KINETIC STUDY OF PANCREATIC LIPASE INHIBITION

MUHAMMAD HAFIZ BIN ABDUL BASIR, DR MIRADATUL NAJWA

Faculty of Chemical Engineering, Universiti Teknologi Mara

Abstract—Obesity and its associated diseases and coronary heart diseases are a major challenge for our society. An important target for the treatment of obesity includes the development inhibitors of nutrient digestion and absorption. Pancreatic lipase is the enzyme responsible for digestion and absorption of triglycerides, being its inhibition one of the widest studied methods used to determine the potential activity of natural products to inhibit dietary fat absorption. Decrease of energy intake from dietary fat through inhibition of this enzyme may be an excellent strategy to prevent and treat obesity.The inhibitory activity on pancreatic lipase enzyme of *Lawsonia Inermis or henna leaves* was evaluated using the spectrophotometer to get the concentration. Inhibition of pancreatic lipase are found by using the michelis menten equation, lineweaver-burk, hanees wolf plot and eadie hofstee plot. The enzyme kinetics were obtained with different concentration of substrate (cooking oil) and gallic acid, quercetin, pancreatic lipase and buffer were the constant. Then the result was plotted to get the correct mode of inhibition.

Keywords— Lawsonia inermis, obesity, stearic acid, gallic acid, quercetin, pancreatic lipase, substrate, kinetic studies, inhibition

I. INTRODUCTION

Obesity and its associated diseases such as diabetes mellitus and coronary heart diseases are a major challenge for our society. Obesity can lead to a number of health problem and also increase the risk of type 2 diabetes (Rogers et al., 2002). In order to treat the obesity, an approach was taken to use the natural product which is L.inermis (Henna) as anti-obesity agent. The anti-obesity agent provides the mechanism in inhibition of pancreatic lipase. Besides, the use of chemical drug in the treatment of obesity should be reducing because it can contribute towards many adverse side effects. Moreover, by consuming the medicine from natural product can help to reduce the cost of the treatment instead of consuming the chemical drug. The use of naturally occurring inhibitors is considered to be safer (Jamous et al., 2018). The choice of solvent for extraction of plant material depends on the purpose of the final product. This research was done by using water-based solvent for the extraction as the final product is going to consume by human and also it is economical when it comes to cost. Besides, the use of solvents such as acetone and alcohol are highly flammable and required a prove of their absence in the final product. Research on other plant-based as traditional medicine used in treatment of obesity has done by many researchers. However, L.inermis as a source of anti-obesity is still lacking. L.inermis is also believed to have a potential in the obesity treatment due to its antioxidant properties. Inhibition of pancreatic lipase may be an excellent strategy for anti-obesity treatment if energy intake from dietary fat is decrease (Kim et al., 2016). Pancreatic lipase inhibitors prevent the breakdown of dietary fat into fatty acids, thereby reducing their absorption in the gut (Shahu et al., 2017). Therefore, this research was done to find out the new or alternative way to inhibit pancreatic lipase by extract natural compound instead of using modern drugs. An important target for the treatment of obesity includes the development of inhibitors of nutrient digestion and absorption.Inhibition of pancreatic lipase and the associated reduction of lipid absorption is an attractive approach for the discovery of potent agents. The substrate undergoes 4 set of experiment with different inhibitor. That is gallic acid alone, quercetin alone, gallic acid with quercetin the best sample of henna which has been extracted. Type of enzyme inhibition needed to find and the K_m and V_{max} value from the polyphenols extracted from henna. Thus, the inhibition of pancreatic lipase can be determined.

II. METHODOLOGY

A. Materials

L.inermis or henna leaves were collected from a farm located in Selangor. The leaves were washed and dried under the sun. Then the leaves were dried in the oven to remove the moisture content. The drying temperature is set to three different temperatures which are 60 , 70 and 80 .The process followed by grinding the dried leaves by using cutting mill in order to obtain a fine powder form. The ground leaves were sieved by using 1000µm particle size of sieve in order to obtain uniform size of powder for further procedures

B. Reagents

Gallic acid, Quercetin, Folin-Ciocalteau reagent, Sodium Carbonate, Aluminum Chloride, Stearic acid, Sodium Hydroxide, Cooking oil, Porcine pancreatic lipase, Dipotassium Phosphate, Potassium dihydrogen Phosphate.

C. Buffer Preparation

800 mL of distilled water was prepared inside a 1 mL beaker. 16.284 g of Dipotassium Phosphate (K2HPO4) and 0.888 g of Potassium dihydrogen Phosphate (KH2PO4) was added into the solution. Then, distilled water was added until the volume is 1 L. The solution was adjusted to obtain final desired pH of 7.4 by using Sodium hydroxide (NaOH)

D. Preparation of plant extract

The henna extract was prepared by grinding the dried leaves into a fine powder. The powdered henna with three different temperatures of 60 , 70 and 80 was being extracted using two different extraction methods which are heating process in hot boiling water and soaking in warm water. The ratio of the powdered henna leaves to distilled water was 1:100 in g/mL. The extract was filtered using filters paper to obtain a clear stock solution.

E. Preparation of kinetic studies of pancreatic lipase

There are 4 sets of experiment that need to be shown. Using gallic acid, quercetin, gallic acid with quercetin and the best extract from henna. In order to the experiment, added 1 ml of gallic acid with 1 ml of phosphate buffer (pH 7.4), add 1 ml of porcine pancreatic lipase to the test tube. The cooking oil with range from 0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml are used as substrate. The experiment are repeated with quercetin, gallic acid with quercetin and best extract of henna. Wait for incubation time for 30 minutes before put into the spectrophotometer machine to get the concentration.

III. RESULTS AND DISCUSSION

A. The kinetic studies of pancreatic lipase

To determined the kinetic studies of pancreatic lipase, the composition in henna mainly used in our experiment are gallic acid and quercetin. the gallic acid and quercetin were measure by 1ml each and mixed with 1ml of lipase and 1ml of buffer (7.4pH) and different volume (0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml) of substrate used (cooking oil). the chemical is mixed in the test tube and put into spectrophotometer machine to get reading concentration. There 4 reading need to be measured. Then the enzyme activity will be calculated using the following equation

$$\text{Enzyme activity (v)} = \frac{molar\ of\ product}{30\ mins\ of\ incubation\ X\ volume\ of\ solution}$$

B. Comparison enzymatic kinetic using non-linearized method between gallic acid, quercetin, combine of gallic acid with quercetin and best extract from henna.

Below shows the graph of michelis-menten for all the 4 enzyme. the graph were plotted with velocity versus different substrate concentration (0.5ml – 2.5 ml)

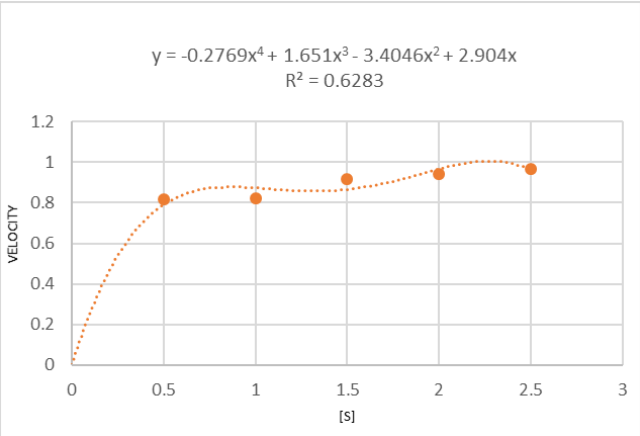


Figure 1: Graph of Michelis Menten for Gallic acid

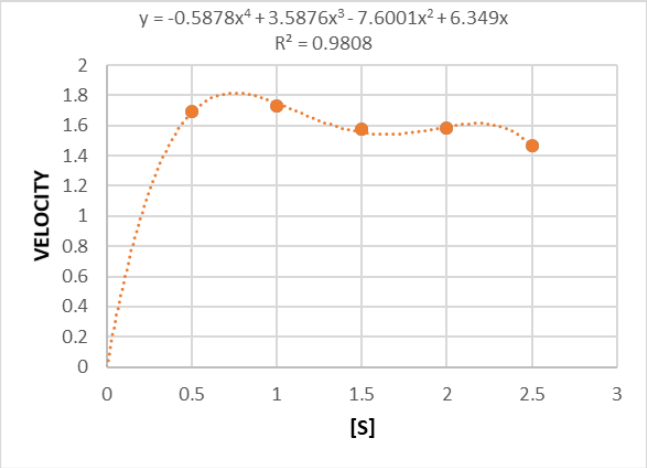


Figure 2 : Graph of Michelis-Menten for Quercetin

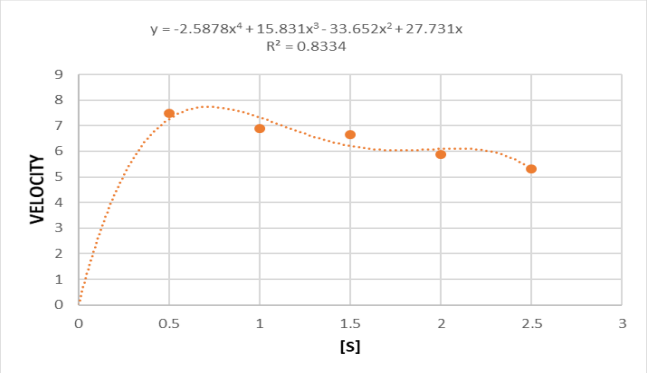


Figure 3: Graph of Michelis-Menten for combination of gallic acid and quercetin

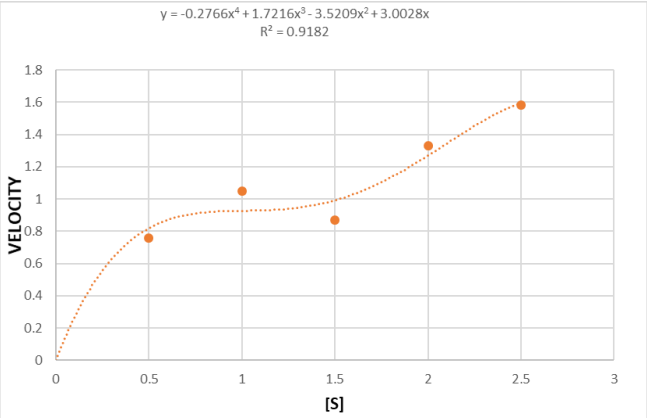


Figure 4: Graph of Michelis-Menten for best extract from henna

Figure 1 presents the michelis menten graph of gallic acid, as shown above the value of R^2 0.6283. Based on the calculation, the value of V_m is 0.99129 and K_m is 0.126854. The velocity of gallic acid become maximum when the substrate concentration becomes very large which is known as V_m point. The enzyme that bound to the substrate which produce product is higher during this point.

As same with the figure 4 which the V_m higher when the substrate concentration is also higher. The value of V_{max} for graph of michelis menten with the best extract from henna is 2.05641 and K_m is 1.10245. The value of R^2 is 0.9182. The initial velocity of both this graph is not 0 as present in the graph above.

Whereas, for figure 2, the graph of michelis menten for quercetin shows that the V_{max} are not linearly increase with the the increasing of substrate concentration but the value of R^2 is 0.9808

shows that the data is fitted with the graph. The value for K_m is 0 and V_m 1.6101. For the graph of michelis menten with combination of gallic acid and quercetin shows that V_{max} value is decreasing when the substrate concentration is increasing. But the high R^2 is 0.8334 values indicated that the data fitted the graph. The K_m value is 0 and V_m value is 6.44092. It can conclude that the quercetin will affect the V_{max} value and can influence the K_m value also. Based on the comparison of 4 graph above, the highest value of R^2 is0.9808 which is quercetin but the V_m and K_m value of quercetin is smaller compared to the graph with the best extract from henna plant which is 2.05641 and 1.10245. Compared with quercetin 1.6101 and 0.

C. Comparison enzymatic kinetic by using linearized method between gallic acid, quercetin, combine of gallic acid with quercetin and best extract from henna.

Graphs of linearized method for gallic acid, quercetin, combine of gallic acid with quercetin and best extract from henna were show below which are Lineweaver Burk plot, Langmuir plot and Eadie-Hofstee plot. The graphs were plotted with velocity versus different substrate concentration.

C.a Lineweaver-Burk plot

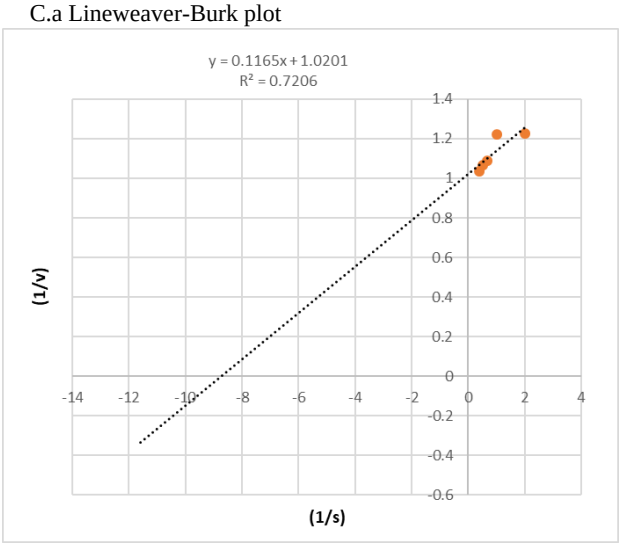


Figure 5: Graph of Lineweaver-Burk plot for Gallic acid

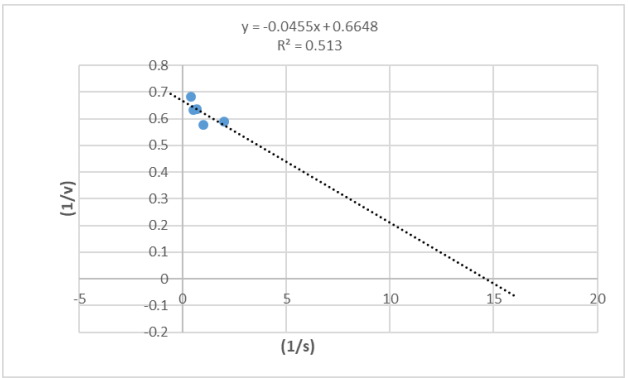


Figure 6 : Graph of lineweaver-Burk plot for Quercetin

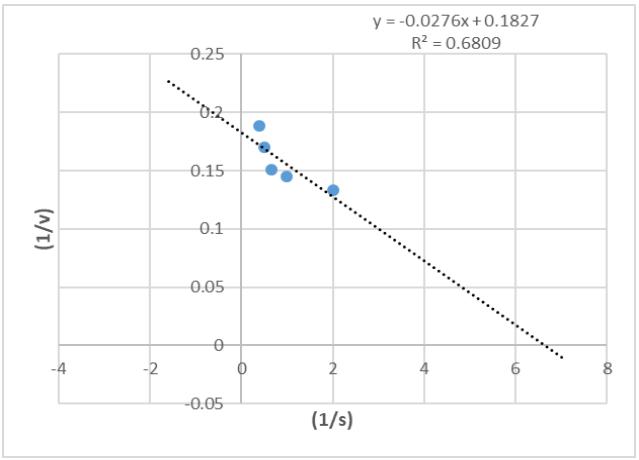


Figure 7: Graph of Lineweaver Burk plot for combination of gallic acid and quercetin

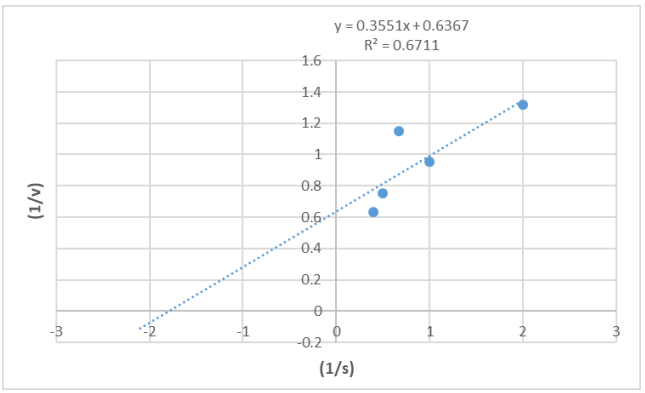


Figure 9: Graph of Lineweaver Burk plot of best extract from henna plant.

Graph in figure 5 above shows the Lineweaver burk plot of gallic acid enzyme which constructed by plotted (1/v) versus (1/s). the values of R^2 for this lineweaver-burk plot is 0.7206. By using the equation, the value of V_{max} was calculated by $1/V_{max}$ which is equal to slope of the graph while K_m was determined by K_m/V_{max} which is equal with the gradient of the graph. Thus the value of K_m and V_{max} are 0.08416 and 0.76545 respectively. Figure 6 present the lineweaver-burk plot with quercetin. For this graph, the values of R^2 is 0.513, on top of that the graph are not the standard then the actual lineweaver-burk standard graph. The values of K_m and V_{max} are determined using K_m/V_{max} and $1/V_{max}$. . Thus the values are -0.12408 and 1.79551 respectively. The figure 7 shows that the graph of lineweaver-burk for the combination of gallic acid and quercetin. The values for R^2 is 0.6809. this graph is also not up to lineweaver burk standard. The values of K_m and V_{max} are -1.1029 and 8.09757 respectively. Lastly, for the figure 9 which is the graph of lineweaver-burk of best extract from the henna plant. The value of R^2 is 0.6711. the values for K_m and V_{max} values are 0.32428 and 0.60257 respectively.

By comparing the R^2 from all the 4 graphs we can concluded that the lineweaver-burk for gallic acid and the best extract from henna plant are the correct graph with 0.7206 and 0.6711 respectively. The difference of R^2 values is a lot. We can see that the enzyme that has quercetin affect the K_m value which can get negative value because the best feed trendline is also shifted. The higher V_{max} value are the graph with the combination of gallic acid and quercetin enzyme. Whereas the highest for the K_m value are from the best extract from henna plant.

C.b Langmuir plot

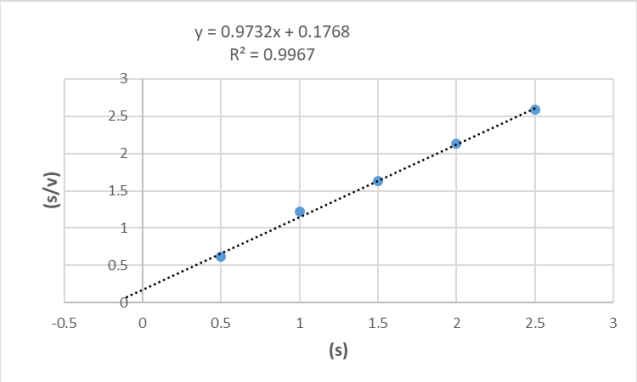


Figure 9: Graph of Langmuir plot for Gallic acid

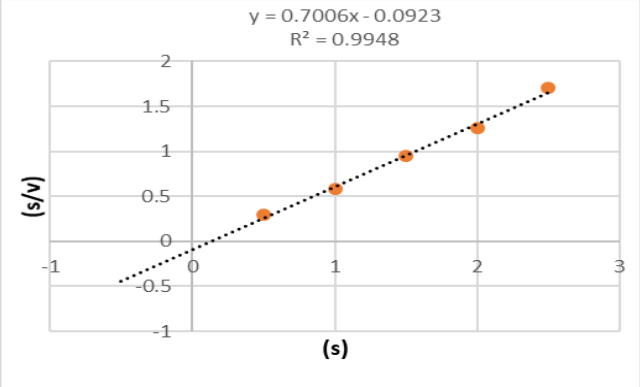


Figure 10: Graph of Langmuir plot of Quercetin

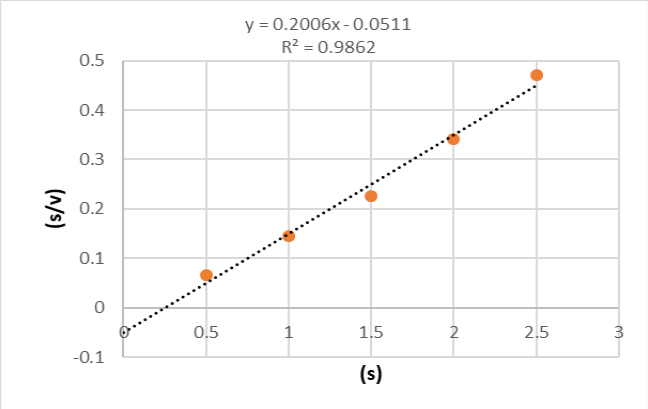


Figure 11: Graph of Langmuir plot of combination of Gallic acid and Quercetin

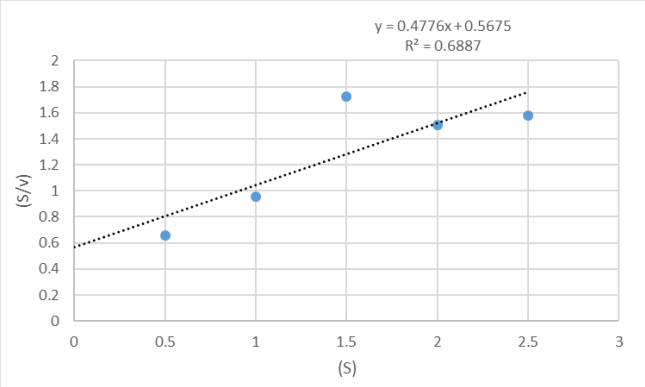


Figure 12: Graph of Langmuir plot of best extract from henna plant

Figure 9 above shows Langmuir plot by plotted (s/v) versus (s). The value of R^2 for gallic acid enzyme is 0.9967. In order to calculate the values of V_{max} and K_m Langmuir plot, the equation is used in the excel to be more precise and systematic. The value of V_{max} calculated by gradient of the graph which represent $1/V_m$. in order to determined K_m the value of slope of the graph used since represents K_m/V_m. As in results, the values of V_m and K_m of gallic acid is 0.07806 and 0.77708 respectively. For the graph 10 represent quercetin, based on the graph the R^2 is 0.9948. After done the calculation by using the equation of Langmuir plot to determined the values of V_{max} and K_m, the results shows that the V_{max}, is 0.14246 and K_m is 1.56649. For the combination between gallic acid and quercetin in figure 11 shows that the R^2 is 0.9862. After calculation the V_{max} and K_m value is 0.10343 and 0.59293 respectively. Lastly, the graph from the best extract from henna plant in figure 12, shows that the R^2 value is 0.6887. after calculation in Langmuir equation shows that the value of V_{max} and K_m is 0.39008 and 0.50654.

As shown in the graph above, all the enzyme has higher R^2 value than standard R^2 value which is 0.95. But the gallic acid has highest value of R^2 from other enzyme. Although the gallic acid has the highest value of R^2 it has the lowest V_m value with 0.07806 compared with the others enzyme. the highest value for K_m is from the quercetin with 1.56649 whereas the lowest ones is from the best extract from henna plant with 0.50654 values.

C.c Eadie-Hofstee plot

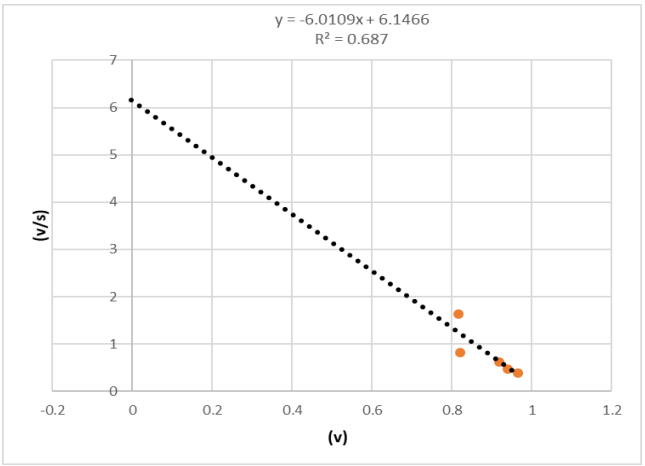
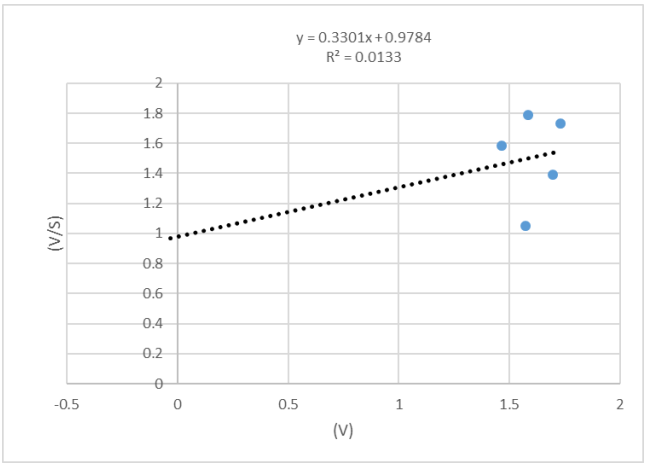
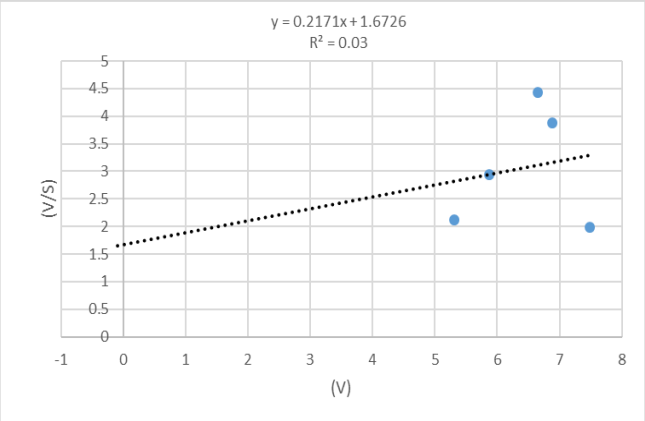


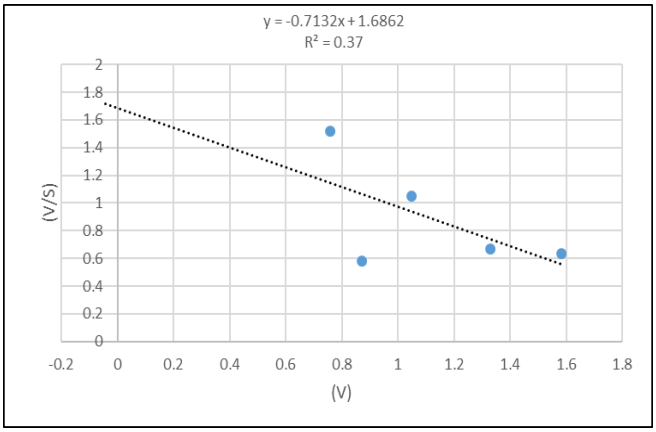
Figure 13: Graph of Eadie-Hofstee of Gallic acid



Graph 14: Graph of Eadie-Hofstee plot of quercetin



Graph 15: Graph of Eadie-Hofstee plot of combination of gallic acid and quercetin



Graph 16: Graph of Eadie-Hofstee plot of best extract from henna plant

Graph in figure 13 present the Eadie-Hofstee plot of gallic acid, this plot was constructed by (v/s) versus (v). the value of R^2 for gallic acid is 0.687. Based on the equation, K_m was determined by the slope of the graph which is equal to $-1/K_m$ while V_{max} was calculated by V_{max}/K_m which represent the gradient of the graph. Thus the values of K_m and V_{max} are 1.31301 and -0.1143 respectively. Figure 14 shows that the eadie-hofstee plot of quercetin, the graph is not well fitted thus the value of R^2 is low which is 0.0133, the values of V_{max} and K_m were calculated using the same equation which represent the gradient. As a results, values of K_m and V_{max} are -1.26168 and 2.84474 respectively. Figure 15 shows the graph plot with combination of gallic acid and quercetin enzyme, the data is well not fitted as a result the R^2 is 0.03 which is low. The values of K_m and V_{max} was also calculated which represent of the slope and gradient of the graph, as a result the values of K_m and V_{max} are -0.18032 and 1.03496 respectively. Last for the graph 16, present the graph for best extract from henna plant, as stated the value of R^2 is 0.37. the values of K_m and V_{max} are calculated from the gradient and the slope from the graph. As a results the values of K_m and V_{max} are 1.81471 and -0.68 respectively.

Between all the four enzyme use, the highest values of R^2 is from gallic acid enzyme with 0.687, with the lowest R^2 are from quercetin enzyme. The highest K_m value is from the best extract from henna plant with value of 1.81471, which affect from the equation of slope ($-1/K_m$). the highest V_{max} value are from quercetin enzyme with 2.84474 from the equation (V_{max}/K_m).

D. Comparison of kinetic enzyme by using non-linearized method and linearized method

Kinetic parameters, V_{max} and K_m are important in studying the kinetic enzyme. Under Michelis-menten condition, K_m is an estimate of the dissociation constant of enzyme (E) from substrate (S). High of K_m means that the binding between enzyme and substrate is weak binding while small of K_m is tight binding. V_m is the theoretical maximal rate of the reaction. All enzyme need to bind tighly with the substrate in order to achieve V_m.

Michelis-menten provides inaccurate value to determined of V_{max} and K_m. this is because Michalis menten plot needed to be hyperbolic. Thus, linearization methods such as Lineweaver-Burk, Langmuir, and Eadie-hofstee plots were used to determined V_{max} and K_m. The accuracy of linearization method are great compared with Michelis menten plot.

	Non-linearized method			
	Michelis-Menten			
	Gallic Acid	Quercetin	Combination Gallic Acid & Quercetin	Best extract from Henna plants
R^2	0.6283	0.9808	0.8334	0.9182
V_{max}	0.99129	1.6101	6.44092	2.05641
K_m	0.12684	0.12	0.10	1.10245

Table 1: Non-linearized method michelis menten

	Linearized method			
	Lineweaver-Burk			
	Gallic Acid	Quercetin	Combination Gallic Acid & Quercetin	Best extract from Henna plants
R^2	0.7206	0.513	0.6809	0.6711
V_{max}	0.76545	1.79551	8.09757	0.60257
K_m	0.08416	-0.12408	-1.1029	0.32428

Table 2: Linearized method Lineweaver-Burk

	Linearized method			
	Langmuir			
	Gallic Acid	Quercetin	Combination Gallic Acid & Quercetin	Best extract from Henna plants
R^2	0.9967	0.9948	0.9862	0.6887
V_{max}	0.07806	0.14246	0.10343	0.39008
K_m	0.77708	-1.56649	0.59293	0.50654

Table 3: Linearized method Langmuir

	Linearized method			
	Eadie-Hofstee			
	Gallic Acid	Quercetin	Combination Gallic Acid & Quercetin	Best extract from Henna plants
R^2	0.687	0.0133	0.03	0.37
V_{max}	-0.11428	2.84474	1.03496	-0.68
K_m	1.31301	-1.26168	-0.18032	1.81471

Table 4: Linearized method Eadie-Hofstee

As shown in table 1 through table 4 above, the highest value of R^2 between Gallic acid, quercetin, combination for gallic acid with quercetin and best extract for henna plant are from the Langmuir plot with 0.9967, 0.9948, 0.9862 and 0.6887 respectively. The second highest is Michelis menten and the lowest is Eadie-hofstee. However, the results from michelis menten is not accurate and should be eliminated.

The highest V_{max} all four enzyme are from the lineweaver-burk plot. This plot gives visual impression of the different form of enzyme inhibition and easy to determine the V_{max} and $K_{m.}$ By referring to the data of lineweaver-burk plot, values of K_m of enzyme that has quercetin is lower than the enzyme that do not have quercetin or has little amount of quercetin.

The highest of V_{max} value are from the Langmuir plot since we have eliminated the Michelis menten plot. The value or V_{max} with the quercetin enzyme appears to be higher than other enzyme and also with the high value of R^2. Eadie-hofstee plot is not give an accurate value because its plotted variables are depending with the experimental error.

IV. Conclusion

The studies showed that *Lawsonia inermis* or henna could have potential in becoming a new natural source of medicine and beneficial in clinical use. The inhibitory rate increases with increase of the polyphenols extracts from the lawsonia inermis concentrations. The kinetic studies stated that the polyphenols does not change the Vmax value of pancreatic lipase but increase the Km value, indicated that polyphenols competitively inhibit pancreatic lipase activity and bind to the active site of pancreatic lipase. Thus, the high activities of pancreatic lipase inhibition show that the enzyme can react well. We can conclude that *Lawsonia inermis* or henna appears to be potential source as a pancreatic lipase inhibitor but still this research is still lacking and further research need to do.

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