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Inhibition of pancreatic lipase by gallic acid and quercetin equivalent in ultrasonicated Malaysian grown *Aquilaria spp.* leaves of different particle size

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Abstract

Pancreatic lipase inhibitory compounds (gallic acid and quercetin) and their inhibition properties were determined in the crude extract of matured leaves from widely grown *Aquilaria spp*. in Malaysia, which are *Aquilaria malaccensis* and *Aquilaria subintegra*. The leaves were dried at 60 °C for 24 hours and undergone a pre-treatment method by using ultrasonication, within 30 minutes of reaction. Dried matured leaves were ground and sieved into particle sizes of 250, 300, 400, 500, and 1000 µm. Each particle size was soaked in distilled water with a ratio of 1% (w/v) for 24 hours, prior to ultrasonication process. Particle size of 250 µm resulted in the highest concentration of gallic acid and quercetin equivalent for both species of *Aquilaria,* wherein 89.99 mg/ml of gallic acid equivalent and 0.0295 mg/ml of quercetin equivalent were obtained in *A. malaccensis* crude leaves extract and in *A. subintegra* crude extract, 101.27 mg/ml and 0.0373 mg/ml were obtained, respectively. Thus, this sample was used in the inhibition study of pancreatic lipase. The concentrations of substrate and enzyme activity prepared were varied in the inhibition kinetics study. Based on the inhibition of pancreatic lipase, the crude leaves extract of both *Aquilaria* species exhibited the mixed-inhibition which is also known to be the non-competitive inhibition because the reaction mechanism showing the binding of inhibitor to the free pancreatic lipase and pancreatic lipase-substrate complex. This research is one of the fundamental works for future studies on the natural source to control obesity, which is a new direction of the key research in lipase inhibition.

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1.0 Introduction

Recently, obesity has become a major cause of health complaint in developed countries like Malaysia and it also associated with excess weight. In New Straits Times (2012), Datin Paduka Dr Santha Kumari, Chairman of The Selangor Branch of the Malaysian Diabetes Association mentioned that Malaysia is leading in the prevalence of obesity among Southeast Asian countries and it is still on the upward trend. Almost one in two Malaysians is overweight or obese which places them at a high risk for diabetes. The excessive activities of pancreatic lipase which are involved in breaking down fat is found to be the cause and the alternatives solution to slow down the enzyme activities is by taking the inhibitors. However, the synthetic drug inhibitors are found to give gastrointestinal side effects which can cause vitamin deficiency. Therefore, the use of natural remedies from plant extracts has increased based on its reliability, safety, and cost compared with synthetic drugs. Yet, proper data collection is required in order to validate that the natural sources are safe and effective to be used.

Aquilaria spp. crude leaves extract was obtained via hydrodistillation method. In the research conducted by Stanojević et al. (2011), it was found that average yield obtained from five hydrodistillation runs was 5.73 cm^3/100 g of essential oil from dry plant material, wherein it was 88.56% of oil recovery compared to the initial oil content in the plant material. Although hydrodistillation is the most common and successful method in obtaining essential oils and extracts from plants, there is still exists a need to enable the production of these extracts at optimum output.

According to Stanojević et al. (2011), with the modification in the process of hydrodistillation, the recovery can be increased to 95.05%. Therefore, this common extraction method required enhancement in order to increase the yield of gallic acid and quercetin equivalent in the crude extract. In this research, pre-treatment process, such as soaking and ultrasonication, were conducted without affecting the properties of the desired compound. The yield of gallic and quercetin equivalent in the crude leaves extract could be increased by the utilization of these pre-treatment methods prior to the hydrodistillation process.

The dried *Aquilaria spp*. leaves were ground into smaller size. This is known as the cell disruption method, which involved the size reduction process, wherein the surface area in contact with the solvent used in the step of extraction is increased. According to Abu Bakar (2010), the particle size has great influence on recovery of the desired compounds, where finer particles enhanced the extraction due to the increasing in surface area which in contact with the solvent during the extraction phases in the process. In the soaking pre-treatment method, the ground leaves were soaked in water or other solvent such as ethanol for a period of time to further open the pores of the plant sample which enabling its intracellular contents to be efficiently released. However, it was found by Deng & Donnelly (1993) that soaking samples in ethanol at room temperature yielded the same amount of desired compounds as that of extraction by boiling ethanol. This indicates that the use of heat during pre-treatment is not necessary to obtain optimal extractions when using organic solvents. Organic solvents such as alcohols, alcohol-water mixtures and chloroform dichloromethane commonly were used, but their adverse effects on the environment as well as food ingredients have raised concern (Joshi & Adhikari et al., 2019). According to Noriham et al. (2015), samples extracted using water showed significantly higher antioxidant activity than ethanolic extract and the differences in concentration of the inhibitory compounds obtained were influenced by the polarity of extracting solvents and the solubility of the compounds in the solvent during extraction process. Nevertheless, water extraction is more favourable owing to its simplicity since no contamination from the solvent is expected (Mohamad et al., 2014).

Another approach to increase the yield of the extract is by integrating the extraction process with ultrasonication method. Ultrasonication is widely used as an extraction technique applied in the laboratory, pilot-scale or in industry especially in the phyto-pharmaceutical industry for a wide range of herbal extracts as it offered a lot of advantages (Vilkhu et al., 2007). This method of extraction was selected because it is considered as an effective and beneficial method due to shorter processing time with lower energy consumption and environmentally friendly (Annegowda et al., 2012). Ultrasonication process using water has shown its effectiveness where the disruption of cells result from the collapse of cavitation bubbles occurs in the solvent. This enhances the mass transfer between the sample and solvent, then allowing for efficient extraction. Ultrasonication process was used in the study done by Husen et al. (2014) and it successfully enhanced the extraction process from the result of significantly higher phenolic values. Furthermore, it was also claimed to reduce the centrifugation time from 2 hours to 30 minutes (75% reduction), which shows that this pretreatment method helps in reducing extraction time. Thus, in this study, the pretreatment was conducted by decreasing the size of *Aquilaria spp*. by soaking in the water and expose the sample to the ultrasonic before hydrodistillation take place, with the aim to increase the yield of extract.

Liu et al. (2020) found that the research on lipase inhibition of plant-based products is widely studied and some of the research shows good inhibitory effects. Nevertheless, it cannot be produced in large quantities due to the low content of active ingredients, complication of extraction procedures and low recovery rate. Consequently, only few of them had undergone the clinical stage and this is a major drawback in commercialization of lipase inhibitors derived from edible plants. One of Liu et al. (2020) suggestion is to study the action mechanism of natural compounds on pancreatic lipase, while the high-activity of pancreatic lipase inhibitor is still continuously screened. Hence, the inhibition kinetic study was done to determine the mode of inhibition reaction, along with the relationship between the inhibitor, enzyme and substrate during the inhibition reaction. This relationship was perceived from the effect of inhibition by *A. malaccensis* and *A. subintegra* crude leaves extract on pancreatic lipase through the value of Michaelis-Menten constant (K_m) and the maximum enzyme activity (V_m) obtained as the activity of pancreatic lipase with inhibitor was determined throughout the reaction using the Lineweaver-Burk plot.

This research is one of the fundamental works for future studies on the natural obesity treatment and also for further development of plant-based medicine. It will also lead to the direction of key research in the lipase inhibition mechanism field, as being suggested by Liu et al. (2020). It is also expected that the scientific data obtained would be beneficial towards the development of agricultural and healthcare, especially in controlling weight gain and obesity.

2.0 Methodology

2.1 Material

The materials required for this research were matured leaves of *A. malaccensis* and *A. subintegra,* Folin-Ciocalteau reagent, Na_2CO_3, gallic acid, quercetin, aluminium chloride ($AlCl_3$), potassium acetate, porcine pancreatic lipase (PPL), tris-HCl buffer, NaOH, *p*-Nitrophenyl Palmitate (*p*-NPP), acetone, and ethanol. All chemicals purchased from Sigma Aldrich were available in the Chemistry Lab at Faculty of Chemical Engineering, UiTM.

2.2 Methods

2.2.1 Collection of samples

The matured leaves of *A. malaccensis* and *A. subintegra* were collected from Jalan Kebun, Shah Alam, Malaysia. The collected leaves were washed and cleaned to remove any dirt and impurities and dried in the oven.

2.2.2 Drying and Grinding

The drying process was carried out at 60 °C for 24 hours in the oven (Memmert, Germany), to remove 82% to 89% of moisture content in matured leaves after cleaning process. The dried leaves were cooled by leaving at room temperature before grinding process was done, by using a Mastar (MAS-160BL(A)-I) blender. Then, it was sieved into different particle sizes using shaker sieve machine with sieve tray at the size of 250 µm, 300, 400, 500, and 1000 µm, as available in the lab. The fibre of the leaves obtained after sieving process were ground again until less amount of fibre was obtained.

2.2.3 Soaking

The ground leaves were soaked at a ratio of 1% (w/v) of dried leaves to distilled water for 24 hours at room temperature. This process is very important in order to promote the enlargement of the pore size thus will enhance the releasing of the intended compound from the leaves.

2.2.4 Pretreatment using ultrasonicator

After 24 hours of soaking process, without discarding the water content, every particle size of soaked leaves was pre-treated using sonicator bath (NEXXsonics NS-A-18H) at a constant sonication frequency of 37 kHz and at the temperature of 60 °C. All ultrasonication processes were carried out for 30 minutes.

2.2.5 Hydrodistillation

The 1% (w/v) of dried leaves to distilled water that was soaked and ultrasonicated was then hydrodistillated at the temperature of 70 °C. The ground dried leaves of *Aquilaria* were hydrodistillated by using the round bottom flask that was heated on the TOPS MS-06 heating mantle at atmospheric pressure until a sufficient amount of hydrodistillate was obtained for about 4 hours of heating. The distillate collected was about 90% of the initial volume. This is to ensure all phenolic compounds in the mixture have been distilled in order to obtain accurate results during analysis. Then, the sample was evaporated using rotary evaporator (Heidolph Laborota 4000 Efficient, Germany) in order to gain more concentrated crude leaves extract. The samples were kept refrigerated before analysis. This can prevent the breeding of any microbial on the samples.

2.2.6 Determination of total phenol contents (TPC)

The total phenol was determined using Folin-Ciocalteau method (Jawad Kadhim et al., 2019; Hatami et al., 2014; Stanković et al, 2011; Singleton et al., 1999). The mix of 0.2 ml of leaves extract and 0.2 ml of Folin-Ciocalteau reagent were left to stand for 4 minutes before 1 ml of 15% Na_2CO_3 was added. The mixture was allowed to stand for another 2 hours at room temperature. The absorbance was read at 760 nm and the absorbance readings were triplicated and compared with gallic acid standards curve.

2.2.7 Determination of total flavonoid contents (TFC)

The total flavonoid content was determined using the aluminium chloride ($AlCl_3$) assay (Shah & Hossain, 2014). Approximately 0.5 ml of leaves extract, 0.1 ml 10% $AlCl_3$, 0.1 ml of potassium acetate, and 4.3 ml of deionized water were mixed and incubated for

30 minutes at room temperature. The absorbance was then measured at 415 nm using spectrophotometer and the absorbance readings were triplicated and compared with quercetin standard curve.

2.2.8 *Evaluation of inhibition functional group*

The active inhibitory functional groups, especially phenolic and flavonoids, presence in leaves extracts of *Aquilaria* were identified by using the FTIR spectroscopy (Perkin Elmer Spectrum 2000, USA). The determination was performed within the measurement band range from 4000 to 400 cm^{-1}, which is equivalent to the wavelength between 2.5 to 10 µm. The resolution of the FTIR was 4 cm^{-1} and the scanning time was 64 second, where the spectrum took about two minutes to complete the whole scanning cycle to be recorded. The range of wavenumber selected was based on the literature review in which the functional groups of the inhibitory compound can be identified and trials were also done. Then, the results of spectrums obtained were compared with the functional groups was obtained by Khalil et al. (2013).

2.2.9 *Determination of pancreatic lipase inhibition activity*

The porcine pancreatic lipase was prepared and suspended in tris-HCl buffer of pH 7.4 with 2.2 mmol NaOH to result in 200 unit/ml and 400 unit/ml lipase solutions (Ozgen et al., 2016; Bustanji et al., 2011). *p*-Nitrophenyl Palmitate (*p*-NPP) was dissolved in water as a substrate. Next, different volumes of *A.malaccensis* and *A.subintegra* leaf extracts were mixed with a ratio of 1:3:6 volumes of PPL suspension, p-NPP solution and tris-HCl buffer. The mixture was incubated in 37 °C water bath for 30 minutes to allow for hydrolytic reaction. The reaction was stopped by adding 1 ml of 1:1 acetone-ethanol mixture. The absorbance was read at 410 nm and the readings were triplicated and averaged. The absorbance was used to obtain the amount of p-nitrophenol (*p*-NP) liberated by using the equation from *p*-NP calibration curve. The calibration curve was constructed from the absorbance and concentration of *p*-NP at the range from 0 to 1000 µmol/ml. The readings were triplicated and averaged. The activity of control which was the standard pancreatic lipase activity without *Aquilaria* crude extract and the activity of sample with *Aquilaria* crude extract were calculated using Eq. (1). Then, the percentage of inhibition was determined by using Eq. (2)

$$Pancreatic\ Lipase\ Activity = \frac{Micromoles\ of\ p-nitrophenyl\ released\ from\ p-NPP\ by\ 1\ ml\ of\ PPL\ at\ 37\ °C}{Reaction\ time} \quad (1)$$

$$Percentage\ inhibition\ (\%) = \frac{Activity\ of\ Control - Activity\ of\ Sample}{Activity\ of\ Control} \times 100 \quad (2)$$

2.2.10 *Evaluation of pancreatic lipase inhibition kinetics*

For kinetic analysis, the graphical methods, Michaelis-Menten and Lineweaver-Burk, plots were generated by using the results of inhibitory activities obtained. The data required to draw the plots are the substrate concentration (S) and enzyme activity (V), wherein a hyperbolic shape and linear line graph were constructed. The mode of inhibition was determined from the Lineweaver-Burk plot. The Michaelis-Menten constant (K_m) and the maximum reaction rate or enzyme activity (V_m), were obtained from the reciprocal of Michaelis-Menten Eq. (3), given by Eq. (4). The V_m was calculated from the interception at y-axis and K_m was calculated from the slope of the linear graph.

$$V = \frac{V_m\,[S]}{K_m + [S]} \quad (3)$$

$$\frac{1}{V} = \frac{K_m}{V_m} \cdot \frac{1}{[S]} + \frac{1}{V_m} \quad (4)$$

3.0 Results and discussion

3.1 *Gallic Acid and Quercetin Equivalent Content in Aquilaria Crude Extracts from Ultrasonic Pretreatment*

The content of gallic acid and quercetin equivalent in *A.malaccensis* and *A.subintegra* crude extracts of different particle size of ground leaves were determined (Fig. 1) by using the TFC and TPC methods. Based on the results obtained, it was found that, for both *Aquilaria* species, the highest concentration of gallic acid and quercetin equivalent were determined in the crude extracts with particle size of 250 µm under ultrasonication temperature of 60 °C, with a soaking ratio of 1% (w/v). The results proved that smaller size of particle produced higher concentration of gallic acid and quercetin in the extraction process. Based on Sumari et al. (2013) research work on the ultrasonication process, the particle size of material was reduced proportionally to the duration of time, where there is a reduction of

particle diameter resulted by the cavitation energy generated by ultrasound. This cavitation energy produced shock waves that raised local pressure changed and shifted in liquid which resulting in damaged on the particle. In a study done by Samavardhana et al. (2015), the total phenolics and flavonoids were significantly increased with the decrease of particle size, wherein it was found that 362.02 mg of gallic acid equivalent and 272.61 mg of catechin equivalent per gram of grape seed dry matter was obtained.

Bucić-Kojić et al. (2007) and Qu et al. (2010) agreed that smaller particle size provides a shorter mass transfer distance and more surface area is available for molecular transport which contributes to a more extensive mass transfer of solute between phases. According to Saleh et al. (2015), grinding into smaller size also shorten the path that the solvent has to travel, which also decreases the time for maximum phytochemical content to be extracted. Norhidayah et al. (2013) mentioned that a sample with larger particle size has a smaller surface area which would restrict the solubility of water-soluble components which then led to the decrease in the values of total phenolic and flavonoid content extracted. Thus, the particle size reduction of plant part has turned into a fundamental viewpoint that must be considered and it has a significant effect in the extraction of active compounds (Saleh et al., 2015). The optimization efforts can be rationally developed with the presence of quantification of such heuristic rules for each plant source (Giao et al., 2009). It means that it can be done through trial and error and assumptions where the parameters range has been specified based on the results that were obtained in this research and others which had done.

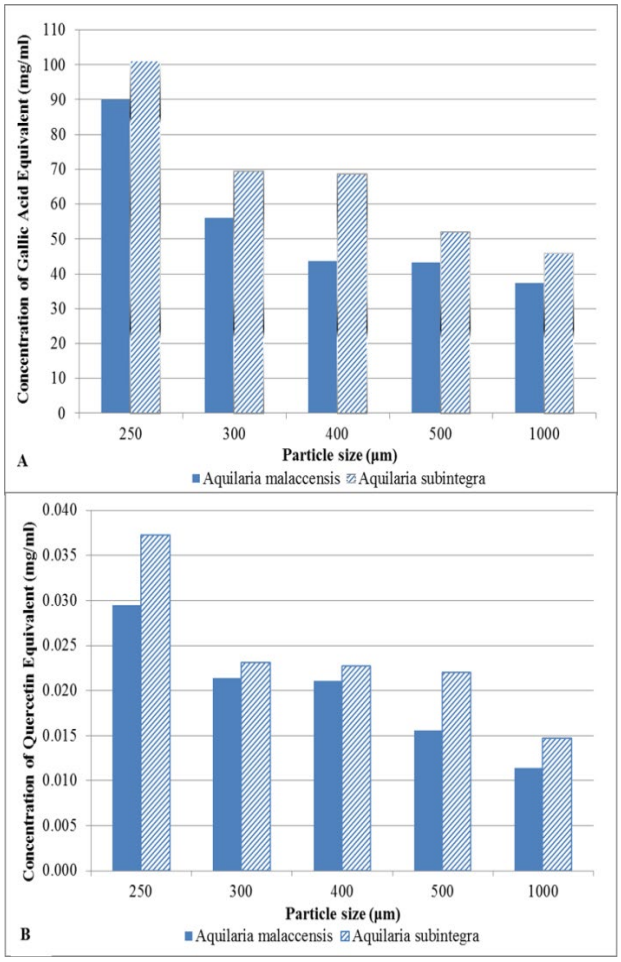

Fig. 1: A) Gallic acid and B) quercetin concentration in *A.malaccensis* and *A.subintegra* at different particle sizes for 60 °C of ultrasonication.

3.2 Evaluation of inhibitory functional group in crude leaves extracts

Pancreatic lipase, an enzyme that is responsible for hydrolysis of 50% to 70% of triglycerides into monoglycerides and fatty acid, thus pancreatic lipase inhibition is a valuable pathway towards the treatment of obesity (Liu et al., 2013). The inhibitor found in crude leaves extract of *A. malaccensis* and *A. subintegra* are under the group of phenolic compounds. Several studies on the potential of phenolic compounds as pancreatic lipase inhibitor were reported, such in a study done by Ahn et al. (2013), where the flavonoid known as flavones without glucose from *Nelumbo nucifera* was able to inhibit pancreatic lipase. Therefore, an FTIR analysis was done to evaluate the existence of the required functional group which exhibits the characteristic of an inhibitor. The FTIR spectrum of functional group presence in the crude leaves extract of *A.malaccensis* and *A.subintegra* is shown in Fig. 2. The functional groups which are present are further described in detail by referring to the study done by Pednekar and Raman (2013) and Jamahseri et al. (2014).

A broad spectrum of O−H bond of hydrogen bonded alcohol/phenol (3600–3200 cm^{-1}) is observed to show that this group frequency was present in gallic acid, quercetin and crude leaves extracts. This is important because the presence of O−H bond indicates the existence of phenolic compound in the extracts and also represents the characteristic of inhibitors. This finding is in agreement with Khalil et al. (2013) and Jamahseri et al. (2014) as they stated that the presence of alcohol/phenol functional group was identified in Agarwood leaves with frequency of 3600 to 3200 cm^{-1}. Besides, Ahn et al., (2013) also reported

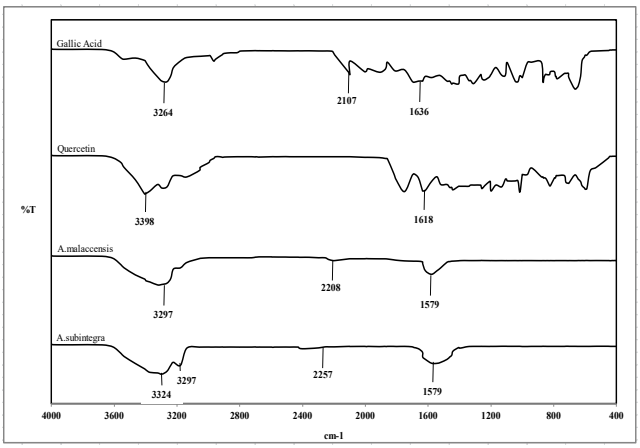

Fig. 2: FTIR spectrum of gallic acid, quercetin, *A.malaccensis* crude extract and *A.subintegra* crude extract.

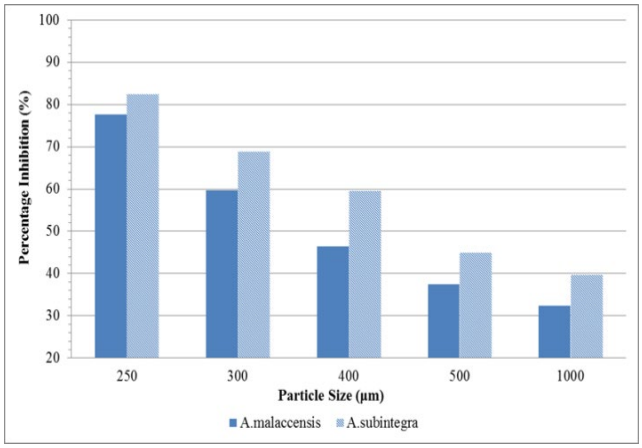

Fig. 3: Percentage inhibition of pancreatic lipase by *A.malaccensis* and *A.subintegra* leaves crude extract for different particle sizes at 60 °C.

that the presence of the hydroxyl group (3338 cm^{-1}) in *Nelumbo nucifera* leaves, was able to inhibit pancreatic lipase. Other than that, C≡C bond of Alkyne group at frequency between 2260 to 2100 cm^{-1} was also found in gallic acid and crude leaves extracts. N−H bending of amines (1650–1560 cm^{-1}) group frequency was also identified in gallic acid, quercetin and leaves crude extracts.

3.3 In-vitro inhibitory effect of crude extracts on pancreatic lipase

The percentage inhibition of pancreatic lipase was identified for all particle size of *Aquilaria spp*. Matured leaves, which had to undergo the pre-treatment process at the temperature range of 60 °C and soaking ratio of 1% (w/v). Fig. 3 shows the percentage inhibition of pancreatic lipase by *A. malaccensis* and *A. subintegra* leaves crude extract for particle sizes range of 250, 300, 400, 500, and 1000 µm at ultrasonication temperature of 60 °C. The highest percentage of pancreatic lipase inhibition by the crude extracts was determined at the particle size of 250 µm. The results indicate that the percentage of pancreatic lipase inhibition increased as the content of gallic acid and quercetin equivalent obtained increased. It was found that the highest percentage of pancreatic lipase inhibition was given by the *A. subintegra* crude extract due to the content of gallic acid and quercetin equivalent was higher in this species compared to *A. malaccensis*..

Results on inhibition depend on the content of phenolic and flavonoid obtained from different particle size of *Aquilaria spp*. leaves, in which this is in line with the research done by Zaiter et al. (2016) where the results revealed that antioxidant activity was dependent on particle size of green tea powders. The phenolic and flavonoid extraction were strongly influenced by the sample matrix, the particle size and the extraction technique (Khoddami et al., 2013). It has been discussed before that there was an increment of the total phenolic and flavonoid with decrement of sample particle size. Thus, it shows that a smaller particle size of sample leads to a higher percentage inhibition of pancreatic lipase, due to the total phenolic and flavonoid obtained via. pretreatment using ultrasonication process.

3.4 Inhibition kinetics analysis of crude extracts

The inhibition kinetics of crude extracts on pancreatic lipase was done by using Michaelis-Menten Kinetics plot and double reciprocal, Lineweaver-Burk plot. Due to the highest percentage inhibition shown, therefore the crude extracts from the leaves with particle size of 250 µm, which has undergone ultrasonication temperature of 60˚C, and at a soaking ratio of 1% (w/v) was selected. The plots were constructed at different pancreatic lipase activity and substrate concentration for the enzymatic reaction with and without inhibitor. The concentration of pancreatic lipase prepared for this enzyme activity study was 200 Unit/ml and 400 Unit/ml. The substrate concentration was varied from 100 µM to 1000 µM.

Based on the Michaelis-Menten plots shown in Fig. 4, Fig. 5, Fig. 8, and Fig. 9, the maximal reaction rate (V_m) value decreased for both species of *Aquilaria*. This is demonstrated by a lower maximum pancreatic lipase activity on a graph plotting enzyme activity against substrate concentration. According to Lineweaver-Burk plots in Fig. 6, Fig. 7, Fig. 10 and Fig. 11, the pancreatic lipase reaction was affected by *A. malaccensis* and *A. subintegra* leaves crude extract,

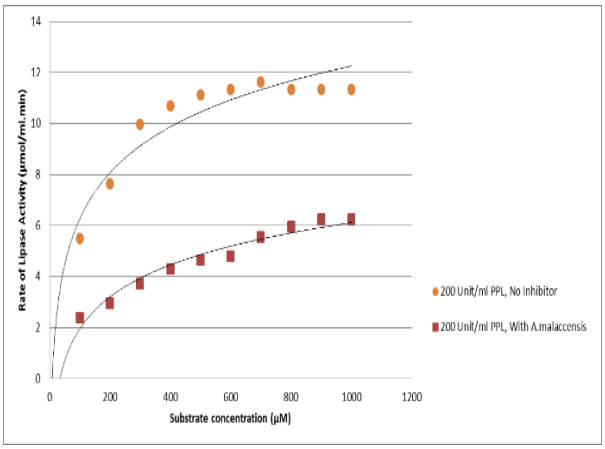

Fig. 4: Michaelis-Menten Kinetics plots of pancreatic lipase activity with and without *A.malaccensis* leaves crude extract at 200 Unit/ml of pancreatic lipase.

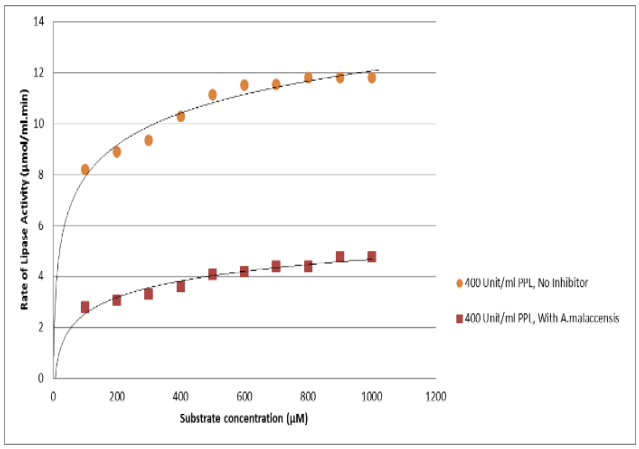

Fig. 5: Michaelis-Menten Kinetics plots of pancreatic lipase activity with and without *A.malaccensis* leaves crude extract at 400 Unit/ml of pancreatic lipase.

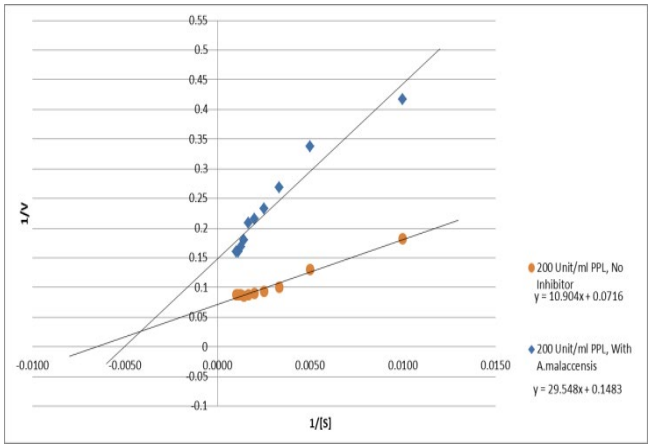

Fig. 6: Lineweaver-Burk plots of pancreatic lipase activity with and without *A. malaccensis* leaves crude extract at 200 Unit/ml of pancreatic lipase.

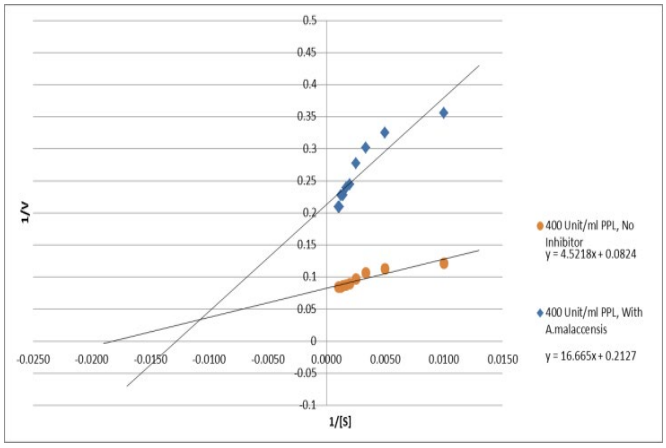

Fig. 7: Lineweaver-Burk plots of pancreatic lipase activity with and without *A. malaccensis* leaves crude extract at 400 Unit/ml of pancreatic lipase.

hence indicating mixed mode of inhibition. Based on the mode of inhibition identified, the overall reaction mechanism of mixed-inhibition represented by *A. malaccensis* and *A. subintegra* leaves crude extract was constructed as Fig. 12. The value of K_m and V_m was calculated from the linear line equation ($y = mx+c$) obtained from the Lineweaver-Burk plots. Based on the equation, the interception at y-axis gave the value of $\frac{1}{V_m}$ and the slope of the graph gave the value of $\frac{K_m}{V_m}$. All calculated values of K_m and V_m were tabulated in Table 1.

In the inhibition study, as compared to the reaction without inhibitor, it was found that the effect of mixed-inhibition was a reduction in V_m and increment in K_m. Referring to Fig.12, the value of V_m in the inhibition changes because of the capability of the inhibitor in preventing catalysis regardless of whether the substrate bound to the free enzyme or enzyme-substrate complex. The increment in the values of K_m indicates that the inhibitors diminished the efficiency of substrate conversion in the reaction, even if the substrate had attached to the enzyme. Thus, it can be concluded that a larger value of K_m shows a weak binding of substrate to the enzyme (Buchholz et al., 2012).

Similar inhibition mode is observed in the pancreatic lipase inhibition study done by Ong et al. (2016) using the extracts of *Eleusine indica (L.) Gaertner* which indicates the possibility of pancreatic lipase-substrate complex formation with the inhibitor binding at a distinct site from the active site resulting in reduction in complex affinity, thus explaining the increase in K_m. According to Sharma (2012), the mixed type inhibitor does not have structural similarity to the substrate but it binds both the free enzyme and the enzyme-substrate complex. The presence of a substrate has no influence on the ability of a non-competitive inhibitor to bind an enzyme and vice versa. Although the binding is away from the active site, it can still alter

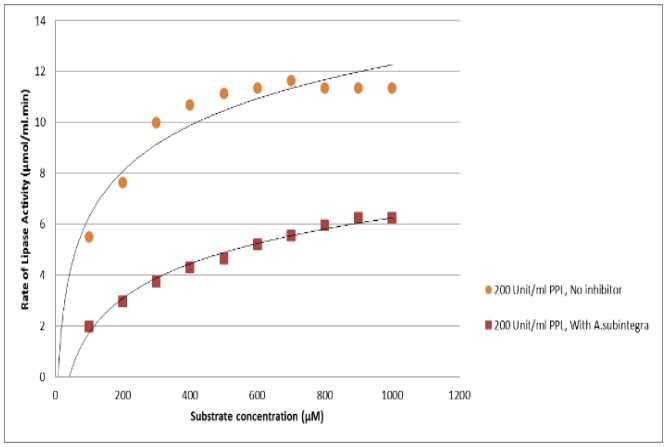

Fig. 8: Michaelis-Menten Kinetics plots of pancreatic lipase activity with and without *A. subintegra* leaves crude extract at enzyme activity of 200 Unit/ml of pancreatic lipase.

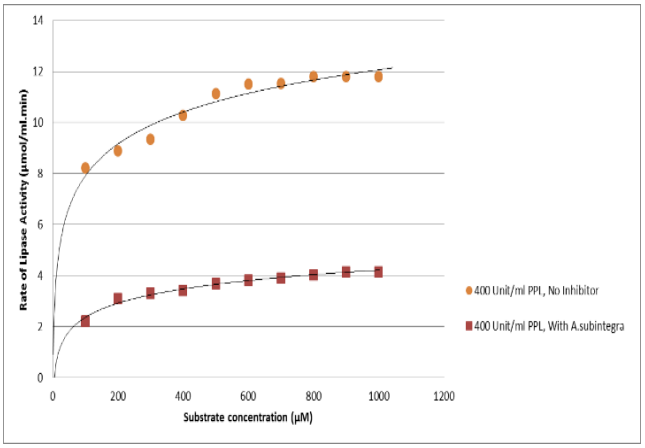

Fig. 9: Michaelis-Menten Kinetics plots of pancreatic lipase activity with and without *A. subintegra* leaves crude extract at enzyme activity of 400 Unit/ml of pancreatic lipase.

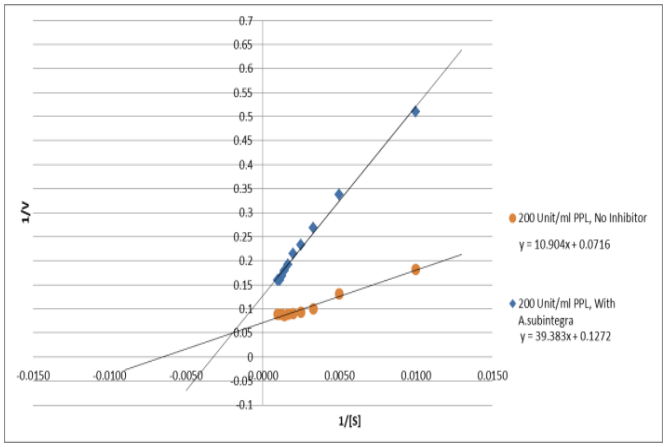

Fig. 10: Lineweaver-Burk plots of pancreatic lipase activity with and without *A. subintegra* leaves crude extract at enzyme activity of 200 Unit/ml of pancreatic lipase.

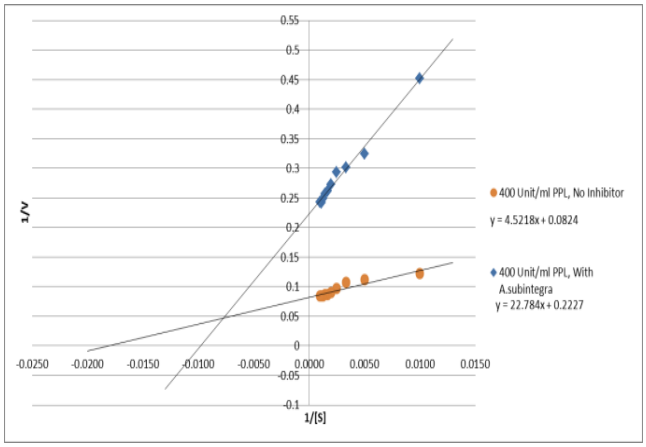

Fig. 11: Lineweaver-Burk plots of pancreatic lipase activity with and without *A. subintegra* leaves crude extract at enzyme activity of 400 Unit/ml of pancreatic lipase.

the conformation of the enzyme and reduce its catalytic activity due to changes in the nature of the catalytic groups at the active site (Sharma, 2012). Thus, the existence of a different site, other than the active site on the enzyme which is known as allosteric site was possible where this site allows any molecule to either activate or inhibit the enzyme activity.

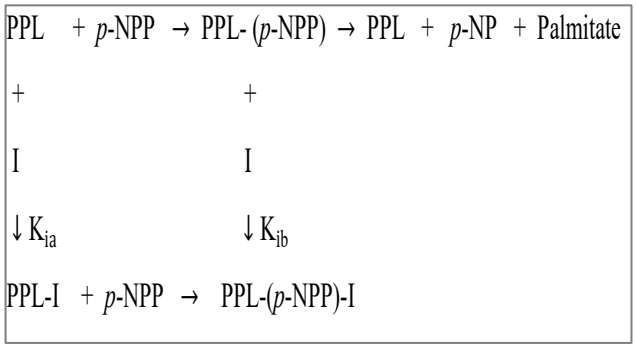

Fig. 12: The overall reaction mechanism of mixed-inhibition for crude extract of ultrasonicated *Aquilaria* leaves (PPL–Porcine pancreatic lipase).

Table 1: Kinetic parameters of inhibition reaction by *A. malaccensis* and *A. subintegra* leaves crude extract.

Enzyme Concentration (Unit/ml)		K_m (µmol)	V_m (µmol/ml·min)
200	No Inhibitor	81.87	12.67
	A. malaccensis	199.25	6.74
	A. subintegra	309.62	7.86
400	No Inhibitor	54.88	12.14
	A.malaccensis	78.35	4.70
	A.subintegra	102.31	4.49

4.0 Conclusions

It was found that particle size of matured leaves played an important role in affecting the recovery of pancreatic lipase inhibitory compound (gallic acid and quercetin) extracted from *A. malaccensis* and *A. subintegra*. In conclusion, the inhibitory compounds obtained were higher at the particle size of 250 µm, with an ultrasonication temperature of 60°C, and

soaking ratio of 1% (w/v). The existence of gallic acid and quercetin in the crude extracts contributes to the inhibition of pancreatic lipase with the highest percentage inhibition of 82% for 1 ml of crude extract used. Therefore, the inhibitory activity determined from *Aquilaria spp*. is expected to benefit the prevention of obesity and other problems associated with excess weight. Besides, it can further increase the potential of widely planted and wildly grown *Aquilaria* species in Malaysia.

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