

Anti Hepatitis C virus activity screening on Harpullia arborea extracts and isolated compound

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AntiHepatitis C virus activity screening on *Harpullia arborea* extracts and isolated compound

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4 INTRODUCTION

Hepatitis C is a major healthcare problem worldwide. Available therapy for hepatitis C treatment is very expensive and probably not be accessible for all patients. Regarding to this reason, the development of safe and inexpensive antiviral drugs is required. Natural products as a source of new drugs are potential to study. Some antiHCV substances from plants were obtained (Wahyuni, 2013; Adianti, 2014; Aoki, 2014).

Harpullia arborea (tulip wood tree) is a member of Sapindaceae family commonly known as kayu pacat in Indonesia (Basuni, 1997). Traditionally, watery exudates from barks and fruits is used as leech repellent, oil extracted from seeds is a source of antirheumatics (Singh, 2011). *Harpullia* seeds extract also shown antibacterial activities against various strains of bacteria. *Harpullia* seeds contain glycosides, steroids, saponins and resins (Gowri, 2009). A norhopane triterpenoid also isolated from the leaves of *Harpullia* (Poovapathanachari, 2008).

This study was conducted to determine antiHCV activity of *Harpullia* extracts and isolated compound.

MATERIALS AND METHOD

Plant material

Harpullia arborea was obtained from Alas Purwo National Park at Banyuwangi, East Java. Sample was authenticated by the authority of Purwodadi Botanical Garden, Pasuruan, East Java.

Extraction dan fractionation

Harpullia was extracted by ultrasonic assisted extraction method using 80% ethanol as a solvent. Liquid fractionation was conducted using dichloromethane, ethyl acetate and buthanol respectively.

AntiHCV activity test

Extract was examined for antiHCV activity against JFH1a and J6/JFH1 in a cell culture system using Huh7 cells at a multiplicity of infection (MOI) of 0.1.

RESULTS DAN DISCUSSION

Anti-Hepatitis C Virus (anti-HCV) activity screening of *Harpullia* leaves and stem extract revealed that leaves extract exhibited anti-HCV with IC₅₀ value of 17.5 µg/ml and 12.4 µg/ml against HCV JFH1a and J6/JFH1 respectively, meanwhile stem extract was found to be not active against both HCV type.

Fractionation of leaves extract resulted in 4 fractions which were dichloromethane, ethyl acetate, buthanol and aqueous fraction. Anti-HCV activity screening at a concentration of 30 µg/ml revealed that buthanol fraction inhibited HCV JFH1a growth by 54% in which other fractions only inhibited by 15-30%. Buthanol fraction contains yellow spot on TLC profile as a major compound. Further separation of buthanol fraction using sephadex LH-20 and methanol 90% as a solvent was obtained 7 fractions (B1-B7). Fraction B5 contain yellow precipitate and by recrystallization process obtained a yellow crystal as a glycosylated flavonoid compound which identified as Kaempferitrin (3,7-di- α -L-rhamnopyranosyl kaempferol). Structure determination of compound was done by nuclear magnetic resonance spectroscopy and data were compared with references (Ouyang Ming-An, 2003; De Souza Menezes, 2007). Kaempferitrin was further tested against JFH1a. Anti-HCV activity test shown that kaempferitrin was not exhibited anti-HCV. It is possible to explain that anti-HCV activity of extract and buthanol fraction was produced by other compounds in the extract and buthanol fraction instead of kaempferitrin or the activity was created

by synergism effect of many compounds. Previous studies were reported some activity of kaempferitrin. Like many flavonols, it has antimicrobial, antioxidant and antiinflammatory activities. It is also mimics insulin in **2**mutulating glucose uptake in diabetic rats, but inhibits insulin-stimulated glucose uptake in 3T3-L1 cells (Jorge, 2004; Prasad, 2009). But no report about antiviral activity of kaempferitrin was found. Further study need to be done to investigate the antiHCV compounds of *H.arborea*.

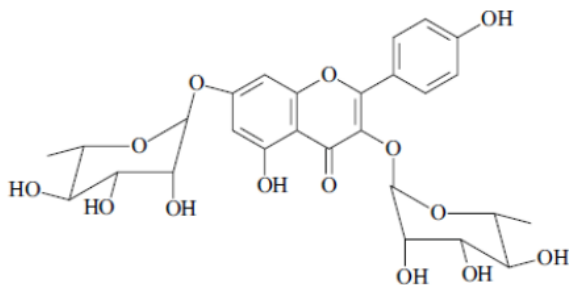


Figure 1. Kaempferitrin (3,7-di- α -L-rhamnopyranosyl kaempferol)

CONCLUSION

In this study, we concluded that *H.arborea* leaves extract and butanol fraction were exhibited anti-HCV activity against JFH1a virus, while the isolated compound, kaempferitrin was not.

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