UNIVERSITI TEKNOLOGI MARA

IN-VITRO INHIBITION OF MALAYSIAN GROWN *AQUILARIA SPP.* EXTRACTS AND KINETIC ON PANCREATIC LIPASE

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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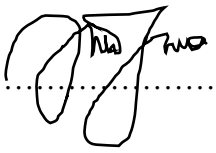
Signature of Student	:	 ……..
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ABSTRACT

Pancreatic lipase inhibitory compounds, which are gallic acid and quercetin, are among the effective inhibition compounds that had been identified in natural remedies. In the current work, the content of gallic acid and quercetin in the crude leaves extract of widely grown *Aquilaria spp.* in Malaysia, which was *Aquilaria malaccensis* and *Aquilaria subintegra*, that was obtained after soaking, ultrasonication and hydrodistillation process were determined. Moreover, the inhibition activity, kinetic mode and behaviour, mechanism, equation and kinetic parameters of in-vitro pancreatic lipase inhibition also were suggested and evaluated based on the linear model of Michaelis–Menten kinetic plots, namely, Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf. The *Aquilaria spp.* leaves were dried for 24 hours at 60^{o}C before it was ground and sieved into a particle size of 250 µm, 300 µm, 400 µm, 500 µm, and 1000 µm. Each particle size was water-soaked at a ratio of 0.5:100, 1.0:100, and 1.5:100 (w/v) for 24 hours and underwent the ultrasonication process (37 kHz), at the temperature of 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C, and 80^{o}C for 30 minutes. The results disclosed that the highest concentration of gallic acid and quercetin equivalent determined was 89.99 mg/ml and 0.0295 mg/ml in *A.malaccensis* and 101.27 mg/ml and 0.0373 mg/ml in *A.subintegra* crude extract of the particle size of 250 µm, respectively, extracted at the ultrasonication temperature of 60^{o}C and soaking ratio of 1.0:100 (w/v). The FTIR spectrum also exhibits the stretching of the O–H groups that were identified at 3297-3224 cm^{-1}, showing that the inhibition functional group exists in the extract, which results in 77.7% inhibition of pancreatic lipase by *A.malaccensis* and 82.34% inhibition by *A.subintegra*. The 50% inhibition concentration of gallic acid and quercetin equivalent calculated from the linear regression method were 54.631 mg/ml and 0.0195 mg/ml in *A.malaccensis* and 57.373 mg/ml and 0.0185 mg/ml in *A.subintegra,* respectively. The Lineweaver-Burk plot of the inhibition activities exhibited the mixed-typed pancreatic lipase inhibition, which indicates that the inhibitors were attached to the free pancreatic lipase and also binding to the pancreatic lipase-*p*-NPP complex. The reaction mechanism was similar to the non-competitive inhibition; however, the value of dissociation constant (K_i) which denoted by K_{ia} and K_{ib}, of the diverse pathways of gallic acid and quercetin binding to pancreatic lipase was different, where the value of K_{ia} was lower than K_{ib}, which indicates that this mixed-inhibition was predominantly towards competitive inhibition. The value of cooperativity coefficient (n) of this inhibition was above one which presents that the multiple ligand binding occurred and this proved the existence of allosteric sites for the mixed-inhibition to take place. The kinetic parameters determined in the inhibition at various concentrations of *p*-NPP and pancreatic lipase using the Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf kinetic plots gave the increasing value of K_m with the decreasing value of V_m, k_{cat}, and k_{cat}/K_m compared to the non-inhibition reaction. In the comparative study of the experimental and theoretical kinetic plots, the error of R^2 was between 0.07% and 60.62%. The results showed that the data of inhibition activities fit well in the Michaelis-Menten and Hanes-Woolf kinetic plots with an error in R^2 of less than 5%. In conclusion, this study revealed that *Aquilaria spp.* leaves contributed a substantial pancreatic lipase inhibition activity that makes it a potential natural product for diabetes control. The kinetic study conducted can be used as guidance in finding the mechanism and the optimal kinetic inhibition activity of plant-based inhibitors used in diabetes therapy.

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