INHIBITION OF PANCREATIC LIPASE BY MELASTOMA MALABATHRICUM FRUIT EXTRACT IN VITRO

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***Abstract*— Obesity has become global health problems since it is generally associated with many diseases. Currently, anti-obesity treatment used synthetic drugs to overcome obesity but this synthetic drugs has been proven to have adverse side effects to human. Thus, this research was done in order to discover new anti-obesity drug based on natural product that help in slowing down the digestion of lipid in the pancreas and small intestine of humans as alternative to synthetic drugs in order to provide safer and less or no side effects. *Melastoma malabathricum fruit*(*MMF*) or locally known as 'buah senduduk' possess high total antioxidant and high total phenolic content which has potential as PL inhibitor that could treat the obesity. Aims of this research was to determine the content of Gallic Acid(GA) and Quercetin(Q) that present in MMF. This research also was done in order to identify the inhibitory activity of *MMF* extract against pancreatic lipase(PL) in vitro. The study was carried out by using water based extraction method and were prepared by using heat and without heat. From *Fourier Transform Infrared (FTIR) Spectroscopy Analysis*, it was founded that the MMF consist of alcohol, alkynes and alkenes functional group which were confirming the existence of compounds; GA and Q in MMF extract. From phytochemical screening, the highest TPC value obtained were 1.133 mg GAE/g extract and 2.031 mg GAE/g extract for extraction without heat and extraction with heat respectively. As for TFC, the highest TFC value obtained were 0.818 mg QE/g extract and 0.835 mg QE/g extract for extraction without heat and extraction with heat respectively. The results suggested that the inhibition activity more efficient for the sample prepared with heat compared to the sample prepared without heat. The samples of MMF (99.52%) exhibits higher percentage inhibition then Q (98.15%). Thus, it suggested that the natural inhibitor is better than snythetic inhibitor. This result suggests that MMF has a potential role in therapy for obesity-related disorders.**

***Keywords*—Anti-Obesity,Gallic Acid,Quercetin, *Melastoma malabathricum fruit*, Pancreatic Lipase,in vitro**

INTRODUCTION

The global obesity epidemic has lead to the global health crisis. Obesity is defined as a condition or a state of excess adipose tissue mass due to the imbalance energy intake and energy expenditure, or a combination of the two[1]. According to the previous studies, health complications such as type 2 diabetes, cardiovascular diseases and certain cancers are linked to the overweight and obesity [2]. According to the recent report in the New Straights Time 2018, Malaysia is suspected as highest obesity prevalence in Southeast Asia. Currently, obesity and overweight statistic in Malaysia are 13.3% and 38.5% respectively [3].

Current scenario, people use drug like orlistats as anti-obesity treatments. Orlistat is an anti-obesity drug that content a potent competitive inhibitors of pancreatic lipase. Based on report, orlistat enhanced both long-term and short-term weight loss and minimized weight gain to the obese people. Unfortunately, orlistat drug have side effects such as steatorrhea, abdominal cramping and fat-soluble vitamin deficiencies [4]. Besides, drug treatment of obesity is also often associated with rebound weight gain after the cessation of drug use and the potential for drug abuse. Due to the increasing frequency of anti-obesity drug use and their common side effects, there is an urgent need to identify natural products with minimal or no side effects.

In this study, peripherally acting is used in modulation of mechanisms for energy homeostasis for obesity treatment. It is done by using an inhibitor which is; an agent that interfere with the hydrolysis and absorption of dietary lipids. Pancreatic lipase is the principal lipolytic and glycosidic chain hydrolysis enzyme synthesized and secreted by the pancreas play a key role in the efficient digestion of triglycerides and starch and glycogen. By disturbing or inhibits the digestion and absorption of lipids or fat by pancreatic lipase in digestive system, the amounts of fats absorb by the body is reduced. Thus, it can reduced the body weight gain. Many reported natural products, particularly the phenolics, terpenes and saponins have already shown profound inhibition of pancreatic lipase. Although, research is continually going on in the development of pancreatic lipase inhibitors from nature, unfortunately none has reached to the clinical use.

Melastoma malabathricum fruit (M. malabathricum fruit) or locally known as 'buah senduduk' is classified as a type of berries. It possess high total antioxidant and high total phenolic content that act as anti-obesity activity and anti-cancer. Based on acute toxicity test, this fruit is safe to administer orally [5]. *M.malabathricum fruit* is mainly consumed by tribal, forest dwellers and rural people of Odisha,India. This fruit is editable and contain essential vitamins, minerals and fibres which can provide necessary dietary supplements and therapeutic usage as source of functional foods [6]. It also use as medicinal herb traditionally in Malay and Indonesian folk since long ago. As example, the fruit juice is used for dry lips treatment [7].

Since *M.malabathricum fruit* have phytochemical constituents that can be used as therapeutic drugs pharmaceutical agents, *M.malabathricum fruit* is selected in this study in order to determine the inhibitory compounds (gallic acid and quercetin) and identifying the inhibitory activity in *M.malabathricum fruit* extract against pancreatic lipase in vitro.

METHODOLOGY

A. Materials and Methods

Preparation of Fruit Extract

The M.malabathricum fruit was collected from Kampung Seberang Pintasan, Dungun on the month of March.
The *M.malabathricum fruits* were washed using water before rinsed using a filter. After the water was removed, the fruits were peeled to obtain the fruit content. The fruits were mashed using a mortar to make fruit paste before stored in the refrigerator. The fruit extract was prepared by using water based extraction. The fruits paste were weighted using electronic balance while the distilled water was measured using measuring cylinder. By using the ratio of 1:100 of 1g fruit paste to the 100g distilled water, the dilution samples were prepared. The samples were prepared by using two different temperature. The fruit extract without heat
was prepared under room condition; 25 ^{0}C temperature while fruit extracts with heat was heated until reached temperature at 60 ^{0}C for 30 minutes.

Determination of Chemical Compound Functional Group

Functional group of the samples can be identified by using Fourier Transform Infrared (FTIR) Spectroscopy where the wavelength is ranging from 4000 to 400 cm-1 as phenolic compounds functional groups will be identified at this wavelength. First, clean the plate by wiping the plate with acetone. Once the acetone has evaporated from this paltes, a drop of sample is added onto the plate and the reading is taken. Lastly, the comparison between the resultant spectrums with the standard for entirely functional groups was conducted.

Phytochemical Screening

a) Determination of Total Phenol Contents (TPC)

The total phenols content is determined by using Folin-Ciocalteau method. The mixture of 0.2 mL of MM fruit extracts and 0.2 mL of Folin- Ciocalteau reagent were stand for 4 minutes before it was added with 1 mL of 15% Na2CO3. The mixture was allowed to stand for another 2 hours at room temperature. The absorbance was read at 760 nm and the readings were triplicated and compared with Gallic Acid standard curve.

b) Determination of Total Flavonoid Contents (TFC)

The total flavonoids content was determined by using the Aluminium Chloride assay. 0.5 mL of MM fruit extract, 0.1 mL 10% AlCl3, 0.1 mL of Potassium Acetone 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. The readings were triplicated and compared with Quecertin standard curve.

Determination of Pancreatic Lipase (PL) Inhibition Activity

Porcine pancreatic lipase was suspended in phosphate buffer at pH 7.4. The buffer is being used to sustain the pH condition in the process. First, 1mL of gallic acid was added with 3mL of substrate solution which is p-Nitrophenyl Palmitate (p-NPP) and 1mL of porcine pancreatic lipase assay. The reaction of the hydrolytic was done in water bath at temperature of 37 ^{0}C. The solvent used for this study was water since it will be consumed by human as an edible product. Then, the procedure was repeated by using quercetin, gallic acid and quercetin, and sample of melastoma malabathricum fruits. The sample also used spectrophotometer to analyze the pancreatic lipase inhibitory activities.

II. RESULTS AND DISCUSSION

Determination of Chemical Compound Functional Group

Fourier Transform Infrared (FTIR) Spectroscopy Analysis was used to identify the functional group present in *M.malabathricum fruit*. Fig.1 and Fig.2 below shows the FTIR spectrum of *M.malabathricum fruit* with heat and without heat and Table 1 and Table 2 shows the summarize of results from the FTIR spectrum. A further detail of the functional groups present in the *M.malabathricum fruit* is being discussed. The group frequency of O-H bonded hydogen-alcohol or also known as hydogen-phenol bond was detected in the both fruit extract at the wavelength 3305.77cm^{-1}and 3322.46cm^{-1} for extraction without heat and extraction with heat respectively. This bonded presented alcohol functional group or also typically known as hydroxyl group and it was detected at the wavelength ranges from 3200-3550 cm^{-1}. Both extracts has strong H-bond since the lower the frequency, the stronger the H bond. The presence of O-H bond proven the existance of phenolic compound in the extracts [8]. This finding is supported by Rajan & Muthukrishnana (2013) that reported the characteristics of Gallic Acid present at peak of 3409.38 cm^{-1} which represent polymeric OH stretch in pseudarthria viscida root extract [9]. Ahn et al., (2013) also had reported *Nelumbo nucifera* leaves was able to inhibit pancreatic lipase at wavelenght 3338 cm^{-1} that indicated the present of hydroxyl group [10]. Phenolics are considered as efficient antioxidants mainly because of the structure that has the ability to donate the electron from the hydroxyl (-OH) group.

Meanwhile, the R-C≡C-H was detected at the wavelength 2097.07 cm^{-1} and 2123.37cm^{-1} for extraction without heat and extraction with heat respectively. The results indicated that the alkynes group was presented in the fruit since it was founded at the wavelength ranges from 2100-2140 cm^{-1}. The present of alkynes group in the *M.malabathricum fruit* extract shows that the extract has possiblity with pharmaceutical value as anticancer drugs. It was agreed by Ott et.al (2008) that reported Hexacarbonyl dicobalt complexes that contains various alkynes group is used in biomedical research for development of anticancer drug [11]. RCH=CH_2 bond of alkene group has wavelength ranging from 1626-1662 cm^{-1}. RCH=CH_2 bond was identified at the wavelength 1638.06 cm-1 and 1635.97 cm-1 for extraction without heat and extraction with heat respectively. By supported by the previous report by Rajan & Muthukrishnana (2013), they confirmed that the presence of functional group to Quercetic include the polymeric OH stretch (hydroxyl group) and and C=O stretch (alkene) in pseudarthria viscida root extract [9].

From this FTIR analysis for *M.malabathricum fruit* extract without heat and with heat, it was founded that the fruit extract consist of alcohol, alkynes and alkenes functional group. From this functional group, it was confirmed the existence of compounds; Gallic Acid and Quercetin in the *M.malabathricum fruit* extract.

Table 1: Functional group in *M.malabathricum fruit* extract without heat

Wavelength number (cm-1)	Group Bond (cm-1)	Bond	Functional group
3305.77	3200-3550	O-H	Alcohol
2097.07	2100-2140	R-C≡C-H	alkynes
1638.06	1626-1662	RCH=CH2	alkenes

Wavelength number (cm^{-1})	Group Bond (cm^{-1})	Bond	Functional group
3305.77	3200-3550	O-H	Alcohol
2097.07	2100-2140	R-C≡C-H	Alkynes
1638.06	1626-1662	RCH=CH_2	Alkenes

Table 2: Fuctional group in *M.malabathricum fruit* extract with heat

Wavelength number (cm-1)	Group Bond (cm-1)	Bond	Functional group
3322.46	3200-3550	O-H	Alcohol
2123.37	2100-2140	R-C≡C-H	alkynes
1635.97	1626-1662	RCH=CH2	alkenes

Wavelength Number (Cm^{-1})	Group Bond (Cm^{-1})	Bond	Functional Group
3322.46	3200-3550	O-H	Alcohol
2123.37	2100-2140	R-C≡C-H	Alkynes
1635.97	1626-1662	RCH=CH_2	Alkenes

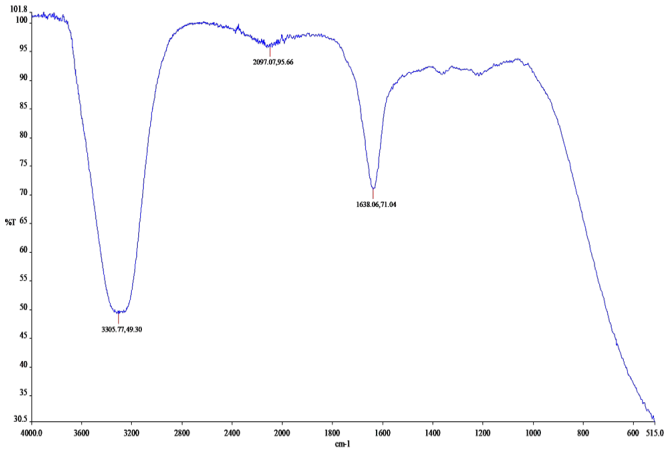

Fig. 1: FTIR spectrum of *M.malabathricum fruit* extract without heat

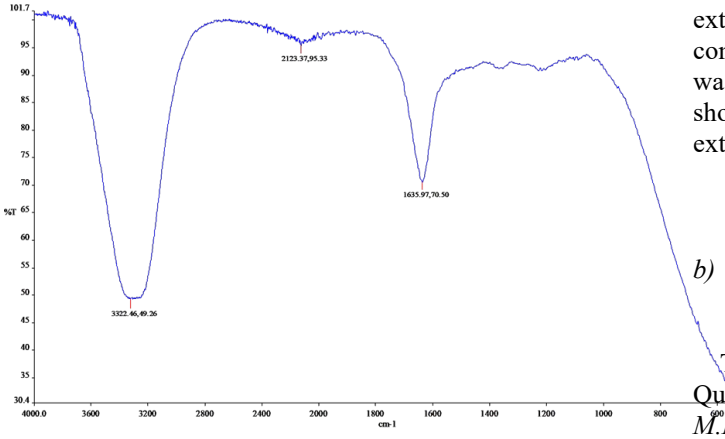

Fig. 2: FTIR spectrum of *M.malabathricum fruit* extract with heat

Phytochemical Screening

a) Determination of Total Phenol Contents (TPC)

The total phenolic compound was expressed as mg of Gallic Acid equivalent per grams (mg of GAE/g) in *M.Malabathricum Fruit* Extract (MMF).This was determined from the equation y=0.7961 x+0.0028,R^2=0.9956 from calibration curve that was prepared from Gallic Acid concentrations or also known as Gallic Acid Standard Curve (Fig.4).

Based on the graph in Fig. 3, the graph indicates that the directly proportional pattern for extraction with heat while fluctuated pattern for extraction without heat. Based on the previous research finding, the TPC increased as the extract concentration increased and the highest TPC was founded at the highest extraction concentration. However, only extraction with heat was fitted with the previous research finding. Since the correlation values, R^2 of the graph for extraction with heat is higher than extraction without heat, it can be seen that there were errors occur during extraction without heat analysis was conducted.

The highest TPC value obtained were 1.133 mg GAE/g extract and 2.031 mg GAE/g extract for extraction without heat and extraction with heat respectively. The TPC obtained by using extraction with heat was higher compared to the extraction without heat. This indicates that temperature of the extraction plays significant role in TPC yield. It supported the previous research finding by S. Jokić et al., 2009. The finding reported that stated increasing of extraction temperature had more positive effect on extractability of phenolic compound. Besides, phenolic compound yield the best when the optimum temperature was used during extraction process [12]. Thus, the TPC shows that the MMF extract perform better through extraction with heat compared without heat.

b) Determination of Total Flavonoid Contents (TFC)

The total flavonoid content was expressed as mg of Quercetin equivalent per grams (mg of QE/g) of *M.Malabathricum Fruit* Extract (MMF). This was determined from the equation y=0.995 x+0.0877, R^2=0.9733 from calibration curve that was prepared from Quercetin concentrations or also known as Quercetin Standard Curve (Fig.6). From Fig. 5, the results generally indicated that the TFC increased as the concentration of MMF increased for both graphs respectively. The highest TFC value obtained were 0.818 mg QE/g extract and 0.835 mg QE/g extract for extraction without heat and extraction with heat respectively.

The TFC obtained by using extraction with heat was higher compared to the extraction without heat. This indicates that temperature of the extraction plays significant role in TPC yield. It supported the previous research finding by S. Jokić et al., 2009. The finding reported that stated increasing of extraction temperature had more positive effect on extractability of flavonoid compound [12]. Thus, the TFC shows that the MMF extract perform better through extraction with heat compared without heat.

a) Determination of Total Phenol Contents (TPC)

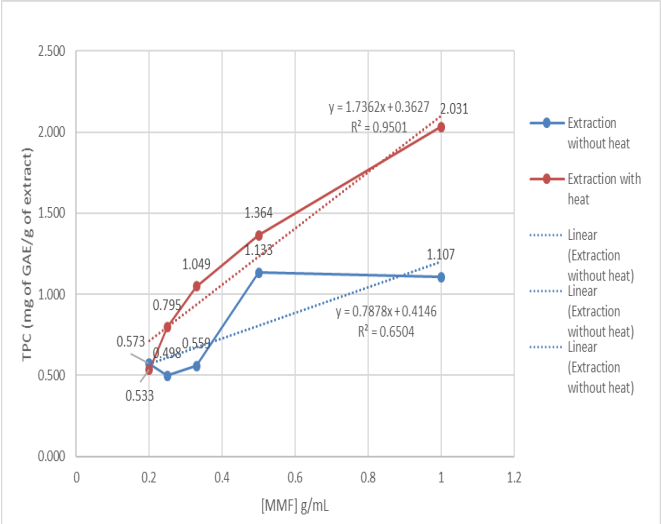

Fig. 3: Total Phenolic Contents (TPC)

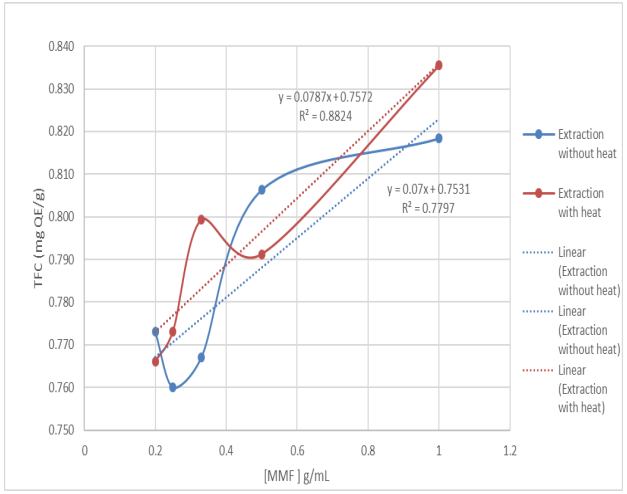

Fig. 5: Total Flavonoid Contents (TFC)

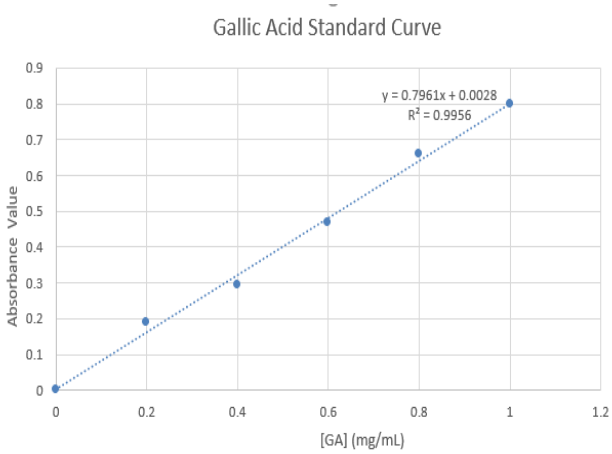

Fig. 4: Gallic Acid Standard Curve

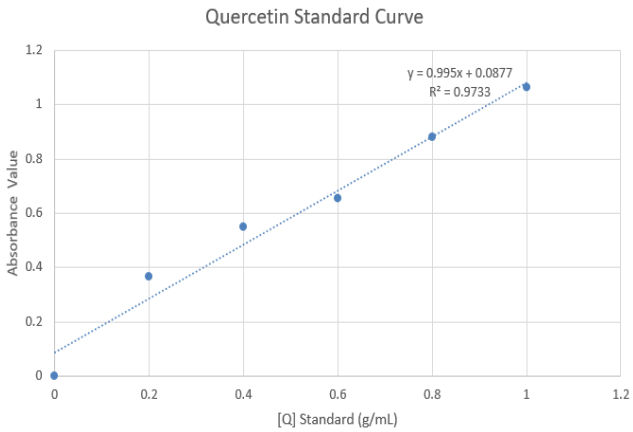

Fig. 6: Quercetin Standard Curve

b) Determination of Total Flavonoid Contents (TFC)

Determination of Pancreatic Lipase (PL) Inhibition Activity

Lipase Activity Sample	Concentration Sample	% Inhibition	
		Without heat	With heat
Gallic Acid	1	36.94	27.46
	0.5	98.99	98.27
	0.33	98.97	98.47
	0.25	99.42	97.93
	0.2	75.53	79.82
Quercetin	1	69.31	94.98
	0.5	99.02	98.15
	0.33	99.60	99.61
	0.25	99.77	99.93
	0.2	73.13	73.22
Gallic Acid + Quercetin	1	77.68	-98.66
	0.5	99.53	99.81
	0.33	99.94	89.48
	0.25	99.03	99.31
	0.2	73.19	87.92
MMF	1	5.13	-329.69
	0.5	96.66	99.52
	0.33	95.24	93.95
	0.25	96.63	98.00
	0.2	88.97	92.17

Inhibition of pancreatic lipase by *M.Malabathricum Fruit* Extract was summarize in Table 3 below. The results obtained shows that the synthetic inhibitor which were; Gallic Acid, Quercetin and combination of Gallic Acid and Quercetin generally have high inhibitory activity againt PL. Gallic Acid shows maximum inhibitory activity against PL at 0.25 mg/mL (99.42%) and 0.33 mg/mL (98.47%) for sample without heat and sample with heat respectively.

Meanwhile, both samples of Quercetin shows the maximum inhibitory activity at 0.25 mg/mL with 99.77% and 99.93% for sample prepared without heat and with heat respectively. As for the sample of the mixture of Gallic Acid and Quercetin, the samples shows maximum inhibitory activity at 0.33mg/mL (99.94 %) and at 0.5 mg/mL (99.81%) for sample without heat and sample with heat respectively.

For the natural inhibitor, both *M.Malabathricum Fruit, MMF* exhibits maximum activity at 0.5 mg/mL with 96.66% and 99.52% for extraction without heat and extraction with heat respectively. From the result obtained, it indicates that inhibition activity more efficient for the sample prepared with heat compared to the sample prepared without heat. The presence of heat in the sample helps to break down the supportive tissue of major component in the *M.Malabathricum Fruit.*

Based on the Table 3 and Fig.7, percentage of inhibition exhibited by *M.Malabathricum Fruit* Extract with heat and Quercetin were compared at 0.5 mg/mL. The results indicates that *M.Malabathricum Fruit* exhibits higher percentage inhibition then Quercetin. This finding reveals that *M. malabathricum* contains several secondary metabolites such as flavonoid and phenolic compounds which are responsible for its therapeutic activities such as anticancer and antioxidant as stated by Zakaria et. Al (2015).

However, percentage of inhibition of *M.Malabathricum Fruit* shows significant drop after concentration 0.5 mg/mL. This is might occurs due to the presence of contaminant or precipitation in the samples that interfered with lipase assay. As for Quercetin, the graph stay stationary after concentration 0.33 mg/mL.

According to the Karupiah and Ismail (2014), they reported about the study of anti-obesity effect of *M. malabathricum* in vivo on rats. They reported that Quercetin exhibits the anti-obesity effects by inhibiting the differentiation of preadipocytes and inducing the apoptosis of mature adipocytes. They also founded that *M. malabathricum* had significantly reduce the total cholesterol, LDL-cholesterol, and total lipids in the rats. [14]. Thus, this discovery may confirmed that *M. Malabathricum* effectiveness as PL inhibitor.

Table 3: Percentage of inhibition

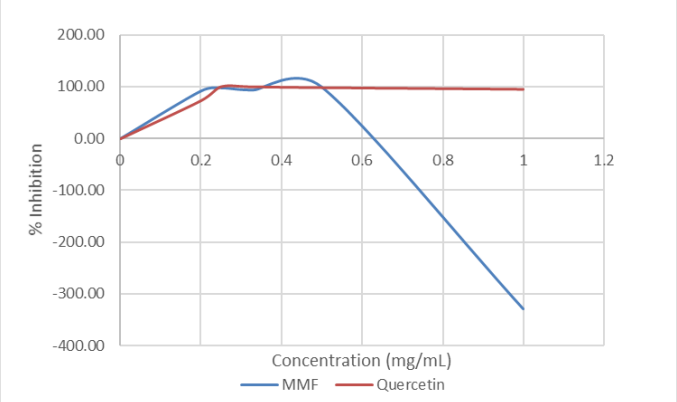

Fig. 7: Porcine Pancreatic Lipase (PPL) inhibitory activities of *M.Malabathricum Fruit* Extract With Heat and Quercetin

III. Conclusion

In this study of inhibition activity in vitro by MMF, the extraction samples prepared with heat shows higher inhibition than samples prepared without heat. The samples of MMF (99.52%) exhibits higher percentage

inhibition then Quercetin (98.15%). Thus, it suggested that the natural inhibitor is better than snythetic inhibitor. As such, the findings through this study provide a basis for developing novel anti-obesity agents using MMF that may have no significant adverse effects. This result suggests that MMF has a potential role in therapy for obesity-related disorders.

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