



Anti-Hepatitis C Virus Activity of Various Indonesian Plants from Balikpapan Botanical Garden, East Borneo

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Abstract

Background: Hepatitis C Virus infection is a serious health problem that leads to chronic liver disease, liver cirrhosis, hepatocellular carcinoma, which causes high morbidity. Direct-Acting Antiviral Agents have been used as anti-hepatitis C Virus therapy. However, it was covered only in limited patients due to the high cost. Moreover, serious side effects and resistance cases were also reported in some HCV genotypes. **Objective:** This research aimed to find new anti-HCV from some Indonesia plants collected from Balikpapan Botanical Garden, East Borneo. **Methods:** Twenty-one leaf and stem barks extracts were successively extracted in n-hexane, dichloromethane, and methanol. Extracts were screened for their anti-HCV activity under in vitro culture cells in the concentration of 30 µg/mL. Plant extracts were inoculated in the Human Hepatocellular 7it and infected with HCV Japanese Fulminant Hepatitis strain 1a. Determination of 50% Inhibitory Concentration (IC₅₀) value was further conducted at concentration of 100; 30; 10; 1; 0.1; 0.01 µg/ml of extracts. **Results:** In vitro anti-HCV activity revealed that among 21 plants extract, 11 extracts, namely, n-hexane extract from *Luvunga scandens* leaves, DCM extract from the leaf of *L. scandens*, *Artocarpus sericicarpus*, *Artocarpus dadah*, *Eusideroxyton zwageri*, *Neolitsea cassiaefolia*, methanol extract from *A. sericicarpus* and *A. anisophyllus* leaves, DCM extract from *A. anisophyllus* and *A. elmeri* stem bark, methanol extract from *A. dadah* stem bark, having potential inhibition with IC₅₀ range 0.08 ± 0.05 to 12.01 ± 0.95 µg/mL. **Conclusions:** These results indicate that the eleven extracts could be good candidates as sources of anti-HCV agents.

Keywords: health, hepatitis C virus, herbal medicine, medicinal plant extracts

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INTRODUCTION

Hepatitis C Virus (HCV) infection is a global problem that is still endemic in several countries (Hajarizadeh *et al.*, 2013). The global prevalence of HCV infection is estimated to be 2 - 3%, equivalent to 130 - 170 million people. As reported by the World Health Organization (WHO) in 2017, 71 million people out of the world's total population were infected with HCV. Each year there are about 3 – 4 million cases of new infections (Morozov & Lagaye, 2018). About 350,000 people die of HCV each year (World Health Organization (WHO), 2019). Hepatitis virus is a significant public health problem in Indonesia, with about 2.5 million infected with HCV (Jonathan *et al.*, 2018). HCV can cause chronic liver disease, cirrhosis of the liver, hepatocellular carcinoma, often called a malignant liver tumor, and complications of other conditions resulting from lipid and glucose metabolic disorders (Morozov & Lagaye, 2018).

HCV treatment has been in place for almost 15 years, with *pegylated interferon-alpha* and ribavirin with an infection recovery rate reaching 45% in the 1.65% in genotype three and about 85% in genotype 2. Development of treatment continues to be undertaken; the WHO recommends *Direct Acting Antivirals* (DAAs) therapy using a combination of sofosbuvir, daclatasvir, and ledipasvir (Morozov & Lagaye, 2018). In 2011, telaprevir and boceprevir were approved for use in the treatment of HCV with a combination of *pegylated interferon-alpha* and ribavirin. This treatment had a *sustained virologic response* (SVR) rate between 75% to 90% while reducing the duration of treatment. Combining sofosbuvir, daclatasvir, and sofosbuvir/ledipasvir, which were standards used according to WHO guidelines, could achieve healing levels above 95% (World Health Organization, 2019). But this therapy was expensive, had side effects, and might give rise to resistance (Ashfaq *et al.*, 2011; Morozov & Lagaye, 2018). Therefore, there was a need to develop a more safe, efficacious, and affordable new drug therapy.

Indonesia is a country that possesses large biodiversity, including plants that can be utilized for treatment. Of the world's approximately 40,000 species of flora, Indonesia owns 30,000 plants, and about 940 species of the plants are known to be effective as medications. Plant species are estimated to include 90% of the number of medicinal plants circulating in Asia (Hafid *et al.*, 2017). It is known that certain medicinal plants have antiviral activity, including anti-HCV (Wahyuni *et al.*, 2016).

Our previous studies reported several ethanol extracts from some Indonesian medicinal plants which have been screened and possessed anti-HCV activity. Ethanol extract of *Toona sureni*, *Melicope latifolia*, *Ficus fistulosa* leaves, *Melanolepis multiglandulosa* stem; 13.9; 3.5; 15.0; and 17.1 µg/mL, respectively. Some plants from the family Rutaceae were also reported to be active anti-HCV are *Ruta angustifolia* and *Melicope latifolia*. In Indonesia, *R. angustifolia* has been known as a traditional medicine for liver disease and jaundice. This plant contains coumarin, alkaloid, and flavonoid compounds. The dichloromethane extract of *R. angustifolia* leaves indicates anti-HCV activity with IC₅₀ value of 1,6 µg/mL. Two active compounds of anti-HCV, chalepin, and pseudane IX, are obtained from isolation and identification of dichloromethane fraction of *R. angustifolia* leaves using High Performance Liquid Chromatography (HPLC) (Wahyuni *et al.*, 2013; 2014).

Infusion of *Persea americana* leaves, the family Lauraceae, strongly inhibits type 1 herpes simplex virus (HSV-1), *Aujeszký* virus (ADV), and type 3 adenovirus (AD3) in cell culture. Isolation and identification with the basis of anti-HSV one and ADV testing showed Afzelin and quercetin *3-O-α-D-arabinopyranoside* exhibiting anti-HSV-1 activity resistant to acyclovir (De Almeida *et al.*, 1998). Methanol and water extracts from medicinal plants used in traditional Sudanese medicine, such as *Boswellia carterii* and *Acacia*, exhibit over 90% activity at 100 µg/mL (Hussein *et al.*, 2000).

Several plants of the genus *Artocarpus* have been reported to be active for anti-HCV, such as *Artocarpus heterophyllus*, *Artocarpus altilis*, and *Artocarpus camansi*. The dichloromethane extract of *A. heterophyllus* leaves has an IC₅₀ of as much as 1.5 ± 0.6 µg/mL against the JFH1a virus. The genus *Artocarpus* consists of about 50 species and is widespread in tropical and subtropical regions (Wetprasit *et al.*, 2000).

This study conducted anti-HCV activity from 21 extracts of eight plants. All of the extracts were tested for their anti-HCV activity against the JFH1a virus.

MATERIALS AND METHODS

Materials

Chemicals and reagents

The materials for anti-HCV assay solutions are methanol, dichloromethane, n-hexane, Huh7it hepatocyte cells, JFH1a hepatitis C virus, *Dimethyl Sulfoxide* (DMSO, Merck, Darmstadt, Germany), *Dubelco's Modified Eagle Medium* (DMEM, GIBCO, USA), 10% *Fetal Bovine Serum* (FBS, biowest, USA),

Non-Essential Amino Acid (NEAA, GIBCO, New York), *Dubelco's Phosphate Buffered Saline* (DPBS, SIGMA, USA), *Penicillin Streptomycin* (GIBCO, USA), *Trypsin-EDTA* (GIBCO, USA), *Bovine Serum Albumin* (BSA, Roche, Germany), formaldehyde (Merck, Germany), TritonX-100 (Promega, USA), *thermo staining 3,3'-diaminobenzidine* (DAB, Thermo scientific, USA), hepatitis patient antiserum C, HRP-Goat-anti-human Ig (MBL).

Plant materials

Plant materials were collected from Balikpapan Botanical Garden, East Borneo. The plant was verified by the Indonesian Institute of Sciences Botanical Garden, Purwodadi, Pasuruan Malang. Plant identification certificate number is 0074/IPH.06/HM/XII/2015.

Cells and virus

Huh 7it cells and *Japanese Fulminant Hepatitis strain 1a* (JFH1a) virus were used and developed at the Center for Natural Product Medicine Research and Development, Institute of Tropical Diseases, C Campus of Universitas Airlangga, Surabaya, East Java.

Method

Extraction

Extraction was conducted by ultrasonic method (UAE, *Ultrasonic assisted extraction*). A total of 800 grams of simplicia powder was extracted using the 3000 mL solvent n-hexane, dichloromethane, and methanol. Simplicia powder was extracted for six minutes and put in an ultrasonic machine. Every two minutes, the extract was stirred, then put in an ultrasonic machine again and filtered. The next step is evaporated extract using a rotary evaporator. The concentrated extract was drained inside the oven at a temperature of 40°C until obtaining a constant weight.

In vitro anti-HCV activity test

Anti-HCV activity assays were conducted in the following method. Ten mg of dried extracts were weighed and suspended in 100 µL of dimethyl sulfoxide (DMSO). The sample was diluted to obtain a concentration of 30 µg/mL for screening evaluation, while dose-dependent inhibition assays were also evaluated at 100, 30, 10, 1, 0.1, and 0.01 µg/mL. The mixture of sample and virus were inoculated to the Huh7it cells culture with *Multiplicity of Infection* (MOI) 0.1 at 48-well plates. After two hours of incubation, the cell was washed. The old medium was replaced with a new medium and further incubated in an extract-filled medium of the same concentration for 48 hours. After 48 hours of post-infection, culture supernatants were harvested for virus titration assay (Hafid *et al.*, 2017).

These procedures performed viral titration and immunostaining. First, a virus supernatant was made 30 times dilution in the medium and then inoculated into Huh7it cells. After four hours of incubation, the cells were cultured with a medium for 48 hours. After 48 hours of incubation, the cell was fixated with a formaldehyde solution and 0.5% X-100 triton. Cells were immunostained by adding the primary (human serum) and secondary antibodies (HRP-Goat-anti-human Ig (MBL)). Reagent of 3,3'-diaminobenzidine (DAB) Thermo staining was added, and infected cells were calculated under the microscope. The percent of infected cells was detected under the microscope. The percentage inhibitory was determined by SPSS probit analysis to obtain IC₅₀ values.

RESULTS AND DISCUSSION

This study was conducted to determine the anti-HCV activity of some extracts from plants that originated from the Balikpapan Botanical Garden, East Borneo. The selection of plants is based on the chemotaxonomy approach of the plant. Several studies reported that plants from the family Rutaceae, Moraceae and Lauraceae showed anti-virus activity, including potential anti-virus Hepatitis C (De Almeida *et al.*, 1998; Wahyuni *et al.*, 2013; 2014). Plants from that family are reported to contain various metabolite compounds, such as flavonoids, terpenoids, lignin, sulfide, polyline, thiophene, protein, and peptides that were thought to be able as anti-HCV agents (Wahyuni *et al.*, 2013).

We evaluated 21 plants extract against HCV at 30 µg/mL (Table 1). The result showed that 11 extracts had higher percentage of inhibition ($\geq 80\%$). Therefore, further analysis was conducted to n-hexane extract of *Luvunga scandens* leaves, dichloromethane extract of *L. scandens*, *Artocarpus sericarpus*, *Artocarpus dadah*, *Eusideroxylon zwageri*, *Neolitsea cassiaefolia* leaves, methanol extract of *A. sericarpus*, *Artocarpus anisophyllus* leaves, dichloromethane extract of *A. anisophyllus*, *Alseodaphane elmeri* stem bark and methanol extract of *A. dadah* stem bark for evaluating their 50% inhibitory effect. The IC₅₀ values of the 11 plants extract were demonstrated to possess potential activities with the IC₅₀ value of 0.08 to 12.01 µg/mL (Table 2).

Some flavonoid compounds reported having anti-HCV activity are *Epigallocatekin-3-gallate* (EGCG) (Ciesek *et al.*, 2011), quercetin, luteolin, apigenin, and ladanein (Calland *et al.*, 2012), naringenin, silymarin/silibin (Wagoner *et al.*, 2011). A wide variety

of chemical components of medicinal plants, such as flavonoid, terpenoid, lignin, sulfide, polyphenolic, coumarin, saponin, furyl compound, alkaloid, and polyline, thiophene, protein, and peptide, was identified to inhibit various viruses (Wahyuni *et al.*, 2016). In addition, some of them were known to be efficient in limiting the genome replication of RNA or DNA and its antioxidant activity (Ashfaq & Idrees, 2014).

L. scandens is a plant of the family Rutaceae and also mediated strong activity against HCV. The leaves

extracts of this plant showed 100% and 96.15% inhibition against the HCV JFH1a virus both in n-hexane and DCM solvents. It was reported that isobutylglucosinolates were isolated from the roots of *L. scandens*. However, there is no information yet on its antiviral activities (Sirinut *et al.*, 2017). Other plants from the *Rutaceae* genus, such as *Ruta angustifolia* and *Melicope latifolia*, have inhibited HCV. Meanwhile, less attention was given to its antiviral activity study.

Table 1. Percentage inhibition of extracts at a concentration of 30 µg/mL against JFH1a virus

Plant	Sample		Solvent	Infection (%)	Inhibition* (%)
	Familia	Part			
<i>Luvunga scandens</i>	Rutaceae	Leaves	n-Hexane	0.00	100.00 ± 0.00
<i>Artocarpus sericicarpus</i>	Moraceae	Leaves	n-Hexane	84.62	15.38 ± 21.76
<i>Neolitsea cassiaefolia</i>	Lauraceae	Leaves	n-Hexane	88.46	11.54 ± 1.81
<i>Luvunga scandens</i>	Rutaceae	Leaves	Dichloromethane	3.85	96.15 ± 5.44
<i>Artocarpus sericicarpus</i>	Moraceae	Leaves	Dichloromethane	17.95	82.05 ± 7.25
<i>Artocarpus dadah</i>	Moraceae	Leaves	Dichloromethane	1.28	98.72 ± 1.81
<i>Eusideroxylon zwageri</i>	Lauraceae	Leaves	Dichloromethane	2.56	97.44 ± 3.63
<i>Neolitsea cassiaefolia</i>	Lauraceae	Leaves	Dichloromethane	12.82	87.18 ± 7.25
<i>Melicope glabra</i>	Rutaceae	Leaves	Methanol	29.49	70.51 ± 9.07
<i>Artocarpus sericicarpus</i>	Moraceae	Leaves	Methanol	3.85	96.15 ± 5.44
<i>Artocarpus anisophyllus</i>	Moraceae	Leaves	Methanol	8.97	91.03 ± 9.07
<i>Artocarpus dadah</i>	Moraceae	Leaves	Methanol	30.77	69.23 ± 7.25
<i>Neolitsea cassiaefolia</i>	Lauraceae	Leaves	Methanol	55.13	44.87 ± 16.32
<i>Melicope glabra</i>	Rutaceae	Stem bark	Dichloromethane	58.97	41.03 ± 10.88
<i>Artocarpus sericicarpus</i>	Moraceae	Stem bark	Dichloromethane	21.79	78.21 ± 9.07
<i>Artocarpus anisophyllus</i>	Moraceae	Stem bark	Dichloromethane	2.56	97.44 ± 3.63
<i>Alseodaphane elmeri</i>	Lauraceae	Stem bark	Dichloromethane	0.00	100.00 ± 0.00
<i>Luvunga scandens</i>	Rutaceae	Stem bark	Methanol	100.00	0.00
<i>Artocarpus sericicarpus</i>	Moraceae	Stem bark	Methanol	55.13	44.87 ± 1.81
<i>Artocarpus anisophyllus</i>	Moraceae	Stem bark	Methanol	57.69	42.31 ± 9.07
<i>Artocarpus dadah</i>	Moraceae	Stem bark	Methanol	0.00	100.00 ± 0.00

*Data represent means ± SD of data from two independent experiments

Table 2. Anti HCV activity (IC₅₀) of extracts against HCV JFH1a

Plant	Sample			IC ₅₀ * (µg/mL)
	Familia	Part	Solvent	
<i>Luvunga scandens</i>	Rutaceae	Leaves	n-Hexane	2.53 ± 0.55
<i>Luvunga scandens</i>	Rutaceae	Leaves	Dichloromethane	0.08 ± 0.02
<i>Artocarpus sericicarpus</i>	Moraceae	Leaves	Dichloromethane	0.08 ± 0.05
<i>Artocarpus dadah</i>	Moraceae	Leaves	Dichloromethane	0.58 ± 0.18
<i>Eusideroxylon zwageri</i>	Lauraceae	Leaves	Dichloromethane	6.23 ± 0.30
<i>Neolitsea cassiaefolia</i>	Lauraceae	Leaves	Dichloromethane	7.73 ± 0.92
<i>Artocarpus sericicarpus</i>	Moraceae	Leaves	Methanol	8.04 ± 2.90
<i>Artocarpus anisophyllus</i>	Moraceae	Leaves	Methanol	2.71 ± 0.14
<i>Artocarpus anisophyllus</i>	Moraceae	Stem bark	Dichloromethane	4.62 ± 0.99
<i>Alseodaphane elmeri</i>	Lauraceae	Stem bark	Dichloromethane	4.65 ± 0.72
<i>Artocarpus dadah</i>	Moraceae	Stem bark	Methanol	12.01 ± 0.95

*Data represent means ± SD of data from two independent experiments

E. zwageri is a plant of the family Lauraceae; DCM extracts of leaves of this plant have a percent inhibition of the HCV JFH1a virus of 97.44 ± 3.63 . So in traditional medicine, it possesses potential as a natural antibacterial (Mariani *et al.*, 2020). Methanol extract of *E. zwageri* leaves was thought to have chemical components such as alkaloids, flavonoids, steroids, phenolics, and saponins that were also owned by the extract of parts of its stem (Wila *et al.*, 2018). According to Radulović *et al.* (2013), such compounds generally owned by plants influenced bacterial growth or might take a role as an antibacterial. Dichloromethane extracts of *N. cassiaefolia* leaves from family Lauraceae have a percent inhibition to the JFH1a HCV virus of 87.18 ± 7.25 (Radulović *et al.*, 2013).

In addition, three coumarins were isolated and characterized from *Viola yedoensis* using various chromatographic procedures. Among the isolated compounds dimer-coumarin 5,5'-bi (6,7-dihydroxycoumarin) significantly inhibited NS3/4A proteases scoring IC_{50} 0.5 g/mL (Zhang *et al.*, 2013).

Diosgenin (3-hydroxy-5-spirostene), a plant-derived saponin, effectively blocked the replication of HCV subgenomic replica systems at mRNA and protein levels. The value of the EC_{50} diosgenin was 3.8 μ mol (Wang *et al.*, 2011). *Silybum marianum* (SM) seeds and their fractions inhibit the HCV core gene of 3a genotype, and a combination of SM and its fractions with interferon will be a better option to treat HCV infection (Ashfaq *et al.*, 2011). Silibinin, a combination of two diastereoisomers, was the primary component of silymarin responsible for anti-HCV activity (Polyak *et al.*, 2007).

Naringenin was the primary flavanone found in grapefruit and tested on being able to suppress the activity of core proteins in Huh7 cells and also effectively block the formation of HCV particles (Nahmias *et al.*, 2008). *Epigallocatechin-3-gallate* (EGCG) was found in green tea extracts and can inhibit HCV activity (Ciesek *et al.*, 2011). Quercetin present in vegetables, fruits, grains, and leaves acted as an anti-HCV agent (Gonzalez *et al.*, 2009). Ladanein was a flavon isolated from *Marrubium peregrinum* L. (Lamiaceae). Ladanein had an IC_{50} of 2.5 μ mol (Haid *et al.*, 2012).

This study identified 11 extracts from eight plant species that exhibited anti-HCV activity. The active substances from extracts were not identified yet. Therefore, those extracts were the potential to be studied, especially on isolation and identification of anti-HCV active compounds.

CONCLUSION

A total of 11 extracts can be candidates for anti-HCV drug material sources. They are n-hexane extract of *Luvunga scandens* leaves, dichloromethane extract of *Luvunga scandens*, *Artocarpus sericarpus*, *Artocarpus dadah*, *Eusideroxylon zwageri*, *Neolitsea cassiaefolia* leaves, methanol extract of *Artocarpus sericarpus*, *Artocarpus anisophyllus* leaves, dichloromethane extract of *Artocarpus anisophyllus*, *Alseodaphne elmeri* stem bark, and methanol extract of *Artocarpus dadah* stem bark.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.W., T.S.W.; Methodology, A.W., T.S.W.; Validation, A.W., T.S.W.; Formal Analysis, R.P., A.A.P.; Investigation, R.P., A.A.P., L.T.; Resources, A.W., T.S.W.; Data Curation, R.P., L.T.; Writing - Original Draft, R.P.; Writing - Review & Editing, R.P., T.S.W., A.A.P., L.T., A.W.; Visualization, R.P., T.S.W., A.A.P., L.T., A.W.; Supervision, A.A.P., L.T.; Project Administration, A.W., T.S.W.; Funding Acquisition, A.W., T.S.W.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

- Ashfaq, U. A. & Idrees, S. (2014). Medicinal Plants Against Hepatitis C Virus. *World Journal of Gastroenterology*; 20; 2941–2947. doi: 10.3748/wjg.v20.i11.2941.
- Ashfaq, U. A., Masoud, M. S., Nawaz, Z. & Riazuddin, S. (2011). Glycyrrhizin as Antiviral Agent Against Hepatitis C Virus. *Journal of Translational Medicine*; 9; 1-7. doi: 10.1186/1479-5876-9-112.
- Ashfaq, U. A., Javed, T., Rehman, S., Nawaz, Z. & Riazuddin, S. (2011). Inhibition of HCV 3a Core Gene Through Silymarin and Its Fractions. *Virology Journal*; 8; 1-7.

- Calland, N., Dubuisson, J., Rouillé, Y. & Séron, K. (2012). Hepatitis C Virus and Natural Compounds: a New Antiviral Approach?. *Viruses*; 4; 2197–2217. doi: 10.3390/v4102197.
- Ciesek, S., von Hahn, T., Colpitts, C. C., Schang, L. M., Friesland, M., Steinmann, J., Manns, M. P., Ott, M., Wedemeyer, H., Meuleman, P., Pietschmann, T. & Steinmann, E. (2011). The Green Tea Polyphenol, Epigallocatechin-3-Gallate, Inhibits Hepatitis C Virus Entry. *Hepatology (Baltimore, Md.)*; 54; 1947–1955. doi: 10.1002/hep.24610.
- De Almeida, A. P., Miranda, M. M. F. S., Simoni, I. C., Wigg, M. D., Lagrota, M. H. C. & Costa, S. S. (1998). Flavonol Monoglycosides Isolated from the Antiviral Fractions of *Persea americana* (Lauraceae) Leaf Infusion. *Phytotherapy Research*; 12; 562–567. doi: 10.1002/(SICI)1099-1573(199812)12:8<562::AID-PTR356>3.0.CO;2-6.
- Gonzalez, O., Fontanes, V., Raychaudhuri, S., Loo, R., Loo, J., Arumugaswami, V., Sun, R., Dasgupta, A. & French, S. W. (2009). The Heat Shock Protein Inhibitor Quercetin Attenuates Hepatitis C Virus Production. *Hepatology (Baltimore, Md.)*; 50; 1756–1764. doi: 10.1002/hep.23232.
- Hafid, A. F., Aoki-Utsubo, C., Permanasari, A. A., Adianti, M., Tumewu, L., Widyawaruyanti, A., Wahyuningsih, S. P. A., Wahyuni, T. S., Lusida, M. I., Soetjipto & Hotta, H. (2017). Antiviral Activity of the Dichloromethane Extracts from *Artocarpus heterophyllus* Leaves Against Hepatitis C Virus. *Asian Pacific Journal of Tropical Biomedicine*; 7; 633–639. doi: 10.1016/j.apjtb.2017.06.003.
- Hafid, A. F., Puliansari, N., Lestari, N. S., Tumewu, L., Rahman, A. & Widyawaruyanti, A. (2017). Skrining Aktivitas Antimalaria Beberapa Tanaman Indonesia Hasil Eksplorasi dari Hutan Raya Cangar, Batu-Malang, Jawa Timur. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*; 3; 7-11. doi: 10.20473/jfiki.v3i12016.7-11.
- Haid, S., Novodomská, A., Gentsch, J., Grethe, C., Geuenich, S., Bankwitz, D., Chhatwal, P., Jannack, B., Hennebelle, T., Bailleul, F., Keppler, O. T., Poenisch, M., Bartenschlager, R., Hernandez, C., Lemasson, M., Rosenberg, A. R., Wong-Staal, F., Davioud-Charvet, E., & Pietschmann, T. (2012). A Plant-Derived Flavonoid Inhibits Entry of All HCV Genotypes Into Human Hepatocytes. *Gastroenterology*; 143; 213-222e5. doi: 10.1053/j.gastro.2012.03.036.
- Hajarizadeh, B., Grebely, J. & Dore, G. J. (2013). Epidemiology and Natural History of HCV Infection. *Nature Reviews. Gastroenterology & Hepatology*; 10; 553–562. doi: 10.1038/nrgastro.2013.107
- Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N. & Shimotohno, K. (2000). Inhibitory Effects of Sudanese Medicinal Plant Extracts on Hepatitis C Virus (HCV) Protease. *Phytotherapy Research: PTR*; 14; 510–516. doi: 10.1002/1099-1573(200011)14:7<510::aid-ptr646>3.0.co;2-b.
- Jonathan, S., Jack, W. & Suzanne, W. (2018). Situation Analysis of Viral Hepatitis in Indonesia : A Policy Report. *Coalition Eradicate Viral Hepatitis in Asia Pacific (CEVHAP)*; 3; 1-23.
- Mariani, Y., Yusro, F. & Wardenaar, E. (2020). Aktivitas Ekstrak Metanol Daun Ulin (*Eusideroxylon zwageri* Teijsm & Binn) Terhadap Empat Jenis Bakteri Patogen. *Jurnal Biologi Tropis*; 20; 94–101. doi: 10.29303/jbt.v20i1.1642.
- Morozov, V. A. & Lagaye, S. (2018). Hepatitis C Virus: Morphogenesis, Infection and Therapy. *World Journal of Hepatology*; 10; 186–212. doi: 10.4254/wjh.v10.i2.186.
- Nahmias, Y., Goldwasser, J., Casali, M., van Poll, D., Wakita, T., Chung, R. T. & Yarmush, M. L. (2008). Apolipoprotein B-dependent Hepatitis C Virus Secretion is Inhibited by the Grapefruit Flavonoid Naringenin. *Hepatology (Baltimore, Md.)*; 47; 1437–1445. doi: 10.1002/hep.22197.
- Polyak, S. J., Morishima, C., Shuhart, M. C., Wang, C. C., Liu, Y. & Lee, D. Y.-W. (2007). Inhibition of T-cell Inflammatory Cytokines, Hepatocyte NF-kappaB Signaling, and HCV Infection by Standardized Silymarin. *Gastroenterology*; 132; 1925–1936. doi: 10.1053/j.gastro.2007.02.038.
- Radulović, N. S., Blagojević, P. D., Stojanović-Radić, Z. Z. & Stojanović, N. M. (2013). Antimicrobial Plant Metabolites: Structural Diversity and Mechanism of Action. *Current Medicinal Chemistry*; 20; 932–952.
- Sirinut, P., Petchkongkeaw, A., Romsaiyud, J., Prateptongkum, S. & Thongyoo, P. (2017). Phytochemical Constituents from the Root of *Luvunga Scandens* and Biological Activity Evaluation. *Natural Product Communications*; 12; 1483-1484. doi: 10.1177/1934578X1701200926.
- Wagoner, J., Morishima, C., Graf, T. N., Oberlies, N. H., Teissier, E., Pécheur, E.-I., Tavis, J. E. & Polyak, S. J. (2011). Differential In Vitro Effects

- of Intravenous Versus Oral Formulations of Silibinin on the HCV Life Cycle and Inflammation. *PloS One*; 6; 1-9. doi: 10.1371/journal.pone.0016464.
- Wahyuni, T. S., Tumewu, L., Permanasari, A. A., Apriani, E., Adianti, M., Rahman, A., Widyawaruyanti, A., Lusida, M. I., Fuad, A., Soetjipto, Nasronudin, Fuchino, H., Kawahara, N., Shoji, I., Deng, L., Aoki, C. & Hotta, H. (2013). Antiviral Activities of Indonesian Medicinal Plants in the East Java region Against Hepatitis C Virus. *Virology Journal*; 10; 1-9. doi: 10.1186/1743-422X-10-259.
- Wahyuni, T. S., Utsubo, C. A. & Hotta, H. (2016). Promising Anti-Hepatitis C Virus Compounds from Natural Resources. *Natural Product Communications*; 11; 1193-1200.
- Wahyuni, T. S., Widyawaruyanti, A., Lusida, M. I., Fuad, A., Soetjipto, Fuchino, H., Kawahara, N., Hayashi, Y., Aoki, C. & Hotta, H. (2014). Inhibition of Hepatitis C Virus Replication by Chalepin and Pseudane IX Isolated from *Ruta angustifolia* Leaves. *Fitoterapia*; 99; 276–283. doi: 10.1016/j.fitote.2014.10.011.
- Wang, Y. -J., Pan, K. -L., Hsieh, T. -C., Chang, T. -Y., Lin, W. -H. & Hsu, J. T. -A. (2011). Diosgenin, a Plant-Derived Sapogenin, Exhibits Antiviral Activity In Vitro Against Hepatitis C Virus. *Journal of Natural Products*; 74; 580–584. doi: 10.1021/np100578u.
- Wetprasit, N., Threesangsri, W., Klamklai, N. & Chulavatnatol, M. (2000). Jackfruit Lectin: Properties of Mitogenicity and the Inhibition of Herpesvirus Infection. *Japanese Journal of Infectious Diseases*; 53; 156–161.
- Wila, H., Yusro, F. & Mariani, Y. (2018). Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Kulit Batang (*Eusideroxylon zwageri*) terhadap *Escherichia coli* dan *Salmonella typhi*. *Jurnal Tengkadang*; 8; 38-49. doi: 10.26418/jt.v8i1.30147.
- World Health Organization (WHO). (2019). Hepatitis C. <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c>. Accessed: 21 April 2021.
- Zhang, L., Li, M. -Y., Wang, L. -W., Gao, J. & Ma, C. -M. (2013). Isolation, Identification, Quantification and Inhibitory Activity on HCV Protease of Coumarins from *Viola yedoensis*. *Canadian Chemical Transactions*; 1; 157–164. doi: 10.13179/canchemtrans.2013.01.03.0025.