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AntiHepatitis C Virus Activity of *Alectryon serratus* Leaves Extract

Lidya Tumewu[a], Evhy Apryani[a], Mei Ria Santi[c], Tutik Sri Wahyuni[a,b], Adita Ayu Permanasari[a], Myrna Adianti[a], Chie Aoki[d], Aty Widyawaruyanti[a,b], Achmad Fuad Hafid[a,b]*, Maria Inge Lusida[a], Soetjipto[a], Hak Hotta[d]

[a]*Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115, Indonesia*
[b]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia*
[c]*Department of Microbiology, Faculty of Medicine, University of Indonesia, Jakarta 10430, Indonesia*
[d]*Division of Microbiology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan*

Abstract

Hepatitis C Virus (HCV) has infected approximately 2-3% (130-170 million) of the world's population. No vaccine is available to prevent HCV infection. Investigation of anti-HCV agent is thus deemed necessary. Various plants have been explored for their anti-HCV activity. *A. serratus* is a member of Sapindaceae family, which fruit and seed were traditionally used as insecticide. Anti-HCV activity tested on *A.serratus* leaves extract has been done. The result showed that leaves extract exhibited anti-HCV with IC_{50} value of 14.9 μg/ml and 9.8 μg/ml against HCV J6/JFH1 and JFH1a, respectively. The cytotoxicity assay results showed that *A.serratus* leaves extract was not toxic and has CC_{50} >100 μg/ml. Mode of action experiment results suggested that *A.serratus* extract inhibited HCV at the post-entry step. Further fractionation of leaves extract by open column chromatography resulted in 4 fractions. Only Fraction 1 (AP-5F.1) exhibited anti-HCV with IC_{50} value of 1.2 μg/ml against HCV JFH1a. Separation of AP-5F.1 by open column chromatography resulted in 15 fractions. Fraction number 13 (AP-5F.1.13) exhibited anti-HCV with IC_{50} value of 0.43 μg/ml against HCV JFH1a. Separation of AP-5F.1.13 by semi preparative-HPLC resulted in isolate identified by TLC and LC-MS method as chlorophyll derivate. There was a possibility that chlorophyll derivate has participated in performing the anti-HCV activity of fractions and extract besides the other compounds contained. In this study, we concluded that *A. serratus* leaves extract, AP-5F.1, and AP-5F.1.13 exhibited anti-HCV activity against JFH1a virus.

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Keywords: Alectryon serratus; leaves extract; antiHepatitis C Virus

* Corresponding author. Tel.: +62-31-5992445; fax: +62-31-5992445.
E-mail address: achmadfuad@yahoo.com

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Nomenclature

HCV	Hepatitis C Virus	HPLC	High Pressure Liquid Chromatography
IC_{50}	Inhibitory Concentration 50	CC_{50}	Cytotoxicity Concentration 50

1. Introduction

Hepatitis C Virus (HCV) has infected approximately 2-3% (130-170 million) of the world's population. No vaccine is available to prevent HCV infection. Treatment for HCV has been developed and rapidly evolving. However, several obstacles remain in the current treatment of HCV[1]. Development of anti-HCV agent is thus deemed necessary. Various plants have been explored for their anti-HCV activity. Anti-HCV activity screening of 21 samples from 17 species of medicinal plants explored in the East Java Region, Indonesia revealed that extract of *Toona sureni* leaves (TSL), *Melicope latifolia* leaves (MLL), *Melanolepis multiglandulosa* stem (MMS), *Ficus fistulosa* leaves (FFL) showed antiviral activity with IC_{50} value at a range of 2.0 – 17.1 μg/ml. Time of addition experiments revealed that TSL and MLL inhibited both at the entry and post-entry steps, while MMS and FFL principally at the entry step[2]. Several numbers of isolated compounds were also reported to exhibit antiviral activity against HCV.

Glycycoumarin, glycyrin, glycyrol and liquiritigenin isolated from *Glycyrrhiza uralensis* were identified as anti-HCV compounds with IC_{50} value of 8.8, 7.2, 4.6 and 16.4 μg/ml, respectively. Those compounds acted on the post-entry step[3]. Quercetin and gallic acid isolated from *Kalanchoe pinnata* also inhibited HCV production in a dose-dependent manner, with IC_{50} value of 1.5 and 6.1 μg/ml, respectively, without exhibiting cytotoxicity. Quercetin acts at the post-entry step, whereas gallic acid at both the entry and post-entry steps[4]. In this study, the anti-HCV activity of *Alectryon serratus* has been determined. *A. serratus* is a member of Sapindaceae family, which fruit and seed were traditionally used as insecticide. It was reported that *A.serratus* leaves extract has antimalarial activity with IC_{50} value of 12.3 μg/ml and antioxidant activity with IC_{50} value of 1.96 μg/ml[5,6], but no reference was found regarding the antiviral activity of this plant. This study aims to determine the active substances of *A.serratus* extract which play a role in anti-HCV activity.

2. Methods

2.1. Plant material

Alectryon serratus leaves were collected from Alas Purwo National Park, Banyuwangi, East Java, Indonesia. Authentication and identification of plant were carried out at The Purwodadi Botanical Garden, East Java.

2.2. Extraction and fractionation

Leaves of *A.serratus* were dried at room temperature and pulverized. Dried leaves powder were extracted using ethanol 80% as solvent by ultrasonic assisted extraction for two minutes to three times replications. The ethanol extract was filtered and the obtained filtrates were concentrated using rotary evaporator to obtain ethanol extract of *A.serratus* (AP-5F). Further separation of AP-5F was done by open column chromatography using silica gel as stationary phase and gradient of chloroform-methanol system as development solvent. The separation resulted in 4 fractions (AP-5F.1–4) which were later continued for bioassay. A bioactivity-active fraction(s) was further fractionated under open column chromatography with sephadex LH-20 and 90% mobile phase methanol. The separation of AP-5F.1 resulted in 15 fractions (AP-5F.1.1-1.15). AP-5F.1.13 was further separated by semi preparative HPLC (solvent system: acetonitrile-methanol-water 75:2.5:22.5, column: Water XBridge 10x250 mm 5μm, flowrate: 2.5 ml/min, detection UV 365 nm).