ABSTRACT

Dengue fever (DF) and dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) are caused by four closely related viruses (DENV-1, DENV-2, DENV-3 and DENV- 4) of the *Flaviviridae* family. According to the World Health Organization (WHO), DF and DHF/DSS are endemic in over 100 countries, with more than 2.5 billion people at risk for epidemic transmission and an estimated 50 million infections each year. Epidemics of dengue have been reported regularly, particularly in crowded urbanized areas in tropical and subtropical regions, including Southeast Asia. Therefore, the aim of this study is to produce an antiviral drug compound from *Quercus Infectoria* and purified natural products for dengue virus infection. The inhibition of the cis cleavage by dengue protease on a fluorogenic peptide as substrate was used to screen inhibitors from tropical plant extracts. The biologically active recombinant DENV-2 protease was expressed as a heterodimeric complex of NS2B/NS3. Each assay was performed using the optimum enzyme concentration of 2.0 µM and 100 µM Boc-gly-arg-arg-MCA as the substrate. The aqueous extract of *Quercus Infectoria* showed strong inhibition towards the dengue 2 protease (greater than 90%). The active compounds from *Quercus Infectoria* water extract were isolated and further fractionated using column chromatography and assayed by a thin layer of chromatography. The solvent systems used were n-hexane, ethyl acetate, acetonititrle, methanol and water with an increase of 10% polarity for each step. The compounds, when isolated, have shown some activity against dengue 2 protease, which was identified by HPLC, followed by NMR. Fractions one and two which contain the active compounds ellagic acid and gallic acid, when viewed under UV light using short wavelength (254nm), showed a yellow spot and gave a green spot when stained with ferric chloride-potassium ferricyanide reagent. Ellagic acid and gallic acid were found to be very effective against NS2B/NS3 DENV-2 protease. The Ki value for ellagic acid was found to be 58.64 µM

and the Ki value for gallic acid is 72.62 µM. The data was analyzed using the Michaelis-Menten model under non-linear regression curve fit in GraphPad Prism 5 software and the type of inhibitors were classified according to *Alpha* value. Both inhibitors were determined to be non-competitive.