In Vitro study of *Garcinia celebica* L. Stem Barks against Hepatitis C virus and Hepatocellular Carcinoma

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Abstract. In this study, we are focused on *Garcinia celebica* as anti-HCV activity against JFH1 strain genotype 2a. The extraction process employed the use of maceration method. Starting from n-hexane solvent and another residue. The extracts were then analysed with various doses of 20, 10, 5, 2.5, and 1.25 μg/mL for anti-HCV against JFH1 strain genotype 2a and anticancer against Huh7it-1 cell line. The extracts exhibited anti-HCV on 1.25 μg/mL and no sign of toxicity to Huh7it-1 cells. The extracts also expressed anticancer activity on 20, 10, 5, and 2.5 μg/mL with a high level of toxicity to Huh7it-1 cells. The results from this study suggest that Garcinia *celebica* may be necessary as an add-on therapy candidate for treating HCV infections and HCC.

1. Introduction

Hepatitis C virus (HCV) is a *Flaviviridae* virus that possesses a stranded-positive RNA genome with 9600 bases long. HCV can lead to hepatocellular carcinoma (HCC) which can develop into liver dysfunction. It can result in death if proper preventive measures are not taken. From statistics, over 350,000 people die of HCV-related liver diseases including HCC each day [1].

Some available therapy of HCV which includes pegylated interferon (PEG-IFNα) and ribavirin have many adverse effects. They are highly expensive, and not all patients respond to them [2]. These therapies may have direct-acting antivirals (DAAs) just like simeprevir, boceprevir, telaprevir, daclatasvir, and sofosbuvir can lead to 80%, and 90% sustained virologic response (SVR). Another alternative therapy is the exploration of antiviral drugs and anticancer for Hepatitis C with HCC obtained from medicinal plants.

The *Garcinia* genus, also referred to as mangosteen has been widely studied. It has been observed that it tends to be used as a vitamin. This observation was preceded by the practical experience performed by several communities for generations. Traditionally leaves, roots, and stem barks are widely used to treat fever, diarrhea, dysentery, and skin diseases [3]. The *Garcinia* genus is
antimicrobial, antimalarial, antioxidant, hepatoprotective and antiviral [3]. In a research carried out some time ago, the acetone extract of Garcinia celebica L. stem barks (GC) with a single dose 20 μg/mL for antiviral activity against JFH1 strain genotype 2a and anticancer cytotoxicity. This research was against Huh7-it-1 cell line which expressed 5.7% infection and 99.7% toxicity, respectively [4]. However, the in vitro antiviral activity and anticancer cytotoxicity of GC with lower than 20 μg/mL have not been confirmed. In this research, the acetone extract of Garcinia celebica L. stem barks (GC) with some doses 20, 10, 5, 2.5, and 1.25 μg/mL was examined regarding its inhibitory effect on replication of HCV and hepatocellular carcinoma.

2. Research method

2.1. Plant material

We obtained leaves of stem barks of Garcinia celebica (GC) from the Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Serpong, Indonesia. Some botanists at the Botanical Research Center for Biology, LIPI, Cibinong, Indonesia, identified the plant species. An herbarium specimen was deposited in the Research Center for Chemistry, LIPI.

2.2. Preparation of extract

Dried GC (1.7 kg) were grounded to powdered form and extracted (4-times) with the aid of n-hexane (4 L) under reflux condition. The filtrate result was evaporated using an evaporator at temperature 40ºC. The filtrate was obtained by n-hexane extract; the residue hexane was extracted (4-times) using acetone respectively in 4 L solvent. The extracts were combined and concentrated under vacuum at 40ºC with a rotavapor to obtain the crude extract. The crude extract was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL and stored at a temperature of -30ºC.

2.3. Cells and viruses

The cells used were cloned from a human hepatocellular carcinoma-derived cell line (Huh7-it-1) [5]. They were cultured in Dulbecco's modified Eagle's medium (Gibco-Invitrogen) supplemented with 10% fetal bovine serum (Biowest), non-essential amino acids (Gibco-Invitrogen) and kanamycin (Sigma-Aldrich) with the condition at 37ºC in a 5% CO2 humidified chamber. The viruses used a JFH1 strain of HCV with genotype 2a. They were propagated as described previously [6].

2.4. Antiviral activity

An antiviral activity was performed as discussed previously [5]. In summary, Huh7-it-1 cells were inoculated with HCV with a multiplicity of infection 0.1 in GC (20, 10, 5, 2.5, and 1.25 μg/mL) for two h at 37ºC. After removing all residual virus by washing, the cells were incubated for 46 h with the same GC. The Cell culture supernatants were collected for virus titration.

2.5. Virus titration

The virus titrations were carried out as described previously [5]. The Cells were kept in 96-well plate and inoculated with culture supernatants serially diluted in culture medium. After virus adsorption was carried out for 4 hours at a temperature of 37ºC, the cells were incubated for 40 h in medium with 0.4% methylcellulose (Sigma-Aldrich). The Titer of HCV were generated using a focus-forming assay [6]. Virus-infected cells were stained anti-HCV serum from human patients followed by horseradish peroxidase (HRP)-conjugated goat anti-human IgG (MBL, Nagoya, Japan). Infectious focus-forming were visualized using the metal enhanced DAB substrate kit (Thermo Fisher). focus-forming images were captured with Olympus digital camera DP21 attached to an Olympus CKX41 microscope (Olympus, Tokyo, Japan), counted using a Katikati counter and normalized with untreated controls to calculate the titers of HCV.
2.6. Cytotoxicity assay
The MTT assay examined cytotoxicity of GC against Huh7it-1 cells. The cells were briefly seeded in 96-well plates and treated in the presence of extracts (20, 10, 5, 2.5, and 1.25 μg/mL) for 48 hours. The MTT assay was carried out as described previously [5].

2.7. Statistical analysis
Results were expressed as the mean ± SD. The differences between the two data sets were analyzed using Student's two-tailed t-test. A P-value (P) of <0.05 was considered statistically significant.

3. Results and discussion
The anticancer cytotoxicity of GC against Huh7it-1 cell line was analyzed by MTT assay. The GC cytotoxicity expressed itself at 20, 10, 5, and 2.5 μg/mL concentrations, while the cytotoxicity exhibited concentrations of 1.25 μg/mL via the cell of 27.19% (figure 1). The CC50 (the concentration of the GC needed to achieve a 50% cytotoxicity against Huh7it-1 cell line) was 1.6 μg/mL.

The anti-HCV of GC against JFH1 strain genotype two was examined by the Immunohistochemical assay. The GC inhibition was exhibited at concentrations of 20, 10, 5, 2.5, and 1.25 μg/mL of 100%, 100%, 100%, 100%, and 93.4%, respectively (figure 1). The IC50 (the concentration of the GC required to achieve a 50% inhibition replication against JFH1 strain genotype 2) was < 1.25 μg/mL.

From the data obtained on cytotoxicity and inhibitory of the GC, the potential of antiviral hepatitis C with selectivity index (SI) to potentiate antiviral hepatitis C was derived from CC50 divided by IC50 obtained (1.6 μg/mL / <1.25 μg/mL) to get the Selectivity index value of > 1.28. The potential of antiviral hepatitis C activity is shown at a concentration of 1.25 μg/mL which has inhibition of 93.4% with low cytotoxicity of 27.19%, while the high toxicity of GC at concentrations of 20, 10, 5, and 2.5 μg/mL is possible for a potential anticancer compound against Huh7it-1 cell line which is a type of hepatocellular carcinoma cell line.

![Figure 1](image.png)

Figure 1. Anti-HCV activity and cytotoxicity of GC. Inhibition and toxicity of GC (20, 10, 5, 2.5 and 1.25 μg/mL) or cyclosporin A (CyA 1 μg/mL as an inhibitor control) were shown. Values represent means ± SD of data from triplicate.

The phytochemical study of stem bark Garcinia celebica consists of various secondary metabolites including triterpenoids, coumarin, tannins, glycosides, flavonoids, and saponins [7]. Another research conducted on secondary metabolite content from the stem Garcinia celebica also contains epicatechin [8], garcinisidone H, and six triterpenoids [9]. The studies of epicatechin against shows that activity decreased NS5B HCV protein and HCV RNA and inhibited hepatocarcinogenic by inhibition of cyclooxygenase-2 (COX-2) [10].
A previous study was performed on the secondary metabolites of the bark of Garcinia celebica containing garcinisidone H, and six triterpenoids indicating a potent anticancer activity against breast cancer cell (MCF-7) [9]. The GC has a potent antiviral activity of Hepatitis C and anticancer of hepatocellular carcinoma. Hence this preliminary study should be considered for further fractionation and isolation studies. The reason for the initial research is to ascertain the type of secondary metabolite active against Hepatitis antiviral activity.

4. Conclusions
In conclusion, the present study explains that GC shows anti-HCV activity and anticancer of hepatocellular carcinoma. Furthermore, GC can be considered for further fractionation and isolation of the active compounds required to know the antiviral compound against HCV and anticancer of hepatocellular carcinoma.

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