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Activities of *Ficus fistulosa* Leave Extract and Fractions Against Hepatitis C Virus

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Abstract

Hepatitis C Virus (HCV) is a major global disease which often leads to chronicity and is potential to liver failure. There is no anti-HCV vaccine and the high diversity of viral genotypes will probably make it very difficult to develop a vaccine. Therefore, the development of new drugs for HCV treatment is highly required. It is commonly known that numerous important modern drugs have been developed from molecules originally isolated from natural sources. In this study, we tested the leave extract and fractions of *Ficus fistulosa* for their anti-HCV activities by cell culture method using Huh7it cells and HCV JFH1a. The result showed that ethanol extract of *Ficus fistulosa* (FFL) inhibited HCV JFH1a with IC_{50} value of 20.43±4.51 µg/ml. Toxicity test also indicated that FFL was not toxic with CC_{50} value of >200 µg/ml. The extract was further fractionated using chloroform (FFLC) and butanol (FFLB) successively. FFLC showed anti-HCV activity with IC_{50} value of 5.67±1.54 µg/ml and CC_{50} value of >100 µg/ml (Selectivity index >17.65). Further separation of FFLC by open column chromatography resulted in 12 subfractions (FFLC1-C12). Two subfractions, FFLC10, and FFLC11 showed high selectivity index (>100) with IC_{50} value of 0.60±0.30 µg/ml and 0.43±0.29 µg/ml, respectively. Therefore the leave extract (FFL) and fractions (FFL10, FFL11) of *Ficus fistulosa* would be a good candidate to develop antiviral against HCV.

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Keywords: Hepatitis C Virus; antiviral activity; *Ficus fistulosa*

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1. Introduction

Hepatitis C was first identified in 1989 as a disease that infects the liver, which is commonly caused by a virus. An estimated 130 to 170 million people, 2% to 3% of the world's population, are living with HCV infection and almost 350,000 people die of HCV each year. The prevalence of HCV in Indonesia is still more than 2 percent (based on serosurveys of voluntary blood donors). Three out of four people with Hepatitis C are potential to suffering from chronic hepatitis C and 10% up to 40% of people with untreated chronic hepatitis C will be likely to develop scarring of the liver (cirrhosis). Approximately 20% of people with cirrhosis will then develop liver failure, and 5% will develop liver cancer, both of which can be fatal[1-4].

HCV is a positive single stranded RNA virus of approximately 9,6 kB that encodes a long polyprotein precursor of ~3,000 amino acids, which is posttranslationally processed by host and virus proteases into mature proteins, including structural proteins (C, E1, E2, and p7) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B)[5,6]. The therapy using pegylated interferon alpha/ribavirin was only effective in approximately 80% and 40–50% of patients infected with HCV genotypes 2–3 and 1, respectively[7]. It has severe side effects such as headache, pyrexia, myalgia, and rigors and high cost[8-10]. Thus, the development of safe and inexpensive antiviral drugs is highly required.

More than 40% of all currently prescribed drugs is derived from chemicals that have been initially identified in plants. Many phytochemicals that have been identified display considerable inhibition of HCV at some stages of the life cycle[11,12]. *Ficus fistulosa* is locally known as *Beunying* and traditionally used to treat wounds. Ficus species are reported as anti Herpes Simplex Virus -1 and 2 (HSV-1, HSV-2), Varicella-Zoster Virus (VZV), Murine Sarcoma Virus (MuSV), anti Moloney Murine Leukemia Virus (MuLV)[13], antimicrobes[14], and antioxidant[15]. Our last publications also reported that *Ficus fistulosa* extract had Anti-HCV activities against 9 different genotypes (1a to 7a, 1b and 2b)[16]. The purpose of this study was to determine the active fractions of *Ficus fistulosa* againts HCV.

2. Methods

2.1. Cells and viruses

Huh7it cells were cultivated in DMEM-Dulbeco's Modiffied Eagle Medium (GIBCO Invitrogen) supplementing 10% Fetal Bovine Serum (FBS, GIBCO-Invitrogen), 1x Non-Essential Amino Acids (NEAA, GIBCO-Invitrogen), and 0.15 mg/ml Kanamycin solution (SIGMA) in 5% CO_2 at 37°C. The culture condition of Huh7it cells was observed under a microscope every day. JFH1a virus (50µl) was propagated by using Huh7it cells. It was suspended in 4 ml medium containing Huh7it ($1.8x10^7$ cells) and incubated at 37°C in 5% CO_2 for 4 hr with agitation for every 30 min. The infected cells were divided into eight T-75 flasks by supplying 10 ml culture medium per flask and incubated for 3 days. We harvested supernatant and removed cell debris by centrifugation at 1,500 rpm, 10 min, 4°C. The supernatant was concentrated by using Amicon-Ultra-15 centrifuge filter. Concentrated supernatant was aliquoted and stored at -80°C until use.

2.2. Extraction and fractionation of Ficus fistulosa

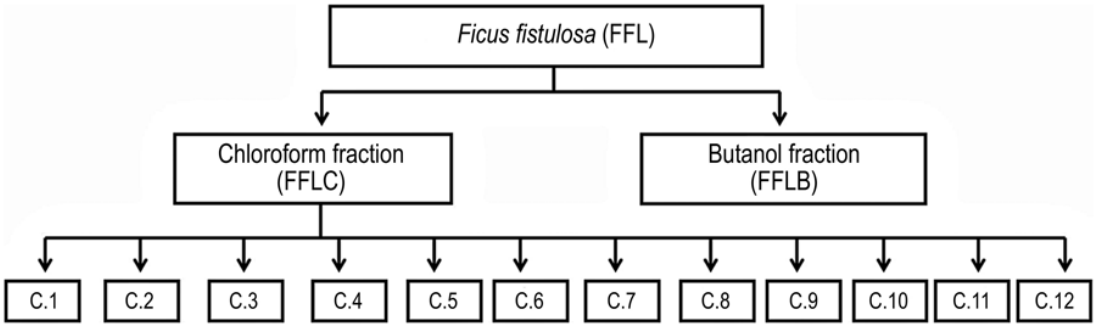

Fig. 1. Extraction and fractionation of *Ficus fistulosa*