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

Gusti Rizaldi,[1] Achmad Fuad Hafid,[2,3] Tutik Sri Wahyuni[2,3]

[1]Master Program in Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia; [2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia; [3]Center for Natural Products Medicine Research and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

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 IC_{50} 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.

Correspondence: Tutik Sri Wahyuni, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, 60115, Surabaya, Indonesia.
Tel.: +62.812 41437599
E-mail: tutik-s-w@ff.unair.ac.id

Acknowledgments: Universitas Airlangga through Mandat project review with the project grand number206/UN3.15/PT/2022.

Key words: Alkaloid, Flavonoid, Hepatitis C Virus, Alternative and Complementary Medicine, Mechanism Action.

Contributions: GR, writing the original draft, data collecting, draft editing; AFH, conceptualization, writing review and editing, supervision; TSW, conceptualization, methodology, data curation, funding acquisition, writing review and editing, supervision. All the authors approved the final version to be published.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Availability of data and material: Data and materials are available by the authors.

Informed consent: The manuscript does not contain any individual person's data in any form.

Received for publication: 1 November 2022.
Revision received: 28 December 2022.
Accepted for publication: 20 January 2023.

Journal of Public Health in Africa 2023; 14(s1):2514
doi:10.4081/jphia.2023.2514

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 diameter[4] 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 properties[18] 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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. (±)-β-Myrifabral A and (±)-α-myrifabral A generated an inseparable anomer mixture (cluster A) (17), as did (±)-β-myrifabral B and (±)-α-myrifabral B (cluster B) (18). These compounds inhibit HCV replication with IC_{50} 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 IC_{50} values of 8.49±0.2 mg/ml.[30] Aloperine (20) inhibit HCV in Huh7it hepatocytes with IC_{50} 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 IC_{50} 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

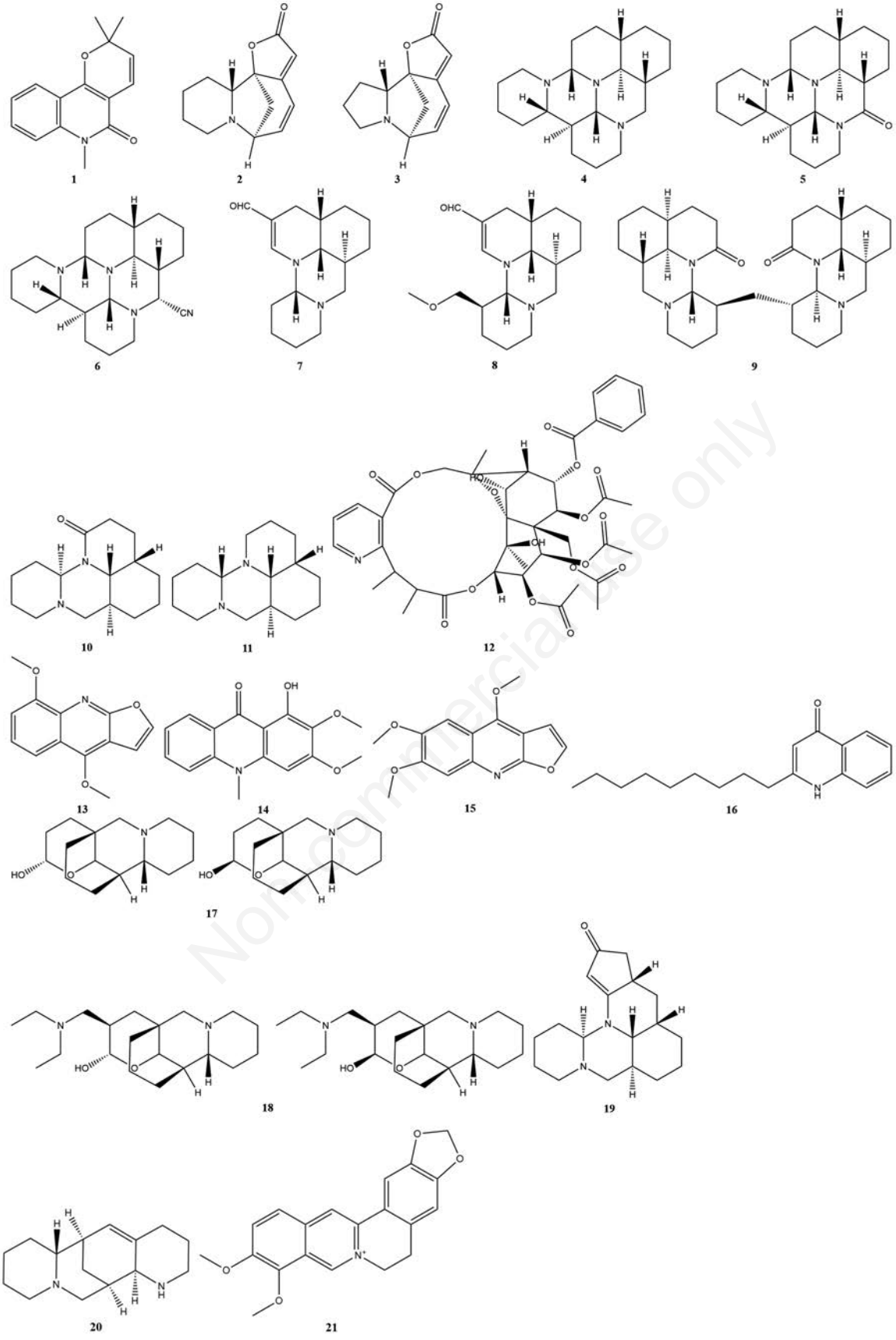

Figure 1. Molecular structure of alkaloids that possess anti-hepatitis C virus activity.

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-methylflindersine (1)	*Melicope latifolia* (Fruits)	3.8±2.7 µg/ml	HCV replication and supressed NS3 expression	(23)
	(-)-securinine (2)	*Flueggea virosa* (Twigs and Leaves)	7.7 µM	HCV replication	(24)
	(-)-norsecurinine (3)		27.6 µM		
	Myritonine A (4)	*Myrioneuron tonkinensis* (Leaves and Stems)	16.27±1.49 µM	HCV replication	(25)
	Myritonine B (5)		17.72±4.20 µM		
	Myritonine C (6)		>66.70 µM		
	Myrifamine A (7)		3.27±2.21 µM		(26)
	Myrifamine B (8)	*Myrioneuron faberi* (Leaves and Stems)	0.92±0.26 µM		
	Myrifamine C (9)		10.53±3.14 µM		
	Myrionamide (10)		30.61±2.44 µM		
	Schoberine (11)		7.35±0.56 µM		
	APS (12)	*Maytrenus ilcilifolia* (Root bark)	2.3 µg/ml	HCV replication	(27)
	γ-Fagarine (13)	*Ruta angustifolia* (Leaves)	20.4±0.4 µg/ml		(28)
	Arborinine (14)		6.4±0.7 µg/ml		
	Kokusaginine (15)		6.4±1.6 µg/ml		
	Pseudane IX (16)		1.4±0.2 µg/ml	HCV replication and NS3 protein synthesis	
Quinolizidine	(±)-β-Myrifabral A & (±)-α-Myrifabral A (cluster A) (17)	*Myrineuron faberi* (Leaves and Stems)	4.7 µM	HCV replication	(29)
	(±)-β-Myrifabral B & (±)-α-Myrifabral B (cluster B) (18)		2.2 µM		
	Myriberine A (19)	*Myrineuron faberi* (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	11.34±3.83 µg/ml	NS3-NS4A Protease	(35)
	Isosakuranetin (23)	*Taxillus sutchuenensis* (Herba)	234.4±2.2 µM	NS3 Protease	(36)
	Homoeriodictyol-7-O-β-D-glucopyranoside (24)		68.9±0.4 µM		
	Viscumneoside I (25)		62.3±2.0 µM		
	Eriodictyol 7-*O*-(6″-caffeoyl)-*β*-*D*-glucopyranoside (26)	*Elsholtzia bodinieri* (Aerial)	0.041 nM	HCV Replication	(37)
Flavonol	Rhamnazin 3-*O*-di-rhamnosyl-glucoside (27)	*Sarcocornia fruticosa* (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±6.2 µM		
	Kaempferol-3,7-bisrhamnoside (32)	*Taxillus sutchuenensis* (Herba)	19.4±0.5 µM		(36)
Flavone	Sorbifolin (33)	*Pterogyne nitens* (*Leaves*)	37.93 µM	HCV Entry by direct action on virus particles	(39)
	Pedalitin (34)		51.97 µM	HCV entry by direct action on virus particles and interference on the host cells	
	Diosmetin 7-*O*-β-glucoside-2″-sulphate (35)	*Thalassia hemprichii*	16 µM	NS3-NS4A Protease	(40)
	Diosmetin 7-*O*-β-glucoside (36)		37 µM		
	Quercetin 3,3',4'-trimethyl ether (37)	*Taxillus sutchuenensis* (Herba)	154.6±1.9 µM	NS3 Protease	(36)
	Luteolin (38)	*Elsholtzia bodinieri* (Aerial)	0.56 nM	HCV Replication	(37)
	Ladanein (39)	*Marrubium peregrinum* (Aerial)	2.79 µmol/L	HCV entry at post-binding	(41)
Flavan-3-ol	EGCG (40)	*Commercial*	10.6±2.9 µM	HCV entry, modify the morphology of HCVpp	(43)
Flavonolignan	Silibinin (Silibinin A & Silibinin B) (41)	*Silybum marianum* (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	HCV fusion	
	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		
	Silidianin (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±24.1 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		
	Silidianin (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)	*Cinnamomum zeylanicum* (Bark)	2.06 µM	HCV Entry at post-binding	(46)

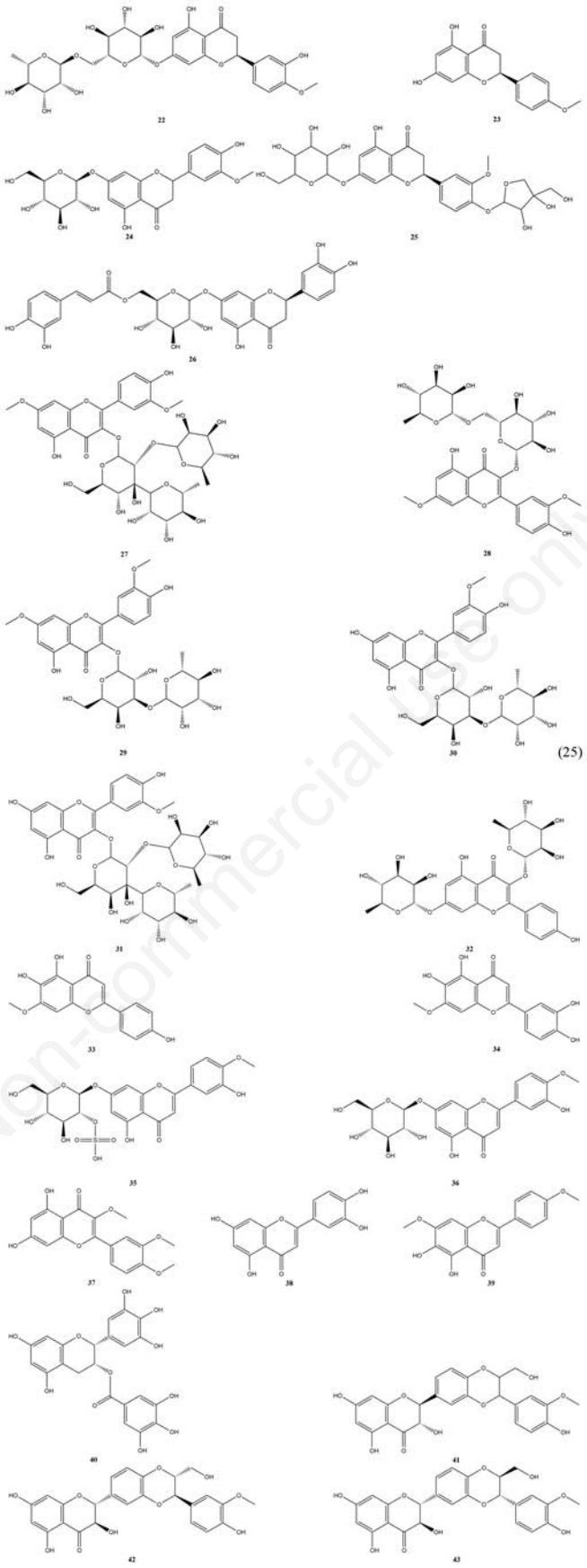

Figure 2. Structure of flavonoids compounds with anti-hepatitis C virus activities

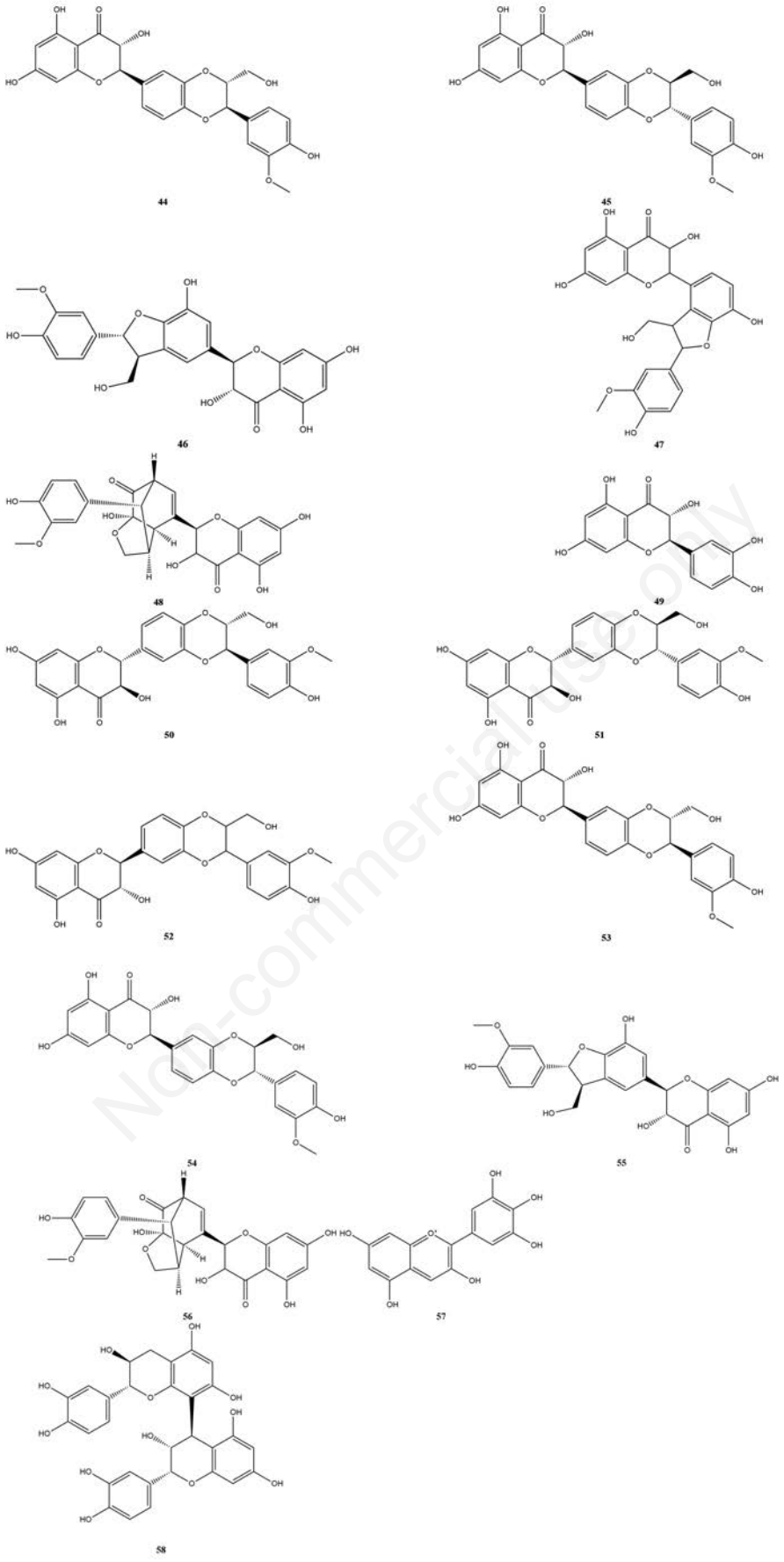

Figure 2. Structure of flavonoids compounds with anti-hepatitis C virus activities

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 IC_{50} 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 IC_{50} value of 19.4 ± 0.5 mM.[36]

Flavones

Sorbifolin (33) and pedalitin (34) isolated from *Pterogyene nitens* leaves has virucidal activity. Nevertheless, pedalitin (34) inhibits interference on the host cells and direct on viral particles. IC_{50} 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 IC_{50} 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 IC_{50} value of 154.6±1.9 mM.[36] Isolation of aerial parts of *Elsholtzia bodinieri* yielded luteolin (38). It inhibit HCV replication with IC_{50} value of 0.56 nM.[37] Ladanein (39) was identified from aerial parts *of Marrubium peregrinum*. Ladanein was synthetized to compare the activity between ladanein form natural source and synthetic ladanein. These compounds suppress HCV genotype 2a infection with IC_{50} value and 2.54 mmol/L. It also active against the most prevalent HCV genotypes with IC_{50} values of 0.74-4.14 mM. Moreover, it acted synergizstically with cyclosporine A to suppress HCV infection.[41]

Flavan-3-ols

The major component of green tea extract, EGCG (40) inhibit HCV entry[42] acting directly on viral particles and inhibiting their adhesion to the cell surface with IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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.

References

1. Antonio ADS, Wiedemann LSM, Veiga-Junior VF. Natural products' role against COVID-19. RSC Adv 2020;10:23379–93.
2. John A, Ibrahim ZG, Magaji SY, et al. Hepatitis B and C: A Twin Silent Killer. World J Pharma Med Res 2017;3:20–8.
3. Mohamed AA, Elbedewy TA, El-Serafy M, et al. Hepatitis C virus: A global view. World J Hepatol 2015;7:2676–80.
4. Alhetheel AF. Impact of Hepatitis C Virus Infection of Peripheral Blood Mononuclear Cells on the Immune System. Frontiers in Virology 2022;1:45.
5. Morozov VA, Lagaye S. Hepatitis C virus: Morphogenesis, infection and therapy. World J Hepatol 2018;10:186–212.
6. Shiryaev SA, Thomsen ER, Cieplak P, et al. New details of HCV NS3/4A proteinase functionality revealed by a high-throughput cleavage assay. PLoS One 2012;7.
7. Pockros PJ. New direct-acting antivirals in the development for hepatitis C virus infection. Therap Adv Gastroenterol 2010;3:191.
8. Yin C, Goonawardane N, Stewart H, Harris M. A role for domain I of the hepatitis C virus NS5A protein in virus assembly. PLoS Pathog 2018;14.
9. O'Boyle DR, Sun JH, Nower PT, et al. Characterizations of HCV NS5A replication complex inhibitors. Virology 2013;444:343–54.
10. Ferrero DS, Buxaderas M, Rodríguez JF, Verdaguer N. The Structure of the RNA-Dependent RNA Polymerase of a Permutotetravirus Suggests a Link between Primer-Dependent and Primer-Independent Polymerases. PLoS Pathog 2015;11:e1005265.
11. Ahmed A, Felmlee DJ. Mechanisms of Hepatitis C Viral Resistance to Direct Acting Antivirals. Viruses 2015;7(12):6716.
12. Geddawy A, Ibrahim YF, Elbahie NM, Ibrahim MA. Direct

Acting Anti-hepatitis C Virus Drugs: Clinical Pharmacology and Future Direction. J Transl Int Med 2017;5:8.
13. Chen SL, Yu H, Luo HM, et al. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med 2016;11:37.
14. Kingston DGI. Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod 2011;74:496–511.
15. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012;75:311–35.
16. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv 2015;33:1582–614.
17. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 2015;14:111–29.
18. Abookleesh FL, Al-Anzi BS, Ullah A. Potential Antiviral Action of Alkaloids. Molecules 2022;27:903.
19. Wang L, Song J, Liu A, et al. Research Progress of the Antiviral Bioactivities of Natural Flavonoids. Nat Prod Bioprospect 2020;10:271–83.
20. Ahmed S, Ullah N, Parveen S, et al. Effect of Silymarin as an Adjunct Therapy in Combination with Sofosbuvir and Ribavirin in Hepatitis C Patients: A Miniature Clinical Trial. Oxid Med Cell Longev 2022;2022.
21. Gu XB, Yang XJ, Hua Z, et al. Effect of oxymatrine on specific cytotoxic T lymphocyte surface programmed death receptor-1 expression in patients with chronic hepatitis B. Chin Med J (Engl) 2012;125:1434–8.
22. Ferreira MJU. Alkaloids in Future Drug Discovery. Molecules 2022;27.
23. Widyawaruyanti A, Tanjung M, Permanasari AA, et al. Alkaloid and benzopyran compounds of Melicope latifolia fruit exhibit anti-hepatitis C virus activities. BMC Complement Med Ther 2021;21.
24. Wang XF, Liu FF, Zhu Z, et al. Flueggenoids A – E, new dinorditerpenoids from Flueggea virosa. Fitoterapia 2019;133:96–101.
25. Li XH, Zhang Y, Zhang JH, et al. Myritonines A-C, Alkaloids from Myrioneuron tonkinensis Based on a Novel Hexacyclic Skeleton. J Nat Prod 2016;79:1203–7.
26. Cao MM, Zhang Y, Huang SD, et al. Alkaloids with Different Carbon Units from Myrioneuron faberi. J Nat Prod 2015;78:2609–16.
27. Jardim ACG, Igloi Z, Shimizu JF, et al. Natural compounds isolated from Brazilian plants are potent inhibitors of hepatitis C virus replication in vitro. Antiviral Res 2015;115:39–47.
28. Wahyuni TS, Widyawaruyanti A, Lusida MI. Inhibition of hepatitis C virus replication by chalepin and pseudane IX isolated from Ruta angustifolia leaves. Fitoterapia 2014;99:276–83.
29. Cao MM, Zhang Y, Li XH, et al. Cyclohexane-fused octahydroquinolizine alkaloids from Myrioneuron faberi with activity against hepatitis C virus. J Org Chem 2014;79:7945–50.
30. Huang SD, Zhang Y, Cao MM, et al. Myriberine a, a new alkaloid with an unprecedented heteropentacyclic skeleton from myrioneuron faberi. Org Lett 2013;15:590–3.
31. Lv XQ, Zou LL, Tan JL, et al. Aloperine inhibits hepatitis C virus entry into cells by disturbing internalisation from endocytosis to the membrane fusion process. Eur J Pharmacol 2020;883.
32. Hung TC, Jassey A, Liu CH, et al. Berberine inhibits hepatitis C virus entry by targeting the viral E2 glycoprotein. Phytomedicine 2019;53:62–9.
33. Calland N, Dubuisson J, Rouillé Y, Séron K. Hepatitis C virus and natural compounds: A new antiviral approach? Viruses 2012;4:2197–217.
34. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. ScientificWorldJournal 2013;2013.
35. Khan M, Rauf W, Habib F et al. Hesperidin identified from Citrus extracts potently inhibits HCV genotype 3a NS3 protease. BMC Complement Med Ther 2022;22:1–18.
36. Yang L, Lin J, Zhou B, et al. Activity of compounds from Taxillus sutchuenensis as inhibitors of HCV NS3 serine protease. Nat Prod Res 2017;31:487–91.
37. Zhong JD, Feng Y, Li HM, et al. A new flavonoid glycoside from Elsholtzia bodinieri. Nat Prod Res 2016;30:2278–84.
38. Hawas UW, Abou El-Kassem LT, Shaher F, Al-Farawati R. In vitro inhibition of Hepatitis C virus protease and antioxidant by flavonoid glycosides from the Saudi costal plant Sarcocornia fruticosa. Nat Prod Res 2019;33:3364–71.
39. Shimizu JF, Lima CS, Pereira CMH, et al. Flavonoids from Pterogyne nitens Inhibit Hepatitis C Virus Entry. Sci Rep 2017;7.
40. Hawas UW, Abou El-Kassem LT. Thalassiolin D: a new flavone O-glucoside Sulphate from the seagrass Thalassia hemprichii. Nat Prod Res 2017;31:2369–74.
41. Haid S, Novodomská A, Gentzsch J, et al. A plant-derived flavonoid inhibits entry of All HCV genotypes into human hepatocytes. Gastroenterology 2012;143.
42. Ciesek S, von Hahn T, Colpitts CC, et al. The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry. Hepatology 2011;54:1947–55.
43. Calland N, Sahuc ME, Belouzard S, et al. Polyphenols Inhibit Hepatitis C Virus Entry by a New Mechanism of Action. J Virol 2015;89:10053–63.
44. Steinmann J, Buer J, Pietschmann T, Steinmann E. Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. Br J Pharmacol 2013;168:1059–73.
45. Ahmed-Belkacem A, Ahnou N, Barbotte L, et al. Silibinin and Related Compounds Are Direct Inhibitors of Hepatitis C Virus RNA-Dependent RNA Polymerase. Gastroenterology 2010;138:1112–22.
46. Fauvelle C, Lambotin M, Heydmann L, et al. A cinnamon-derived procyanidin type A compound inhibits hepatitis C virus cell entry. Hepatol Int. 2017;11:440–5.
47. Blaising J, Lévy PL, Gondeau C, et al. Silibinin inhibits hepatitis C virus entry into hepatocytes by hindering clathrin-dependent trafficking. Cell Microbiol 2013;15:1866–82.