Determination of Lipase Inhibitory Compound from *Aquilaria subintegra* Matured Leaves Extract via Pretreatment Using Bath Sonicator: Effect of Sonication Temperature

Amanina Abdul Manan, Prof. Dr. Ku Halim Ku Hamid, Miradatul Najwa Muhd Rodhi

Faculty of Chemical Engineering, Universiti Teknologi MARA

***Abstract* – Obesity is recognized as the most widespread metabolic disease. This disease was believed to be treated by reducing fat absorption through the inhibition of pancreatic lipase. The natural resources as polyphenol compound such as phenolic and flavonoid can be used as pancreatic lipase inhibitor. Pancreatic lipase is a key enzyme in dietary triacylglycerol absorption, hydrolysing triacylglycerol to monoacylglycerol and fatty acid. Those compound can be found in *Aquilaria subintegra,* a type of leaf of Gaharu. This study was conducted in two different types of parameter where the leaves were ground to 0.25 mm, 0.5 mm and 1.0 mm and pre-treated by ultrasonification with temperature of 40°C, 50°C, 60°C, 70°C and 80°C. The presence of phenolic and flavonoid compound in *A. subintegra* leaves extract was analyzed by using Masterizer Malvern 2000E and High performance liquid Chromatography (HPLC) to validate the presence of phenolic and flavonoid compound. From the analysis, the best temperature of ultrasonification is 60°C and the sample size 0.25 mm has the largest concentration both in flavonoid and phenolic.**

Keyword – Antiobesity, Aquilaria subintegra,flavonoid, phenolic

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I. INTRODUCTION

In the earliest time, people have been used naturally produced resins by plants in traditional medicine. Treatment of several diseases have been used worldwide by using plants and their exudate continue to be developed through phytochemical research. Following the modern and drug research advancing, chemically synthesized drugs have been replaced plants as the source of most medicinal agents. [1].

In developing countries, the majority of the world population cannot afford pharmaceutical drugs. Then, the plant that contained phytochemical such as *Aquilaria* species was found can be used to replace the drugs. *Aquilaria* species (family: Thymelaecaeae) are known to produce dark resinous heartwood. It's also known as Agarwood, Oud, Oodh and agar. There are 15 tree species in the Indomalesian Agarwood [2]. Agarwood produce *Aquilaria* species includes *Aquilaria beccariana*, *A. crassna*, *A. hirta*, *A. malaccensis*, *A. microcarpa*, *A. sinensis* and *A.subintegra* [3]. Currently, Indonesia, Malaysia, Thailand and Vietnam are the major producers of agarwood as there is huge demand for agarwood there. For the past few years, due to the health benefits, *Aquilaria spp.* leaves have been investigated for use as natural health products. Agarwood is known to have many pharmacological functions such as analgesic, anti-inflammatory, anti-microbial, immunomodulatory, and wound healing properties [4]. Due to cultivation condition and various species of *Aquilaria* are available in the market such as *Aquilaria crassna* and *Aquilaria subintegra*, the source of the plants and their activities are varied.

The phytochemicals present in *Aquilaria* leaves are from a range of chemical classes including phenolic acid, benzophenones, xanthonoids, flavonoids, terpernoids, phytosterols, phenolic, saponins, tannins and fatty acids. Some of phytochemicals showed pharmacological effects that could be candidates for future drug discovery [5].

In this research, *Aquilaria subintegra* will be used. The focus on this research is to determine lipase inhibitory compound from *Aquilaria subintegra* matured leaves extract via pretreatment by using a few of different sonication temperature. The presences of phenolic and flavonoids compound particle were determined by using High Performance Liquid Chromatography (HPLC) and Mastersizer Malvern 2000E.

According to Kavita Sharma *et. al.* (2015) [6], heating has a positive effect on flavonoid. The total flavonoids and phenolic were increased after heating at a certain temperature and magnitude of time. Most of the treatments can affect the content

of flavonoid and polyphenol. Researchers have found that heat can trigger the recovery of phenolic compound [7]. Solvent extraction can be promoted when temperature increase by enhancing diffusion coefficient and the solubility of polyphenol content. The cell membrane permeability will increases when temperature increase as it's favoured the release of bound polyphenol in a sample with the breakdown of cellular constituents of plant cell. It is believed this can lead to reduce the chances of polyphenols coagulating with lipoprotein and trigger the solubility of the polyphenols and diffusion increasing polyphenol yield [7].

For this research, *Aquilaria subintegra* undergo grinding to reduce particle size. The path that the solvent has to travel becomes shorter when the smaller particle size is used. This step also can decrease the time for maximum phytochemical content to be extracted. Moreover, the particle size damages on the plant cells can be reduce when the leaves were grind. This also can lead to increases in extraction of phytochemical compounds. 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 [8]. Nevertheless, the higher extraction yields of phenolic compound were obtained when extraction were performed with the smallest particle size.

II. METHODOLOGY

A. Plants material and pretreatment of leaves

Fresh sample of *A. subintegra* leaves were obtained from a local farm in Jalan Kebun, Shah Alam, Selangor. The leaves of *A. subintegra* were cleaned thoroughly, dried in oven at 60°C for 24 hours and were leaves at room temperature. After that, the dried leaves were ground and sieve into 1.0 mm, 0.5mm and 0.25 mm to improve the surface area. Next, leaves were soaking up to 24 hours in distilled water for 24 hours. The distilled water is added to promote the enlargement of pore size [9]. Then, ultrasonication pretreatment was carried out on soaked powder *A. subintegra* leaves sample for further leaf extraction enhancement. The temperatures used are 40°C, 50°C, 60°C, 70°C and 80°C. The duration of this pretreatment was about 30 minutes. The frequency is set to 37 kHz.

B. Hydrodistillation of leaf extract

Hydrodistillation is a method of extraction in which the leaves is soaked in the water after the sample is heated and volatile materials are carried away in the steam, condensed and separated. The samples were boiled with the distilled water in a round bottom flask. The temperature for this process is maintained at 70°C [10]. The water vapour from the hydro distillation process was collected. The water vapour that consists of steamed and volatile compound rises from the extractor and passes through to condenser. Then, the samples were weight and kept refrigerated before going to analyse to prevent any microbial breeding through the sample.

C. Mastersizer Malvern 2000E

Mastersizer Malvern 2000E laser diffraction equipped with a Micro Precision Hydro 2000µP sample dispersion unit was used in the research. This equipment has been designed to measure the size of particles and the distribution of different size within a sample. The particle refractive index is set to 1.48 as 1.48 is the refractive index for *Aquilaria subintegra*. Distilled water is used as the dispersant of the sample and the pump speed is set to 2000 rpm. After placed the samples in the prepared place in the Masterizer 2000E, the samples will be pump to measuring zone and circulating back again for the continuous measurement. Then, we can see the range of particle size distribution base on the graph in the displayer.

D. High Performance Liquid Chromatography (HPLC)

The samples were analysed by using HPLC on a WATER Millenium 32 system. Flavonoids and Phenolic were separated by gradient HPLC on a µ-Nova-Pak reverse phase C18 (8x10) RCM column with a linear methanol/acetic acid (5%) gradient from 0 to 100% methanol in 50 min at a flow rate of 1.0 ml/min. Moreover, the eluting peaks were monitored and spectra were recorded by using photodiode array detector. Then, phenolic and flavonoids spectra were analysed at the wavelength 280nm and 340 nm. The spectra eluted peaks were compared with those standard gallic acid and quercetin.

III. RESULTS AND DISCUSSION

A. Mastersizer Malvern 2000E

The Mastersizer 2000E is used to conduct the analysis of specific surface area particle regarding the temperature of 40°C, 50°C, 60°C, 70°C and 80°C from ultrasonification process and difference size of sample grind that are 0.25 mm, 0.5 mm and 1.0 mm. Mastersizer has been designed to measure the size of this particle or more specifically the distribution of different size within sample. The dispersant refractive index is set to 1.330. Table 1, 2 and 3 shows the result gained from the analysis of *Aquilaria subintegra* size 0.25 mm, 0.5 mm and 1.0 mm.

Table 1: Analysis of *Aquilaria subintegra* size 0.25 mm

Description / Temperature	Weight residual (%)	Obscuration (%)	Specific surface area (m^2/g)	d(0.9) (µm)
40°C	3.039	0.12	0.0468	1412.99
50°C	6.045	0.02	0.0114	1587.54
60°C	2.939	0.03	0.0255	881.415
70°C	7.994	0.04	0.00508	1591.46
80°C	10.744	0	0.0269	1157.21

Table 2: Analysis of *Aquilaria subintegra* size 0.50 mm

Description / Temperature	Weight residual (%)	Obscuration (%)	Specific surface area (m^2/g)	d(0.9) (µm)
40°C	10.866	0.02	0.157	240.414
50°C	8.739	0.07	1.63	19.319
60°C	6.818	0.05	0.0704	883.782
70°C	16.121	0.03	3.9	3.059
80°C	21.271	0.08	2.4	550.06

Table 3: Analysis of *Aquilaria subintegra* size 1.0 mm

Description / Temperature	Weight residual (%)	Obscuration (%)	Specific surface area (m^2/g)	d(0.9) (µm)
40°C	8.396	0.01	0.0061	122.241
50°C	3.943	0.02	0.0808	1325.15
60°C	3.592	0.12	3.52	1455.72
70°C	8.242	0.03	0.566	109.844
80°C	8.396	0.01	0.00611	1494.72

In this research, the goal of the experiment is to find suitable temperature for the extraction process of total flavonoids and phenolic acid without destroy it. By using mastersizer equipment, the results are analysed based on weight residual, obscuration and specific surface area.

The range of weight residual that detect by mastersizer 2000E are between 2 to 21 percent. For the *A. subintegra* size 0.25, the best weight residual is at 60°C that is 2.939%. It is the same go for the both *A. subintegra* size 0.50 and *A. subintegra* size 1.0, the best weight residual is at 60°C that is 6.818% and 3.592%. The lowest the percentage of weight residual is the better [11].

Obscuration is the step to measure the amount of laser light lost due to the introduction of the sample into the analyser beam. The ideal range of obscuration is 3 to 20 percent. However, all the samples have indicated the value of obscuration below than 1. From the table 1, it shows that the highest obscuration is 0.12% at 40°C. For the *A. subintegra* size 0.5, the highest obscuration is 0.08% at 80°C and for size 1.0, the highest obscuration is 0.12% at 60°C. This poor result may due to some problem in experiment such as the stirrer speed is slower and make sample sinks to the bottom, sample swells in dispersant or sample dissolving.

Based on the result and discussion above, it shows that the best ultrasonication temperature is 60°C as the lowest weight residual for *aquilaria subintegra* size 0.25 mm, 0.5 mm and 1.0 mm at the temperature of 60°C. Furthermore, the best obscuration value for *aquilaria subintegra* size 1.0 is at the temperature 60°C. Then at 60°C, the *aquilaria subintegra* for both size 0.25 mm and 0.5 mm get the third best obscuration value that are 0.03% and 0.05%.

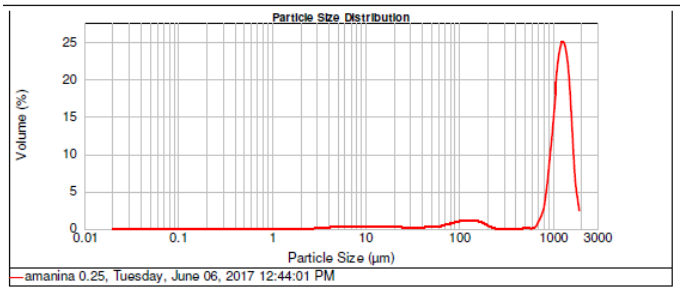

Figure 1: Particle size distribution for 0.25 mm at 60°C

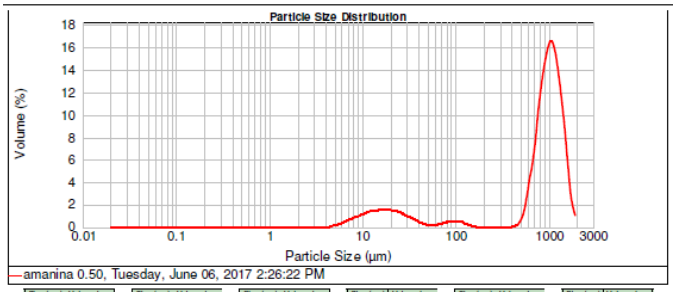

Figure 2: Particle size distribution for 0.5 mm at 60°C

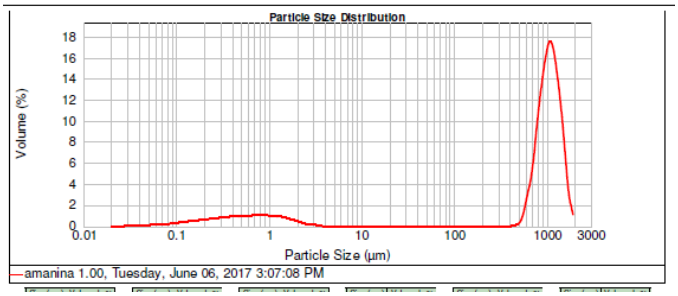

Figure 3: Particle size distribution for 1.0 mm at 60°C

B. High Performance Liquid Chromatography (HPLC)

Gallic acid and Quecertin is used as the standards to detect the concentration of Phenols and Flavonoids in the sample. The results gained then were used to compare the concentration of phenols and flavonoid in the samples. The analysis of HPLC was conduct only to the ultrasonification of the temperature 60°C.

The concentrations of Gallic acid were varied at 0.5 mg/L, 1 mg/L, 2 mg/L and 3 mg/L. The retention time at every highest peak of Gallic acid were recorded and have been used as the indicator for the samples. The acceptable error must be less than 10% from the standards. The same method is used for the Quercetin. However, the concentrations were varied at 2.5 mg/L, 5 mg/L, 20 mg/L, and 25 mg/L. Moreover, the area of the peak for both phenolic and flavonoid content in the samples can be determined by referring the range time obtained from

HPLC analysis. Figure 4 and 5 shows the Gallic Acid and Quercetin standard curve.

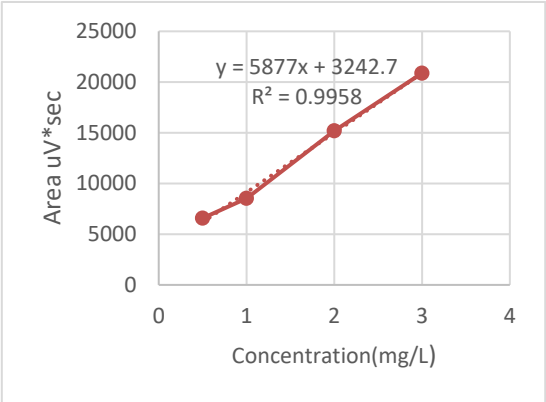

Figure 4: Standard curve of Gallic acid (area under the curve versus concentration) by using HPLC

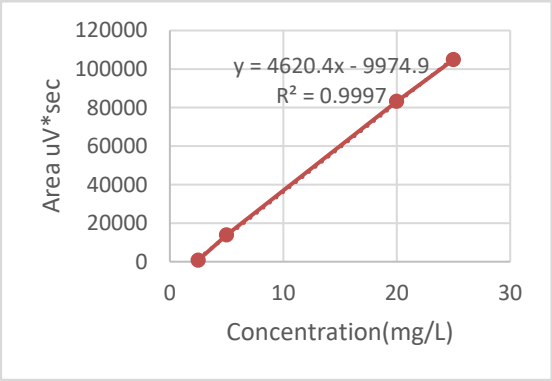

Figure 5: Standard curve of Quercetin (area under the curve versus concentration) by using HPLC

For HPLC analysis the experimental variable (size of samples) which influence the concentration of phenolic acid and flavonoid. Those compounds were successfully identify in the samples according to the retention time and spectral characteristics of their peaks when used gallic acid and quercetin as standard curve.The table 4 and 5 indicated the concentration of phenolic and flavonoid compound in the leaves extract of *Aquilaria subintegra*. As seen in the result, the highest concentration of both phenolic and flavonoid compound were at size 0.25 mm which were 3.14 mg/L and 6.89 mg/L.

Table 4: HPLC result of Phenolic

Size (mm)	Time (s)	Error (%)	Area (uV)	Concentration (mg/L)
0.25	1.952	10.49	21 713.39	3.14
0.50	1.776	1.49	6 859.81	0.62
1.00	1.424	2.83	976.75	0.31

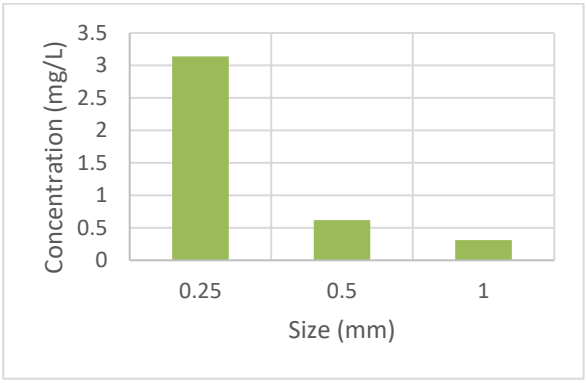

Figure 6: Concentration of Gallic acid in *A. subintegra* versus size of the sample

Table 5: HPLC result of flavonoid

Size (mm)	Time (s)	Error (%)	Area (uV)	Concentration (mg/L)
0.25	3.499	7.87	22 385.81	6.89
0.50	3.514	8.32	10 479.38	4.43
1.00	3.521	6.15	16 432.59	5.11

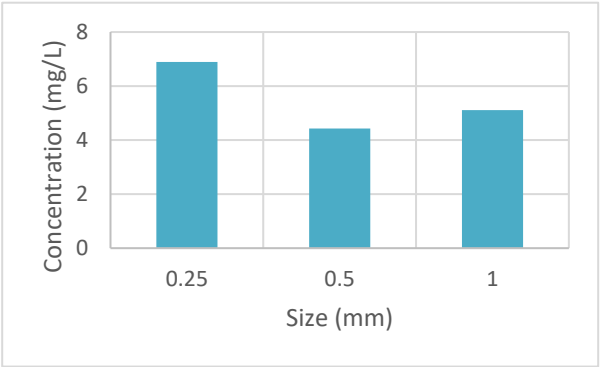

Figure 7: Concentration of Flavonoid in *A. subintegra* versus size of the sample

IV. CONCLUSION

In conclusion, it was found that the temperature of ultrasonification and size of particle played a major role in affecting the recovery of lipase inhibitory compound. From the analysis, the best temperature of ultrasonification is 60°C and the sample size 0.25 mm has the largest concentration both in flavonoid and phenolic. In line with this research, all together samples size 0.25 mm, 0.5 m and 1.0 mm have the smallest percentage of weight residual at 60°C. In addition, the analysis of the samples at 60°C by using gallic acid and quercetin as the standard curve shows that the sample particle size 0.25 mm has biggest concentration that are 3.14 ppm for phenolic and 6.89 ppm for flavonoid compound.

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