ABSTRACT

Inhibition Effect of Faloak (Sterculia quadrifida R.Br) Stem Bark Extract on CD81 Huh7it Cell Line Hepatocytes and NS3 helicase Hepatitis C Virus JFH1

Muhajirin Dean

Hepatitis C Virus (HCV) is the cause of a chronic hepatitis disease, cirrhosis, and hepatic cancer. Available medications able to cure HCV infection remain expensive although they have been included in the government program. Alternative medication using herbal ingredients is still rare and vaccine to prevent hepatitis C has not been discovered. Some attempts should be made to find new medications to treat HCV infection. Recently, scientists around the world have developed plant-based medicinal ingredients. One of these ingredients is flavonoid compound group originating from catechin derivative named epicatechin. Epicatechin has been proved in vitro manner inhibits HCV replication, reduces inflammation caused by HCV, inhibits NS3 protein of HCV, non-toxic, and water soluble. Epicatechin compound is originated from bark of *Sterculia tragacanth, Sterculiaceae family, Sterculia* genus. Based on taxonomical approach, it is assumed that plants from this genus such as *Stercula quadrifida R. Br.* (Faloak) may also contain epicatechin. In East Nusa Tenggara Province especially Kupang City, faloak is empirically used to cure jaundice or hepatitis and has been scientifically proven containing flavonoid.

In this study, the examination in vitro manner on n-hexane, water, and alcohol 70% of faloak stem bark extracts, starting from toxicity examination (CC_{50}), potential inhibition (IC_{50}) against HCV JFH1, time addition test to identify inhibition on HCV JFH1 and ended with western blotting test on CD81 hepatocyte Huh7it cell line and NS3 helicase HCV JFH1. Measurement of epicatechin level was conducted using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS) method of Triple Quad 4000 System.

The CC_{50} examination on hepatocyte Huh7it cell line culture indicates that faloak stem bark extracts with n-hexane, water, and alcohol 70% are low toxicity. The result obtained from IC_{50} examination on HCV JFH1 in hepatocyte Huh7it cell line culture indicates that faloak stem bark extract with n-hexane has poor inhibition on HCV JFH1 while faloak stem bark extracts with water and alcohol 70% are able to inhibit HCV JFH1 and believed to contain epicatechin compound. These two extracts (faloak stem bark extracts with water and alcohol 70%) have been proven to contain epicatechin compound as much as 942 mg/kg and 872 mg/kg. Faloak stem bark extracts with water and alcohol 70% with concentration of 40 µg/ml (twice IC concentration is able to inhibit the entry phase and post-entry phase of HCV JFH1 life cycle. Western blotting test was conducted to identify CD81 protein of hepatocyte Huh7it cell line and NS3 helicase protein of HCV JFH1 exposed to water and alcohol 70% of faloak bark extracts with concentrations of 20 µg/ml and 40 µg/ml. the result indicates that the water extract inhibits CD81 protein of hepatocyte Huh7it cell line while faloak bark extract with alcohol 70 % poor inhibits CD81 protein of hepatocyte Huh7it cell line. Both extracts with water and alcohol 70% inhibit NS3 protein of HCV JFH1. Based on these findings, it can be

concluded that faloak stem bark extracts with water or alcohol 70% provides inhibition on proteins vital in the life cycle of hepatitis C virus. Faloak stem bark extracts can serve as candidate of herbal medicine to prevent and cure hepatitis C virus infection.

Keywords : Sterculia quadrifida RB.r (Faloak), Hepatitis C Virus, Epicatechin.

DAFTAR SINGKATAN

ALT = *Alaninaminotransferase*

APCI = *Atmospheric Pressure Chemical Ionization*

Apo-E = *Apolipoprotein* E

AST = *Aspartate Aminotransferase*

Bcl-x = *B-cell lymphoma-extra*

CC_{50} = *Cytotoxicity Concentration* 50 %

CCG = *Cysteine Cysteine Glycine*

CD81 = *Cluster of Differentiation* 81

CLDN-1 = *Claudin*-1

C-NMR = *Carbon Nuclear Magnetic Resonance*

DMEM = *Dulbecco's Modified Eagle's Medium*

E1 = *Envelope* 1

E2 = *Envelope* 2

EGCG = *Epigallocatechin-3-gallat*

EGFR = *Epidermal growth factor receptor*

EIA = *enzyme immunoassay*

EphA2 = *Ephrin reseptor* A2

ESI = *Electro Spray Ionization*

FT-IR = *Fourier Transform Infrared*

GAG = *Glycosaminoglycan*

HIV = *Human Immunodeficiency Virus*

H-NMR = *Hydrogen* (*Proton) Nuclear Magnetic Resonance*

hVAP-33a = *Human vesicle-associated protein 33a*

IC_{50} = *Inhibitory concentration* 50 %

IFN-α = P*egylated Interferon Alfa*

IRES = *Internal Ribosome Entry Site*

IUDs = *Intravenous Drug Users*

LC MS/MS = *Liquid Chromatography Mass Spectrophotometry/Mass Spectrophotometry*

LDL-R = *Low Density Lipoprotein Reseptor*

LEL = *Large Extracellular Loop*

MAP = *Mitogen-Activated Protein*

MKNK1 = *Kinase interacting serine/threonine kinase 1*

MTT = *Methylthiazolyldiphenyl-tetrazolium bromide*

NCR = *Non Coding Region*

NPC1L1 = *Niemann-Pick C1-Like* 1 *Cholesterol transporter*

NS3 = *Non Structural* 3

NS4A = *Non Structural* 4A

NS4B = *Non Structural* 4B

NS5A = *Non Structural* 5A

NS5B = *Non Structural* 5B

NTPase = *Nucleoside Tri Phosphatase*

OCLN = *Occludin*

ORFs = *Open Reading Frames*

PBS = *Phosphate Buffered Saline*

PCR = *Polymerase Chain Reaction*

PI3K-AKT = *Phophatidyl Inositol 3-Kinase-protein kinase B Active Kinase Tyrosine*

PI4K = *Phophatidyl Inositol 4-Kinase*

PKA = *Protein Kinase A*

RBV = Ribavirin

RhoGTPase = *Rho Guanosin Triphosphatase*

RIPA = *Radio Immune Precipitation Assay*

RNA = *Ribonucleic Acid*

SEL = *Small Extracellular Loop*

SI = *Selectivity Index*

SR-BI = *Scavenger reseptor class B type I*

SVR = *Sustained Virological Response*

TBST = *Tris-buffered saline* 0.1% *Tween* 20

TfR1 = *Transferrin reseptor* 1

VHC = Virus Hepatitis C

WHO = *Word Health Organization*