The Activity of *Sterculia quadrifida* R.br Stembark against Hepatitis C Virus

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Abstract: *Sterculia quadrifida* R.br is commonly known in East Nusa Tenggara, Indonesia as "Faloak". Itsstembark has been traditionally used to cure liver disease. One of the active agentscausing liver disease is theHepatitis C Virus (HCV). Meanwhile, there is no information on the activity of this plant for anti-HCV. This study was aimed to investigate the anti-HCV activity and cytotoxicity of extracts and fractions from *S. quadrifida* R.br stembark. The stembark of S. *quadrifida* R.br was extracted using several different solvents. The stembark was gradually extracted using n-hexane, dichloromethane, and methanol. It was also extracted using 70% ethanol by ultrasonic-assisted extraction method as well. In addition, it was extracted using water by decocta method. All samples were further analyzed for their anti-HCV activity using Huh7it cell and HCV JFH1a virus, while the cytotoxicity was determined by MTT assay. The most active extract was further separated by column chromatography and the fractions were tested for their anti-HCV activity and cytotoxicity. The anti-HCV assay results showed that water, 70% ethanol, and methanol were active against HCV with an IC_{50} value of 6.06 µg/ml, 9.44 µg/ml, and 10.39 µg/ml, respectively. Meanwhile, the hexane and dichloromethane extracts were less active against HCV with IC_{50} values of 51.93 µg/ml and 179.31 µg/ml, respectively. The fractionation of water extract as the most active extract resulted in seven fractions. Fractions 5 and 6 showed highest activity with IC_{50} values of 7.60 µg/ml and 8.87 µg/ml, respectively. Furthermore, the cytotoxicity of these two active fractions exhibited no toxicity with CC_{50} value of >2,000 µg/ml. Methanol extract, 70% ethanol extract, water extract, fraction 5, and fraction 6 of water extract from *S. quadrifida* R.br stem bark had potential activity as anti-HCV.

1. INTRODUCTION

Hepatitis is one of the dangerous diseases caused by Viruses. HCV is a virus include flaviviridae family, a family with dengue virus and yellow fever, and is an RNA virus. HCV, a virus with high envelope heterogeneity and has variations of seven genotypes. Hepatitis C virus spreads through direct contact with blood or blood products infected with HCV [1,2]. In the world, there are more than 170 million people suffering from chronic hepatitis C infection with about 3 million new infections each year and 1-3% global prevalence [3].

Medicinal plants are a promising source of drug candidates for HCV infection. The previous study about natural product that ethanol extract *M. latifolia* as antiviral activity with IC_{50} value of 3.5±1.4µg/ml against HCV JFH1a with CC_{50}>100 µg/ml [4]. In plants that contain various types of active chemical components such as flavonoids, terpenoids, lignin, sulfites, polyphenols, coumarins, saponins, alkaloids, proteins and peptides, tend to inhibit the replication cycle of various types of DNA or RNA viruses [7] . Based on these there is a need to develop safe, cheap, and is well tolerated for HCV infection [13].

Sterculia quadrifida R.br, commonly known by the name Faloak, has been used by the Timorese in East Nusa Tenggara society to cure various diseases. Faloak stembark can cure jaundice, typhoid, ulcers, and hepatitis. The stembark of faloak is commonly used for healing various diseases. In East Nusa

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Tenggara (NTT), people consume Faloak by cutting the stembark into small pieces and then boiled it with water for as much as 3 cups and then take them regularly after eating. The use of faloak stembark as a traditional medicine was commonly found in Timor NTT.

Previous studies reported isolated naptokuinon derivate compound from faloak stembark, which was identified as 2,3-dihydro-6-hydroxy-2 methylenenaphtho [1,2-b] furan-4,5-dione active as an anticancer with IC50 value in breast cancer cells of 9.88 µg/mL and with an index selectivity value of 30.23 [6]. The family *sterculiceae* contains chemical compounds of alkaloids, phenyl propanoids, flavonoids, terpenoids and other types of compounds, including hydrocarbons, sugars, quinones, phenolic acids, lactones, lignans, amines and amides. Test results by the Institute of Integrated Research and Testing (LPPT) UGM showed secondary metabolite compounds in faloak containing phenolic acids, flavonoids, alkaloids, and terpenoids. However, further studies to identify the active extract and fraction which are responsible for anti HCV activities have not been conducted yet. Therefore, this study was conducted to identify active compound from *Sterculia quadrifida* R.Br and analyzed it for anti-HCV.

2. EXPERIMENTAL

2.1 Plant Material

S.quadrifida stembark was collected from Penfui Kupang East Nusa Tenggara, Indonesia. Authentication and determination of plants were carried out at Purwodadi Botanical Garden-Indonesia Institute of Science, East Java.

2.2 Extraction and Fractionation of

S,quadrifida stembark were dried at room temperature and grinded for as much as 800 grams. The stembark of S. *quadrifida* R.br was extracted using several different solvents. The stembark was gradually extracted using n-hexane (sqsh), dichloromethane (sqsd), and methanol (sqsm). It was also extracted using 70% ethanol (sqse) by ultrasonic assisted extraction method as well. In addition, it was extracted using water (sqsw) by decocta method. All samples were further analyzed for their anti-HCV activity using Huh7it cell and HCV JFH1a virus, while the cytotoxicity was determined by MTT assay. The most active extract was further separated by column chromatography, and the fractions were tested for their anti-HCV activity and cytotoxicity.

2.3 Cells and Viruses

Huh7it hepatocyte cells cultured in the medium Dulbecco's modified Eagle (Invitrogen, Carlsbad, CA, USA) with an additional 10% Fetal bovine serum (Biowest, Nuaille, France), kanamycin (SigmaAldrich, St. Louis, MO, USA) and non-essential amino acids(Invitrogen). Every cell growth in the petridish reaches >80% passage cell. Viral culture is done with collecting supernatants from Huh7it cell cultures infected by HCV JFH1. Supernatants collected on the 3th to 5th days after infection are then concentrated using Amicon filters and stored at -80°C.

2.4 Cytotoxicity Test

Toxicity testing of anti-HCV assay material was performed on Huh7it hepatocyte cells with the addition of the sample without inoculation HCV JFH1a being visible from the ingredients for hepatocyte cells without the presence of HCV infection. The toxicity test results were obtained by measurement absorbance at wavelengths 560 nm and 750 nm. The cytotoxicity test was performed on extract and fraction.

2.5 Analysis of Anti-HCV Activities

S.quadrifida extract and fraction were dissolved in dimethyl sulfoxide (DMSO) to obtain stock solution at a concentration of 100 mg/ml. The stock solution was stored at-20°C until it was used. Huh7it cells were seeded in 48-well plates ($5x10^4$ cells/well). A fixed amount of JFH1a, with multiplication infection (MOI) of 0.1 was infected on Huh7it cell then treated with the presence of extract and fraction of *S.quadrifida*.The virus titer was counted after being stained with DAB thermo staining.

3. RESULTS

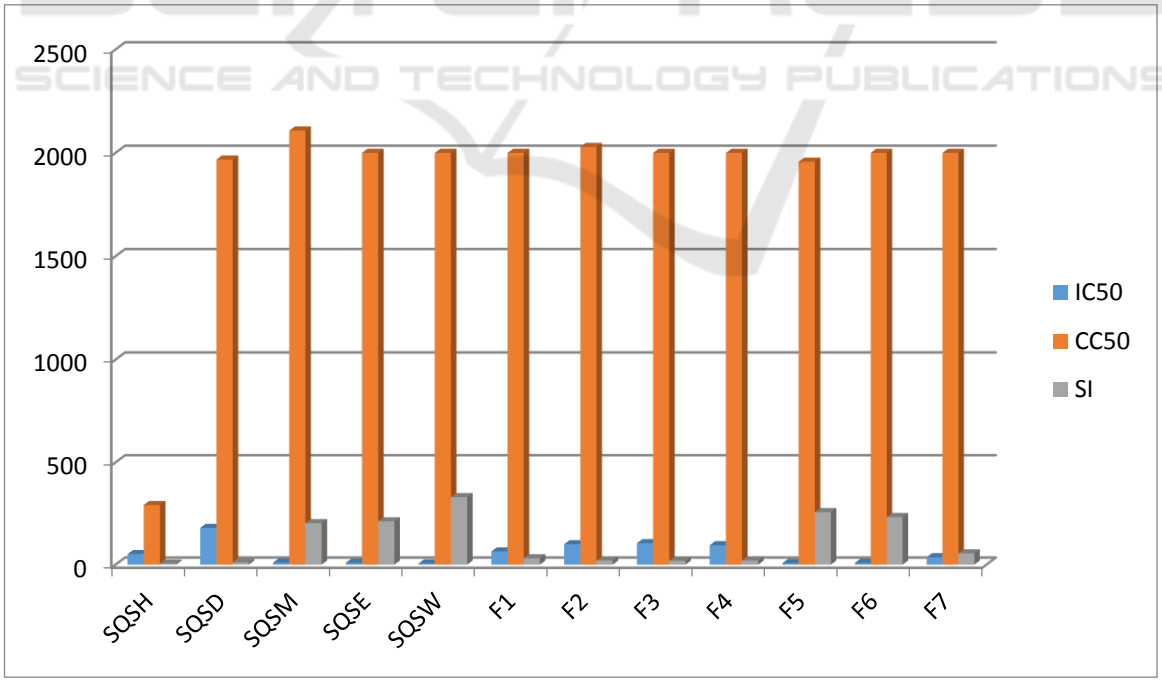
The anti-HCV assay results showed that water (sqsw), 70% ethanol (sqse) and methanol (sqsm) were active against HCV with IC_{50} values of 6.06±0.09 µg/ml, 9.43±0.12 µg/ml, and 10.37±0.96 µg/ml, respectively. Meanwhile, the hexane (sqsh) and dichloromethane (sqsd) extracts were less active against HCV with IC_{50} values of 51.94±0.62 µg/ml

and 179.63±1.88 µg/ml, respectively. The results were then treated with further separation. The fractionation of water (sqsw) extract as the most active extract resulted in seven fractions. Fractions 5 and 6 showed highest activity with IC_{50} values of 7.62±0.04 µg/ml and 8.6±0.21 µg/ml, respectively.

Tests were conducted on Water Extract, Methanol, Ethanol 70%, Dichloromethane, Hexane, and Water Faction 1-7. Furthermore, the water extracts and two active fractions exhibited no toxicity with CC_{50} value of >2,000 µg/ml.

Table 1. The anti-HCV activity (IC_{50}), toxicity (CC_{50}), and selectivity index (SI) of extracts and fractions

Sample	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI (CC_{50}/IC_{50})
Sqsh	51.94±0.62	291,4	5,611
Sqsd	179.63±1.88	1967,9	10,97
Sqsm	10.37±0.96	2108	202,88
Sqse	9.43±0.12	>2000	211,86
Sqsw	6.06±0.09	>2000	330,03
Sqsw fraction 1	64,67±0.53	>2000	30,92
Sqsw fraction 2	100.04±1.37	2028,6	20,27
Sqsw fraction 3	105.08±2.44	>2000	19,03
Sqsw fraction 4	95.05±4.13	>2000	21,04
Sqsw fraction 5	7.62±0.04	1957,2	256,82
Sqsw fraction 6	8.6±0.21	>2000	232,58
Sqsw fraction 7	36.38±3.83	>2000	54,97



4. DISCUSSION

The development of natural materials as drug candidates, extraction, fractionation, and activity testing and toxicity should be performed simultaneously, which is known as bioassay guided

isolation. This means that the subsequent separation is only performed on the selected extract or active fraction as anti-HCV. The anti-HCV antiviral activity screening was conducted on a concentration of 30 µg/ml, which found that the water extract, 70% Ethanol, and Methanol had 100% inhibition value, while the Hexane and DCM extract did not have anti-HCV activity. The results of inhibition percentage were showed, then IC_{50} value was calculated using probit log and the result showed that Methanol had resistance to Hepatitis C antivirus with an IC_{50} value of 10.39 µg/ml. Ethanol 70% had activity against Hepatitis C antivirus with an IC_{50} value of 9.44 µg /ml and Water had activity against Hepatitis C with an IC_{50} value of 6,06µg/ml. The selected water extract was included in the next separation process.

The profile of TLC *S. quadrifida* with stationary phase RP 18 and mobile phase Methanol : Water TFA 0.03% (1:2) showed the presence of blue and green on uv 366, possibly containing phenolic group compounds. Plants containing a wide variety of active chemical components, such as flavonoids, terpenoids, lignins, sulfites, polyphenols, coumarin, saponins, alkaloids, proteins, and peptides, tend to inhibit replication cycles of different types of DNA or RNA [7]. Previous studies on*S. Quadrifide* showed that it contains phenolic acid compounds that can inhibit the growth of *C. albicans* bacteria, with 3-hydroxyoctadecanoic compound is the main compound in the extractive substance of *S. quadrifida* to inhibit the growth of *C.albicans* fungi [8]. Previous study to analyze the antibacterial and antioxidant of ethanol extract of *S. quadrifida* bark. Fraction 3 showed the highest antibacterial activity (IC50) against *B. subtilis* bacteria (90.51 µg/mL), *E. coli* (80.12 µg/mL), *S.aureus* (77.87 µg/mL), and *S. thypi* (61.23 µg/mL). The antioxidant activity test showed that fraction 2 had the highest phenol content (34.16±0.76 mg) and antioxidant activity [9].The main content of phenolic acids and flavonoids in the stembark of *S. quadrifida*had various effects on various organisms, including to cure lumbago, kidney, rheumatic, liver, and other internal diseases [10,11].

The toxicity test was conducted on Water Extract (sqsw), Methanol (sqsm), Ethanol 70% (sqse), Dichloromethane (sqsd), Hexane (sqsh), and Water Faction 1-7. Toxicity testing was performed using an MTT reagent, which was then converted to crystals of purple formazan by succinic dehydrogenase in the mitochondria in living cells. The higher intensity of purple color produced meant more number of living cells [7,12]. From the toxicity test results, it is known the extraction and fraction of water had a good safety value and were safe to use at the separation stage to obtain the active substances contained in faloak.

CONCLUSION

Methanol extract (sqsm), 70% ethanol extract (sqse), water extract (sqsw), fraction 5, and fraction 6 of water extract from *S. quadrifida* R.br stembarkhad potential activity as anti-HCV.

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