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Original article

Anti-Hepatitis C virus activity of *Garcinia lattissima* Miq. stem barks methanol

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Abstract

Herbal medicine treatment for Hepatitis C Virus (HCV) infection is a promising alternative to common medical HCV treatments because it has low unwanted side effects and production costs. This study aimed to evaluate the methanol extract of *Garcinia lattissima* stem barks as an antiviral compound against strain JFH1 (HCV) genotype 2a. *Garcinia lattissima* stem bark methanolic extract (GL-SB) was added to Huh7it-1 cells infected with JFHI. Anti HCV activity was determined by Focus-forming unit (FFU) assay and the cytotoxicity activity was analyzed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. The GL-SB showed its efficacy as the anti-HCV agent with a 50% cytotoxicity concentration (CC50) of 34.2 µg/mL and a 50% effective concentration (EC50) of 4.7 µg/mL. The co-addition and post-infection phases of GL-SB showed an anti-HCV activity, according to a time-of-addition investigation. These findings imply that GL-SB might be a promising candidate for add-on therapy in the treatment of HCV infections.

Keywords: anti-HCV, *Garcinia lattissima*, hepatitis c virus.

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Introduction

The Hepatitis C Virus (HCV) is the cause of hepatitis C disease. HCV infection is a blood-borne virus that is spread when infected blood from one person enters the blood vessels of an uninfected person through intravenous drug use or blood transfusions. In 2015, HCV infection was estimated 1,750,000 people to be anew infected, and numerous chronically infected people are careless of their disease. Chronic HCV infection is approximately more than 71 million people, with 399,000 deaths recorded Worldwide (Renau & Berenguer, 2018; World Health Organization, 2017)

The HCV virus family is extremely heterogeneous, with seven primary genotypes and several subtypes (Bukh, 2016). In addition, there is still no HCV vaccination to prevent infection thus increasing the difficulty to control the infection of HCV. Recently, HCV therapy classified into interferon-based and interferon-free therapies (direct-acting antivirals). However, the therapies are very high-priced and might cause unwanted side effects such as headache, fatigue, diarrhea, and nausea, or worst causing the viral genetic resistance of polymorphisms (Liver, 2017, 2018; Rosenthal & Graham, 2016). Therefore, there is still a necessity to develop harmless and low-cost medications for HCV infections by utilizing plants with medicinal properties.

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Tropical plants of the *Garcinia* genus are used as medicines in Southeast Asia. The Garcinia genus has been reported to perform biological properties, such as antiviral, hepatoprotective, antibacterial, and antimalarial (Magadula & Mbwambo, 2014). *Garcinia lattissima* or Dolomagota (local name) can be found in Seram Maluku and Papua, Indonesia. Traditionally, this plant has been used for reducing itchiness by the local community. There has been few research done for studying the potential effects of *Garcinia latissimi* from Papua, Indonesia, and the information is currently limited (Neneng Siti Silfi Ambarwati, Berna Elya , Amarila Malik, 2017). In this study, the anti-HCV activity of *G. latissima* stem bark (GL-SB) methanol extract was examined against JFH1 strain genotype 2a.

Methods

Plant material

Garcinia latissima stem bark from the National Research and Innovation Agency (BRIN), Serpong, Indonesia's Research Center for Chemistry. The plant has been identified and confirmed by botanists at the BRIN Botanical Research Center in Cibinong, Indonesia. Herbarium specimen were maintained and stored in BRIN's Research Center for Chemistry.

Extraction of GL-SB

About 5.008 kg of dried *Garcinia latissima* stem bark were ground into powder before being extracted five times with methanol (8L) in a refluxing environment. The extracts were assembled with vacuum rotavapor at temperature 40ºC to obtain a Crude Extract from *Garcinia lattissima* Miq. Stem Barks (GL-SB). The GL-

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SB was stored at a temperature of -30ºC after being dissolved in 100 mμg/mL dimethyl sulfoxide (DMSO).

Cells and viruses

The cells were cloned from the human hepatocellular carcinoma (Huh7it-1) cell line (Aoki et al., 2014). The cell was maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco), contained 10% Fetal Bovine Serum (FBS) (Biowest), Non-Essential Amino Acids (Gibco), and Kanamycin (Sigma) under 37ºC with 5% CO_2 conditions. The HCV strain employed by the viruses was JFH1, genotype 2a.(Apriyanto et al., 2016; Apriyanto, Arsianti, et al., 2018; Apriyanto, Aoki-Utsubo, Hartati, Dewi, & Hotta, 2018).

Antiviral activity

The antivirus activity assay was performed according to Apriyanto et al. [15]. The cells were exposed to viruses with a multiplicity of infection (MOI) of 0.1 while being treated with GD-SB at concentrations of 160, 80, 40, 20, 10, 5, and 2.5 μg/mL then incubated at 37 ℃ for 2 h. Moreover, the virus was removed by washing the cells, and the cells were incubated with GD-SB for 46 h at 37 ℃.

According to a previous description, the cell culture supernatants were collected for viral titration (Apriyanto et al., 2016). A time-of-addition study was carried out with 10 μg/mL GL-SB either exclusively during the inoculation phase or after inoculation for the full culture duration till virus collection. Focus-forming unit (FFU) images were captured using an Olympus digital camera DP21 connected to an Olympus CKX41 microscope (Olympus, Tokyo, Japan) in order to measure the titers of HCV. The numbers were then standardized against untreated controls using a Katikati counter.

Cytotoxicity assay

The cytotoxicity of GL-SB against Huh7it-1 cells was tested using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. As previously mentioned, the cells were temporarily seeded in 96-well plates around 2.0×10^4 cells/well and treated with varied extract concentrations (160, 80, 40, 20, 10, 5, and 2.5 μg/mL). The cells were then incubated at 37 ℃ for 48 h. Furthermore, the medium was replaced with an MTT-solution medium, and the cells were further incubated for 4 h (Apriyanto et al., 2016).

Statistical analysis

The student's two-tailed t-test was used to evaluate the differences between the two data sets. Statistical significance was identified as a P-value < 0.05. Results were shown as mean and standard deviation.

Results

We assessed the anti-HCV activity of GL-SB in vitro in a dose-dependent manner. With an EC50 of 4.7 μg/mL, we discovered that GL-SB has successfully prevented the HCV infection (Figure 1). We used the MTT test to assess the cytotoxicity of GL-SB against Huh7it-1. Huh7it-1 cells exposed to GL-SB up to 20 μg/mL, a 50% cytotoxicity concentration (CC50) of 34.2 μg/mL, showed no obvious cytotoxicity (Figure 1A). The selectivity index was 7.28 (CC50/EC50).

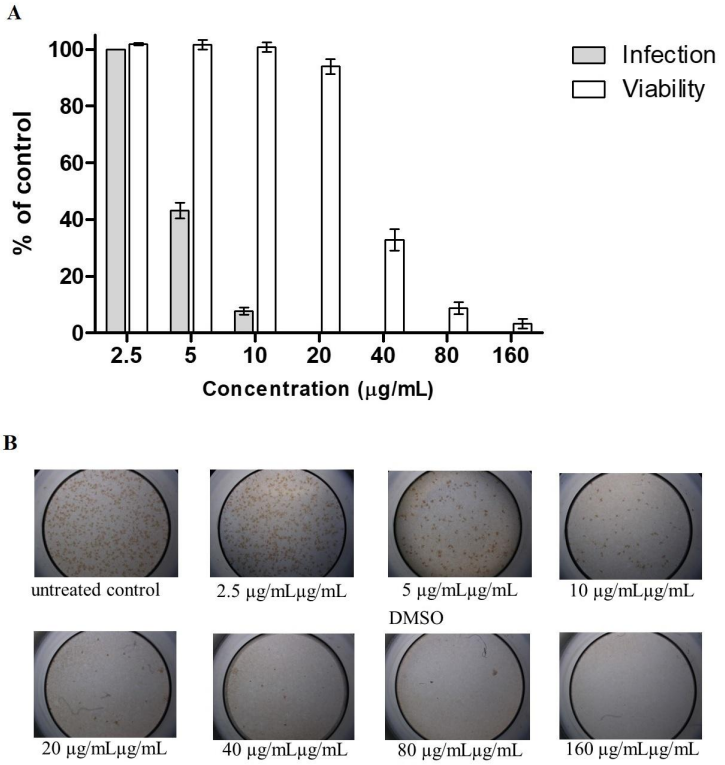

Figure 1. Activity of anti-HCV and cytotoxicity of GL-SB. (A) The percentage of data compared to the untreated control is shown. (B) Focus-forming unit of the virus infection, which appears as brown, was captured using an Olympus CKX41 microscope and an attached DP21 digital camera. Data is presented as mean ± SD of triplicate.

We conducted time-of-addition research of GL-SB to investigate the potential inhibitory step in anti-HCV (Figure 2A). Only 2 h of virus adsorption in cells treated with GL-SB (coaddition) and cells treated with GL-SB following virus adsorption until virus harvest in cells treated with GL-SB (post-infection). As a positive control, cells were infected with a virus mixed with GL-SB for 2 h, and then the entire treatment continued with new medium containing GL-SB for 46 h. With an 86.18% inhibition at coaddition and a 62.76% inhibition at post-infection, GL-SB (10 µg/mL) demonstrated anti-HCV efficacy (Figure 2B).

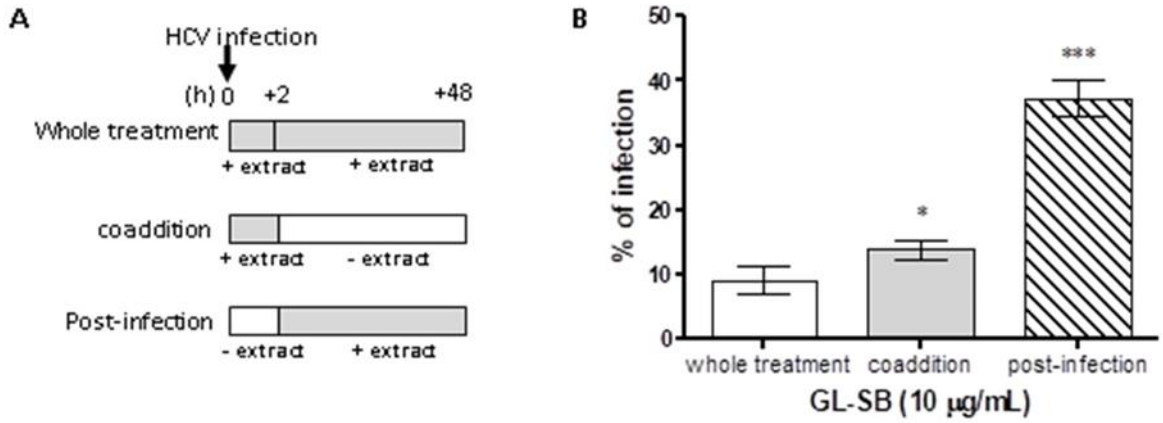

Figure 2. Mode-of-action of GL-SB. (A) Schematic representation of the time-of-addition experiment. (B) The percentage of HCV infection compared to the untreated control (Control) is shown. Data is presented as mean ± SD of triplicate. *, $P < 0.05$; ***, $P < 0.001$ compared with the control.

Discussion

G. lattissima is a member of the *Garcinia* genus that has many sources of secondary metabolites, in which different parts of the plant showed different biological properties to cure various diseases (Khamthong & Hutadilok-Towatana, 2017; Magadula & Mbwambo, 2014; Mitra et al., 2007). The enzymatic study of mangosteen fruit peels extract has shown inhibition towards RNA helicase HCV (Syajarwati, 2013). In another study, the fruit peel extract from mangosteen can suppress replication of HCV genome replication using sub genomic of Bart79I with EC_{50} 5.1 µµg/mL and replicon systems using J6/JFH-1 with EC_{50} 3.8 µµg/mL (Choi, Kim, Lee, Chin, & Lee, 2014), indicating that the plants from the *Garcinia* genus contribute as the resources of medicinal plants against anti-HCV.

The substance or compounds that give GL-SB its anti-HCV activity have not yet been identified. A previous study, analysis of phytochemical revealed that *G. lattissima* steam bark contains flavonoids saponins, alkaloids, and tannins (Neneng Siti Silfi Ambarwati, Berna Elya , Amarila Malik, 2017). The study of flavonoids compounds, such as quercetin, quercetagetin, luteolin, kaemferol, naringenin, and catechin significantly has shown to suppress the replication of HCV (Ahmed-Belkacem et al., 2014; Khachatoorian et al., 2012). HCV replication has been inhibited by studies using saponins such as platycodin D, platycodin D2, platycodin D3, deapioplatycodin D, deapioplatycodin D2, platyconic acid A, and PG saponin mixture (PGSM) (Kim et al., 2013). *Myrioneuron faberi*'s octahydroquinolizine alkaloids have been proved to inhibit HCV replication (Cao et al., 2014). Hydrolysable tannins from the fruits of *Terminalia chebula* have demonstrated an HCV protease inhibition in another investigation (Ajala, Jukov, & Ma, 2014). The time-of-addition study of GL-SB demonstrated inhibition of HCV both at coaddition and post-infection steps (Figure 2B).

Inhibitory coaddition of GL-SB may prevent HCV entry by a direct virucidal action or viral adsorption, according to a time-of-addition investigation. Meanwhile, the potential inhibitory post-infection of GL-SB may involve partial inhibition of virion assembly and direct inactivation of the virion discharged from the infected cells (Apriyanto et al., 2016). GL-SB is an extract with anti-HCV activity that needs further study to identify the compounds involved. It can be used as an additional therapy to minimize the impact of HCV when combined with existing treatments, despite having some side effects. Overall, the study described that GL-SB has a potential candidate as an anti-HCV. Furthermore, the isolation of a specific compound in GL-SB as anti-HCV should be considered for future research.

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