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, 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, Eusideroxylon zwageri, Neolitsea cassiaeolifa, 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, Melanoëpis 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), Aujeszky virus (ADV), and type 3 adenovirus (AD3) in cell culture. Isolation and identification with the basis of anti-HSV one and ADV testing showed Aezelin and quercetin 3-O-a-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), Dulbecco’s Modified Eagle Medium (DMEM, GIBCO, USA), 10% Fetal Bovine Serum (FBS, biowest, USA), P-ISSN: 2406-9388 E-ISSN: 2580-8303
Non-Essential Amino Acid (NEAA, GIBCO, New York), Dulbecco’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, polypeptide, and proteins 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 (≥ 80%). Therefore, further analysis was conducted to n-hexane extract of Luvanga scandens leaves, dichloromethane extract of L. scandens, Artocarpus sericicarpus, Artocarpus dadah, Eusideroxylon zwageri, Neolitsea cassiaefolia leaves, methanol extract of A. sericicarpus, 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 *Melice 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           | Familia         | Part            | Solvent         | Infection (%)   | Inhibition* (%) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| *Lavunga scandens* | Lauraceae       | Leaves          | n-Hexane        | 0.00            | 100.00 ± 0.00   |
| *Artocarpus sericarcarpus* | Moraceae       | Leaves          | n-Hexane        | 84.62           | 15.38 ± 21.76   |
| *Neolitsea cassiaefolia* | Lauraceae      | Leaves          | n-Hexane        | 88.46           | 11.54 ± 1.81    |
| *Lavunga scandens* | Lauraceae       | Leaves          | Dichloromethane | 3.85            | 96.15 ± 5.44    |
| *Artocarpus sericarcarpus* | Moraceae       | Leaves          | Dichloromethane | 17.95           | 82.05 ± 7.25    |
| *Artocarpus dadah* | Moraceae        | Leaves          | Dichloromethane | 1.28            | 98.72 ± 1.81    |
| *Eusideroxylon zwageri* | Lauraceae      | Leaves          | Dichloromethane | 2.56            | 97.44 ± 3.63    |
| *Neolitsea cassiaefolia* | Lauraceae      | Leaves          | Dichloromethane | 12.82           | 87.18 ± 7.25    |
| *Melico glabra* | Rutaceae        | Leaves          | Methanol        | 29.49           | 70.51 ± 9.07    |
| *Artocarpus sericarcarpus* | Moraceae       | Leaves          | Methanol        | 3.85            | 96.15 ± 5.44    |
| *Artocarpus anisophyllus* | Moraceae       | Leaves          | Methanol        | 8.97            | 91.03 ± 9.07    |
| *Artocarpus dadah* | Moraceae        | Leaves          | Methanol        | 30.77           | 69.23 ± 7.25    |
| *Neolitsea cassiaefolia* | Lauraceae      | Leaves          | Methanol        | 55.13           | 44.87 ± 16.32   |
| *Melico glabra* | Rutaceae        | Stem bark       | Dichloromethane | 58.97           | 41.03 ± 10.88   |
| *Artocarpus sericarcarpus* | Moraceae       | Stem bark       | Dichloromethane | 21.79           | 78.21 ± 9.07    |
| *Artocarpus anisophyllus* | Moraceae        | Stem bark       | Dichloromethane | 2.56            | 97.44 ± 3.63    |
| *Alseodaphane elmeri* | Lauraceae      | Stem bark       | Dichloromethane | 0.00            | 100.00 ± 0.00   |
| *Lavunga scandens* | Rutaceae        | Stem bark       | Methanol        | 100.00          | 0.00            |
| *Artocarpus sericarcarpus* | Moraceae       | Stem bark       | Methanol        | 55.13           | 44.87 ± 1.81    |
| *Artocarpus anisophyllus* | Moraceae        | Stem bark       | Methanol        | 57.69           | 42.31 ± 9.07    |
| *Artocarpus dadah* | 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           | Familia         | Part            | Solvent         | IC₅₀* (µg/mL)   |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| *Lavunga scandens* | Rutaceae       | Leaves          | n-Hexane        | 2.53 ± 0.55     |
| *Lavunga scandens* | Rutaceae       | Leaves          | Dichloromethane | 0.08 ± 0.02     |
| *Artocarpus sericarcarpus* | Moraceae       | Leaves          | Dichloromethane | 0.08 ± 0.05     |
| *Artocarpus dadah* | Moraceae        | Leaves          | Dichloromethane | 0.58 ± 0.18     |
| *Eusideroxylon zwageri* | Lauraceae      | Leaves          | Dichloromethane | 6.23 ± 0.30     |
| *Neolitsea cassiaefolia* | Lauraceae      | Leaves          | Dichloromethane | 7.73 ± 0.92     |
| *Artocarpus sericarcarpus* | Moraceae       | Leaves          | Methanol        | 8.04 ± 2.90     |
| *Artocarpus anisophyllus* | Moraceae        | Leaves          | Methanol        | 2.71 ± 0.14     |
| *Artocarpus anisophyllus* | Moraceae        | Stem bark       | Dichloromethane | 4.62 ± 0.99     |
| *Alseodaphane elmeri* | Lauraceae      | Stem bark       | Dichloromethane | 4.65 ± 0.72     |
| *Artocarpus dadah* | 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. cassiaeefolia 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-dihydroxycumarine) significantly inhibited NS3/4A proteases scoring IC₅₀ 0.5 g/mL (Zhang et al., 2013).

Diosgenin (3-hydroxy-5-spirostene), a plant-derived sapogenin, effectively blocked the replication of HCV subgenomic replica systems at mRNA and protein levels. The value of the EC₅₀ diosgenin was 3.8 µmol (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 IC₅₀ of 2.5 µmol (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 Lavunga scandens leaves, dichloromethane extract of Lavunga scandens, Artocarpus sericarpus, Artocarpus dadah, Eusideroxylon zwageri, Neolitsea cassiaeefolia leaves, methanol extract of Artocarpus sericarpus, Artocarpus anisophyllum leaves, dichloromethane extract of Artocarpus anisophyllum, Alseodaphane 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.

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