, isoquinoline has a different mechanism by interacting with the structural proteins E1/E2 of HCV.

Quinolines

Isolation of N-methylflindersine (1) from the fruits of *Melicope latifolia* exhibits anti-HCV action with an IC₅₀ value of 3.8±2.7 mg/ml and the mechanism were inhibit post-entry and decreased NS3 protein expression level.²³ Isolation of twigs and leaves of *Flueggea virosa* yielded (-)-securinine (2) and (-)-norsecurinine (3) that active against HCV with IC₅₀ values of 7.7 mM and 27.6 mM, respectively.²⁴ Isolation of three quinoline alkaloids, Myritonine A (4), B (5), and C (6) from the leaves and stems of *Myrioneuron tonkinensis* active as anti-HCV with IC₅₀ values of 16.27±1.49 mM, 17.72±4.20 mM and >66.70 mM respectively.²⁵ Isolation of leaves and stems *Myrioneuron faberi* yielded Myrifamine A (7), B (8) C (9), Myrionamide (10) and Schoberine (11). All of these compounds has IC₅₀ values of 0.92±0.26 to 30.61±2.44 mM.²⁶ APS (12) is the first alkaloid compound of *Maytrenus ilcifolia* root bark that has been reported to inhibit HCV replication with IC₅₀ values 2.3 mM.²⁷ Four alkaloids (γ -Fagarine) (13), Arborinine (14), Kokusaginine (15) and Pseudane IX (16) from *Ruta angustifolia* leaves have been isolated and identified. All of these compounds inhibit HCV replication with IC₅₀ values 20.4±0.4 mg/ml, 6.4±0.7 mg/ml, 6.4±1.6 mg/ml and 1.4±0.2 mg/ml, respectively. Moreover, Pseudane IX(16) inhibit HCV at the post-entry step and decreased the HCV RNA replication and viral protein synthesis.²⁸

Quinolizidines

Leaves and stems of *Myrioneuron faberi* yielded four pairs of enantiomers. (\pm)- β -Myrifabral A and (\pm)- α -myrifabral A generated an inseparable anomer mixture (cluster A) (17), as did (\pm)- β -myrifabral B and (\pm)- α -myrifabral B (cluster B) (18). These compounds inhibit HCV replication with IC₅₀ values of 4.7 mM and 2.2 mM, respectively.²⁹ A novel alkaloid, myriberine A (19) was isolated from the leaves and stems of *Myrioneuron faberi*. The structure is similar to the matrine alkaloids, myriberine A (19) inhibit HCV with IC₅₀ values of 8.49±0.2 mg/ml.³⁰ Aloperine (20) inhibit HCV in Huh7it hepatocytes with IC₅₀ values of 7.06±2.17 mM and also inhibit primary human. The mechanism inhibits HCV entry, replication, and cell-to-cell transmission by interfering with the endocytosis-to-membrane fusion pathway.³¹

Isoquinolines

Berberine (21) inhibit HCV attachment, entry/fusion and E1/E2 with an IC₅₀ value of 0.49±0.07 mM. Based on molecular docking, the average binding energy for E1 and E2 were 6.6 kcal/mol and 6.4 kcal/mol, respectively.³²

Flavonoids compounds as anti-hepatitis C agents

Flavonoids are characterized by the presence of flavan nucleus and are known as C6–C3–C6 phenolics.¹⁹ It has been extensively acknowledged to have antiviral and antibacterial properties.³³ Activities of flavonoids were frequently correlated with the kinds

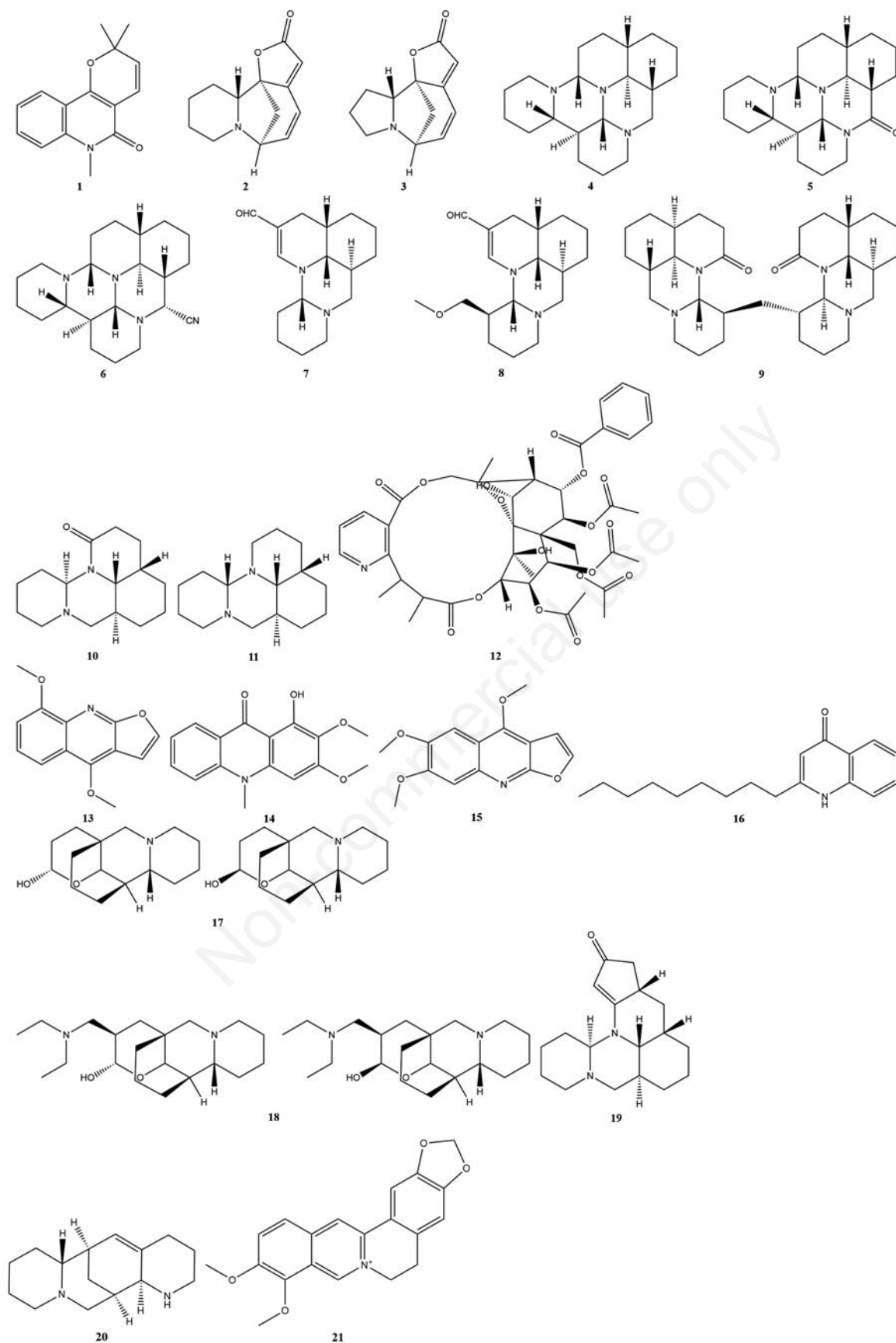


Figure 1. Molecular structure of alkaloids that possess anti-hepatitis C virus activity.

and numbers of functional radical groups present in the chemical structures of various molecules.³⁴ Various flavonoid compounds and its molecular structure were demonstrated to possess anti-HCV activities (Table 2 and Figure 2).

Flavanones

Hesperidin (22), was identified as the main flavonoid of Citrus

extract inhibit NS3-NS4A protease with IC₅₀ value of 11.34±3.83 ng/ml. Molecular docking study found, it inhibit NS3 protease by establishing hydrogen bonds with active site residues (His57, Asp81 and Ser139).³⁵ Isolation from *Taxillus sutchuenensis* herba identified isosakuranetin (23), homoeriodictyol-7-O-β-D-glucopyranoside (24) and viscumneoside I (25). These compounds inhibit NS3 protease with IC₅₀ values of 234.4±2.2 mM, 68.9±0.4 mM

Table 1. Alkaloid compounds demonstrated anti-hepatitis C virus activities.

| Subclass | Compound | Source | IC ₅₀ | Target | Ref |
|---------------|--|---|------------------|---|------|
| Quinoline | N-merhyflindersine (1) | <i>Melicope latifolia</i> (Fruits) | 3.8±2.7 µg/ml | HCV replication and suppressed NS3 expression | (23) |
| | (-) securinine (2) | <i>Froggeniopsis virosa</i> (Twigs and Leaves) | 7.7 µM | HCV replication | (24) |
| | (-) noursecurinine (3) | | 27.6 µM | | |
| | Myritonine A (4) | | 16.27±1.49 µM | | |
| | Myritonine B (5) | <i>Myrionanthe tonkinensis</i> (Leaves and Stems) | 17.72±1.30 µM | HCV replication | (25) |
| | Myritonine C (6) | | >66.70 µM | | |
| | Myrilamine A (7) | | 3.27±2.21 µM | | |
| | Myrilamine B (8) | | 0.92±0.26 µM | | |
| | Myrilamine C (9) | <i>Myrionanthe faberi</i> (Leaves and Stems) | 10.52±3.14 µM | HCV replication | (26) |
| | Myrionamide (10) | | 30.61±2.44 µM | | |
| | Schoberine (11) | <i>Maytenus ilicifolia</i> (Root bark) | 7.35±0.56 µM | HCV replication | (27) |
| | APS (12) | | 2.3 µg/ml | | |
| | γ-Fagarine (13) | | 20.4±0.4 µg/ml | | |
| | Arborinine (14) | | 6.4±0.7 µg/ml | | |
| | Kokusaginine (15) | | 6.4±1.6 µg/ml | | |
| | Pseudane IX (16) | <i>Rhia angustifolia</i> (Leaves) | 1.4±0.2 µg/ml | HCV replication and NS3 protein synthesis | (28) |
| Quinolizidine | (+)-β-Myrifabral A & (-)-α-Myrifabral A (cluster A) (17) | <i>Myrionanthe faberi</i> (Leaves and Stems) | 4.7 µM | HCV replication | (29) |
| | (-)β-Myrifabral B & (=)-α-Myrifabral B (cluster B) (18) | | 2.2 µM | | |
| | Myribenine A (19) | <i>Myrionanthe faberi</i> (Twigs and Leaves) | 8.49±0.2 µg/ml | HCV replication | (30) |
| | Aloperine (20) | Commercial | 7.06±2.17 µM | HCV entry, replication and cell-to-cell transmission through disturbing internalisation from endocytosis to the membrane fusion process | (31) |
| Isoquinoline | Berberine (21) | Commercial | 0.49±0.07 µM | HCV attachment, entry/fusion and E1/E2 Glycoprotein | (32) |

Table 2. Flavonoids compounds demonstrated anti-hepatitis C virus activity.

| Subclass | Compound | Source | IC ₅₀ | Target | Ref |
|------------------|--|--|------------------|--|------|
| Flavanone | Hesperidin (22) | Commercial | 11.74–3.83 µg/ml | NS3-NS4A Protease | (35) |
| | Isosakuranetin (23) | <i>Foxitilus sachalinensis</i> (Herba) | 7.14–7.2 µM | NS3 Protease | (35) |
| | Hemioctodicytol-7-O-β-D-glucopyranoside (24) | | 68.9–0.4 µM | | |
| | Viscumnolide T (25) | | 62.3–2.0 µM | | |
| | Eriodictyol 7-O-(6'-acetyl)-β-D-glucopyranoside (26) | <i>Eriodictyon bodinieri</i> (Aerial) | 0.041 nM | HCV Replication | (37) |
| Flavanol | Rhamnazin 3-O-di-rhamnosyl-glucoside (27) | <i>Sarcocornia frutescens</i> (Leaves) | 8.9–2.6 µM | NS3 Protease | (38) |
| | Rhamnazin 3-O-rutinoside (28) | | 14.3–3.7 µM | | |
| | Rhamnazin 3-O-rhamnosyl-galactoside (29) | | 77.2–5.5 µM | | |
| | Isorhamnetin 3-O-rhamnosyl-galactoside (30) | | 67.6–4.8 µM | | |
| | Isorhamnetin 3-O-di-rhamnosyl-galactoside (31) | | 47.5–6.3 µM | | |
| | Kaempferol-3,7-hishramnoside (32) | <i>Foxitilus sachalinensis</i> (Herba) | 19.4±0.5 µM | | (35) |
| Flavone | Sorbifolin (33) | <i>Pteropogon idios</i> (Leaves) | 37.93 µM | HCV Entry by direct action on virus particles | (39) |
| | Pedalirin (34) | | 51.97 µM | | |
| | Diosmetin 7-O-β-glucoside-2"-sulphate (35) | <i>Parsonsia leopoldi</i> | 16 µM | NS3-NS4A Protease | (40) |
| | Diosmetin 7-O-β-glucoside (36) | | 37 µM | | |
| | Quercetin 3,3',4'-trimethyl ether (37) | <i>Taxillus satchinensis</i> (Herba) | 154.6–1.9 µM | NS3 Protease | (35) |
| Flavan-3-ol | Luteolin (38) | <i>Eriodictyon bodinieri</i> (Aerial) | 0.56 nM | HCV Replication | (37) |
| | Ladanin (39) | <i>Astragalus pterocarpus</i> (Aerial) | 2.79 µmol/L | HCV entry at post-binding | (41) |
| | EGCG (40) | Commercial | 10.6–2.9 µM | HCV entry, modify the morphology of HCVpp | (43) |
| | Silibinin (Silibinin A & Silibinin B) (41) | <i>Silybum marianum</i> (Seeds) | 34.3 µM | HCV fusion and entry by slowing down trafficking through clathrin-coated pits and vesicles | (47) |
| | Silibinin A (42) | | 25–3 µM | | |
| | Silibinin B (43) | | 29–4 µM | | |
| | Isosilibinin A (44) | | 15–3 µM | | |
| | Isosilibinin B (45) | | 31–4 µM | | |
| | Silicristin (46) | | 53–3 µM | | |
| | Isosilicristin (47) | | 81–2 µM | | |
| Flavonolignan | Silidanin (48) | | 100–3 µM | | |
| | Taxifolin (49) | | 60–3 µM | | |
| | Silibinin A (50) | Commercial | 96.4±15.9 µM | NS5B polymerase | (45) |
| | Silibinin B (51) | | 97.2±21.1 µM | | |
| | Silibinin (Silibinin A & Silibinin B) (52) | | 82.0±22.3 µM | | |
| | Isosilibinin A (53) | | 211.6–34.9 µM | | |
| | Isosilibinin B (54) | | 165.5–17.4 µM | | |
| | Silicristin (55) | | > 500 µM | | |
| Silidanin (56) | > 500 µM | | | | |
| | | | | | |
| Anthocyanidin | Delphinidin (57) | | 3.7–0.8 µM | HCV entry at the binding step, modify the morphology of HCVpp | (43) |
| Proanthocyanidin | Procyanidin A (58) | <i>Cissampelos zebrina</i> (Bark) | 2.06 µM | HCV Entry at post-binding | (46) |

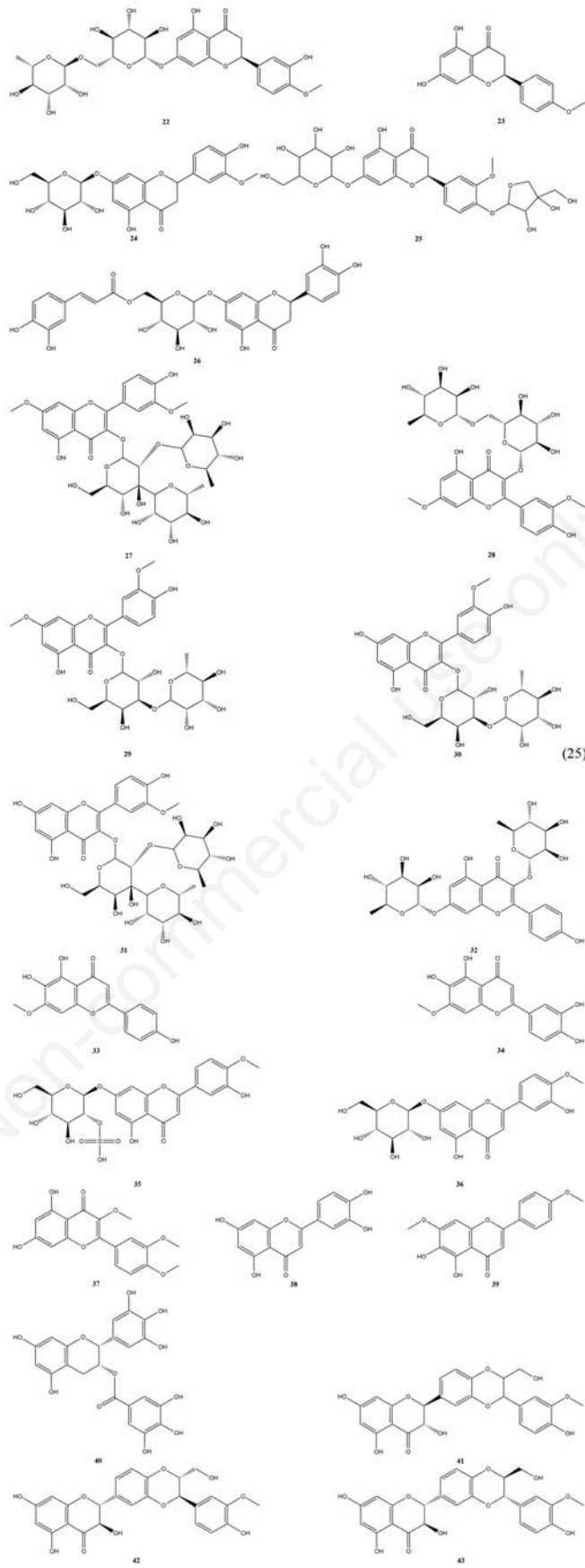


Figure 2. Structure of flavonoids compounds with anti-hepatitis C virus activities

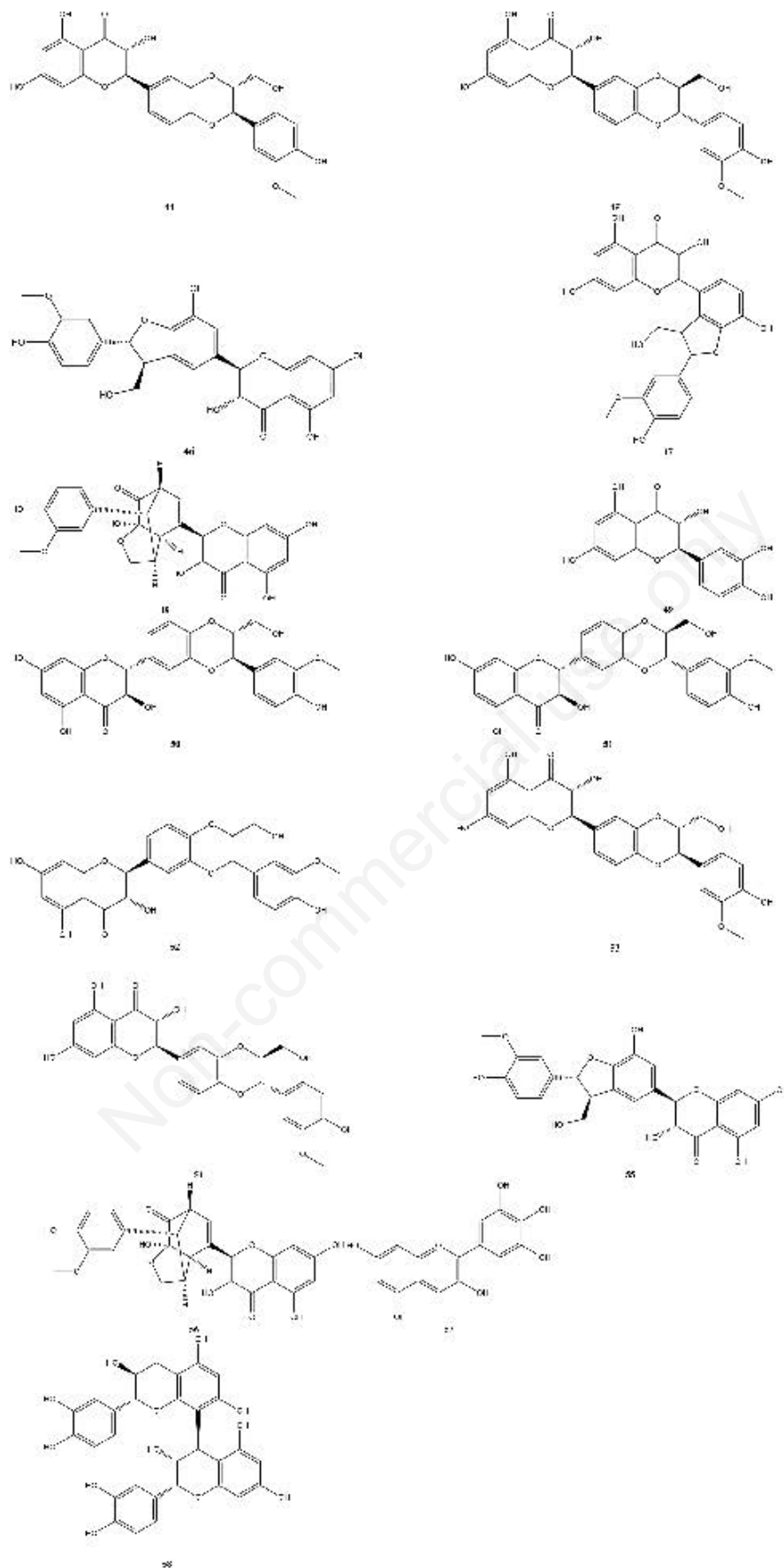


Figure 2. Structure of flavonoids compounds with anti-hepatitis C virus activities

and 62.3±2.0 mM, respectively.³⁶ Isolation of aerial parts of *Elsholtzia bodinieri* yielded flavonoid glycoside, eriodictyol 7-*O*-(6''-caffeoyl)-β-D-glucopyranoside that inhibit HCV replication with IC₅₀ values of 0.041 nM.³⁷

Flavonols

Rhamnazin 3-*O*-di-rhamnosyl-glucoside (27), rhamnazin 3-*O*-rutinoside (28), rhamnazin 3-*O*-rhamnosyl-galactoside (29), isorhamnetin 3-*O*-rhamnosyl-galactoside (30) and isorhamnetin 3-*O*-di-rhamnosyl-galactoside (31) were isolated from leaves of *Sarcocornia fruticosa* inhibit NS3 protease with IC₅₀ values of 8.9±2.6 mM to 67.6±4.8 mM.³⁸ *Taxillus sutchuenensis* herba not only contains flavanones, but also contains flavonol kaempferol-3,7-bisrhamnoside (32) that inhibit HCV NS3 protease with IC₅₀ value of 19.4 ± 0.5 mM.³⁶

Flavones

Sorbifolin (33) and pedalitin (34) isolated from *Pterogyne nitens* leaves has virucidal activity. Nevertheless, pedalitin (34) inhibits interference on the host cells and direct on viral particles. IC₅₀ values of these compounds of 37.93 mM and 51.97 mM, respectively.³⁹ A flavonoid compound diosmetin 7-*O*-β-glucoside-2''-sulphate (35) and Diosmetin 7-*O*-β-glucoside (36) has been isolated from *Thalassia hempricii* inhibit HCV NS3-4A protease with IC₅₀ values of 16 and 37 mM, respectively.⁴⁰ Quercetin 3,3',4'-trimethyl ether (37) isolated from *Taxillus sutchuenensis* herba inhibit HCV NS3 protease with IC₅₀ value of 154.6±1.9 mM.³⁶ Isolation of aerial parts of *Elsholtzia bodinieri* yielded luteolin (38). It inhibit HCV replication with IC₅₀ value of 0.56 nM.³⁷ Ladanein (39) was identified from aerial parts of *Marrubium peregrinum*. Ladanein was synthesized to compare the activity between ladanein form natural source and synthetic ladanein. These compounds suppress HCV genotype 2a infection with IC₅₀ value and 2.54 mmol/L. It also active against the most prevalent HCV genotypes with IC₅₀ values of 0.74-4.14 mM. Moreover, it acted synergistically with cyclosporine A to suppress HCV infection.⁴¹

Flavan-3-ols

The major component of green tea extract, EGCG (40) inhibit HCV entry⁴² acting directly on viral particles and inhibiting their adhesion to the cell surface with IC₅₀ value of 10.6±2.9 mM.⁴³ Additionally, EGCG has pangenotypic activities and antiviral properties against a range of unrelated viruses.⁴⁴

Flavonolignans

HCV fusion is inhibited by silibinin (41), silibinin A (42), silibinin B (43), isosilibinin A (44), isosilibinin B (45), silicristin (46), isosilicristin (47), silidianin (48) and taxifolin (49) with IC₅₀ values of 15±3 mM to 100±3 mM. In addition, silibinin (41) inhibit HCV entry by reducing trafficking through clathrin-coated pits and vesicles. High doses of commercially available silibinin A (50), silibinin B (51), silibinin (52), isosilibilin A (53), isosilibinin B (54), silicristin (55) and silidianin (56) decrease NS5B polymerase activity of HCV with IC₅₀ values of these compounds of 82.0±22.3 mM to >500 mM.⁴⁵

Anthocyanidins

A plant pigment contains anthocyanidin, delphinidin (57), as a novel HCV entry inhibitor at the binding step that modifies the morphology of HCVpp. The infection more efficiently than 40 does with IC₅₀ values of 3.7±0.8 mM and 10.6±2.9 mM.⁴³

Proanthocyanidins

Procyanidin A (58) isolated from *Cinnamomum zeylanicum*

bark showed anti-HCV activity against different HCV strains and genotypes without considerable cellular toxicity and the mechanism were inhibit HCV entry at post-binding with IC₅₀ value of 2.06 mM.^{46,47}

Strength and limitations

This review provides information on alkaloids and flavonoids active against the hepatitis C virus. In addition, this review serves as a basis for further research for discovering and developing alternative and complementary therapies for the hepatitis C virus. The data of compounds mechanism against hepatitis C virus are limited so more studies were needed and provided incomplete information.

Conclusions

Alkaloid and flavonoid compounds isolated from medicinal plants are recently explored for their anti-HCV activity. Alkaloids include quinoline, quinolizidine and isoquinoline, and flavonoids include flavanone, flavonol, flavone, flavan-3-ol, flavonolignan, anthocyanidin and proanthocyanidin were reported to possess anti-HCV activity by various mechanism. The berberine alkaloids and eriodictyol 7-*O*-(6''-caffeoyl)-β-D-glucopyranoside flavonoids had the lowest IC₅₀ with values of 0.49 mM and 0.041 nM. Our results provide information of alkaloids and flavonoids to the researcher for the development of alternative and complementary medicine of hepatitis C.

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