SIRIM Berhad
Industrial Biotechnology Research Centre, Building 19
Tel: 03-55446953/6960
Fax: 03-55446988

TEST REPORT

REPORT NO: R112/18/B19/33	PAGE: 1 of 7

Applicant	:	Mohd Salehuddin Bin Abdul Razak
Manufacturer / Company	:	PIJ Manufacturing Sdn Bhd, Unit F4, POIC SME Factory, PLO 76, Jalan Nibong 4, Tanjung Langsat Industrial Complex, 81700 Skudai, Johor.
Sample	:	One (1) Aloe Vera Juice Aloeshafy
Description of Sample	:	Received one sample with the following identification: The sample is yellow colour liquid
Reference standard / Method of Test	:	1.LWI-238-35: Procedure for Biochemical Antioxidant Assay (ABTS Assay) For Water-Based Substance/ Test Article 2.SIRIM/MOA 3:2017 (Annex G) – Determination of antioxidant free radical scavenging assay (ABTS).
Date Received	:	28th February 2018
Job No.	:	J112/18
Issue Date	:	10th April 2018

Approved signatories,

(SITI KASMARIZAWATY BT SUBOH)
Researcher
Industrial Biotechnology Research Centre
SIRIM Berhad

(DR. NURUL IZZA BT NORDIN)
MJMM0579
Senior Researcher
Industrial Biotechnology Research Centre
SIRIM Berhad

SIRIM Berhad
(No. Syarikat 367474 - V)
1, Persiaran Dato' Menteri
Seksyen 2, Peti Surat 7035
40700 Shah Alam
MALAYSIA
Tel : 60-3-55446000
Hotline : 60-3-55103535
Fax : 60-3-55108095
Website : www.sirim.my

Biochemical Antioxidant Inhibition Assay

1.0 Principle of the assay

ABTS assay is basically a radical cation decolourisation test, which is also a spectrophotometric method widely used for the assessment of antioxidant activity of various substances. A colourless molecule, reduced ABTS, is oxidized to a characteristic blue-green $ABTS^{\cdot+}$. When the blue-green $ABTS^{\cdot+}$ is mixed with any substance that can be oxidized, it is reduced to its original colourless ABTS form again; in contrast, the reacted substance is oxidized. This indicates that the substance possesses antioxidant property.

2.0 Method

2.1 Preparation of Solution

2.1.1 Preparation of standard

L-ascorbic acid was used as standard in this assay and is prepared fresh prior to use. 0.01g of the standard was weighed and dissolved in 1 mL of pure water, to make 1% concentration of the standard. A concentration range (w/v) of 5.00 mg/mL, 2.50 mg/mL, 1.25 mg/mL and 0.63 mg/mL were prepared by serial dilution. Further dilutions were made and tested in order to determine the standard's IC_{50} value of L-ascorbic acid.

2.1.2 Sample

Solubility Determination and Preparation of Samples

2.1.2.1 Solubility Determination of the samples

The physical characteristic of the sample was in aqueous form and can dissolved in pure water.

2.1.2.2 Preparation of test article /sample

The sample was further diluted by serial dilution (1:2) with five concentrations for each run (100%, 50%, 25%, 12.5% and 6.25%). The samples were conducted in triplicate. Suitable range of concentration of the samples were considered and tested for antioxidant assay in

order to obtain the inhibition concentration (IC_{50}) value of the sample. For the aqueous sample, concentration in percentage (%) was used.

2.2 Sample preparation

2.2.1 ABTS Assay

2.2.1.1 Preparation of Phosphate Buffer Solution (PBS), pH 7.4

0.136 g of KH_2PO_4 (potassium dihydrogen phosphate) was weighed and dissolved in 200 mL of pure water (solution A). 2.174 g of K_2HPO_4 (*di*-potassium hydrogen orthophosphate anhydrous) was weighed and dissolved in 200 mL pure water (solution B). Solution B was mixed with solution A using a magnetic stirrer and the pH was adjusted to achieved the pH of 7.4.

2.2.1.2 Preparation of ABTS Stock Reagent

ABTS stock reagent was prepared by dissolving 0.077g ABTS and 0.013 g potassium persulfate in 20 mL of pure water. The stock reagent was kept in the amber bottle for 12 to 16 hours at room temperature before used. The stock reagent was kept in the freezer at -20°C until further used.

2.2.1.3 Preparation of ABTS Working Solution

1 mL of the ABTS stock was added to 19 mL of phosphate buffer saline (PBS), pH 7.4. The ABTS working solution was prepared fresh prior to use.

2.2.1.4 Conduct of the ABTS Assay

200 µL of the working solution was added to 20 µL of the sample in each well of the 96 well plate and agitated for 6 minutes. The absorbance was read at 734 nm after 6 minutes of incubation at room temperature. Pure water was used as the negative control. L-Ascorbic Acid was used as the positive control. This assay was conducted in 96 well plate and performed in triplicates. The antioxidant activity was calculated as percentage of inhibition of free radical in the sample.

2.2.1.5 Calculation of antioxidant activity (inhibition of free radical)

Calculation:

% free radical scavenging activity

$$= \frac{(A_{\text{negative control}} / A_{\text{sample}})}{(A_{\text{negative control}})} \times 100\%$$

A: Absorbance
Negative control: 200 µL ABTS in 20 µL pure water or ethanol
Positive control: Standard; (L (+)-Ascorbic Acid)

The antioxidant activity is based on the efficiency of the sample to inhibit free radical as indicated by the IC_{50} value. The Inhibition concentration (IC_{50}) value of the sample was calculated using equation of trend line plot (microsoft excel) with $R^2>0.9$. The y-axis represents the percentage inhibition of free radical while the x-axis represents the tested concentrations (e.g.mg/ml, %) of the sample. By considering the dilution factor of sample in ABTS solution (Refer to 2.2.1.4). The tested concentration value is 11 times lower to the prepared concentration of the sample. The IC_{50} represent the concentration that reduce half (50%) of the free radical present in the sample. The IC_{50} of sample was compared against IC_{50} of the standard (L-ascorbic acid). The lower the IC_{50} of a sample, the better is the antioxidant activity.

2.2.1.6 Prediction of antioxidant activity

Refering to 2.2.1.5, the absorbance of the sample should be less than absorbance of the negative control. If the absorbance of the sample more than the absorbance of the negative control, it is assumed that the sample do not have antioxidant activity or the data is unacceptable due to the insolubility of the sample.

TEST REPORT

3.0 Results

3.1 Positive control (L-Ascorbic acid)

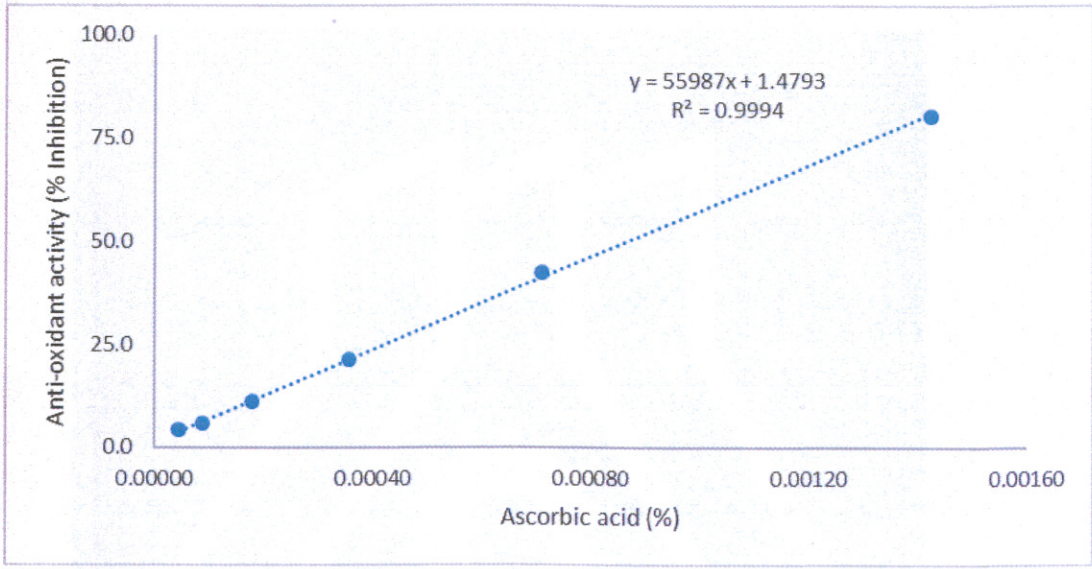


Figure 1: Antioxidant activity of L-Ascorbic acid

Table 1: Data on Antioxidant activity of L-Ascorbic acid

Tested Concentration (%)	Absorbance			INHIBITION %			Average	SEM
	R1	R2	R3	R1	R2	R3		
0.00004	0.922	0.908	0.909	3.35	4.82	4.72	4.30	0.82
0.00009	0.906	0.897	0.895	5.03	5.97	6.18	5.73	0.61
0.0002	0.852	0.846	0.848	10.69	11.32	11.11	11.04	0.32
0.0004	0.756	0.745	0.746	20.75	21.91	21.80	21.49	0.64
0.0007	0.546	0.554	0.548	42.77	41.93	42.56	42.42	0.44
0.0014	0.212	0.176	0.171	77.78	81.55	82.08	80.47	2.34

Negative Control (Absorbance) = 1.03 , where absorbance of the sample less than the absorbance of the negative control (Refer 2.2.1.6)

R1, R2, R3 = replicates

* Data are the mean ± SEM of three separate experiments.
** SEM (standard error mean) is the standard deviation of the sampling distribution
*** The SEM shall be less than 5.0

Assay integrity: Acceptable (R^2>0.9)

The data verify the integrity of the assay

L-Ascorbic acid was used as a standard. Based on figure 1, the IC_{50} value of L-Ascorbic acid (0.000867 ± 0.0002 %) shall indicate at least 50% inhibition of free radical scavengers (oxidant).

3.2 Aloe Vera Juice Aloeshafy

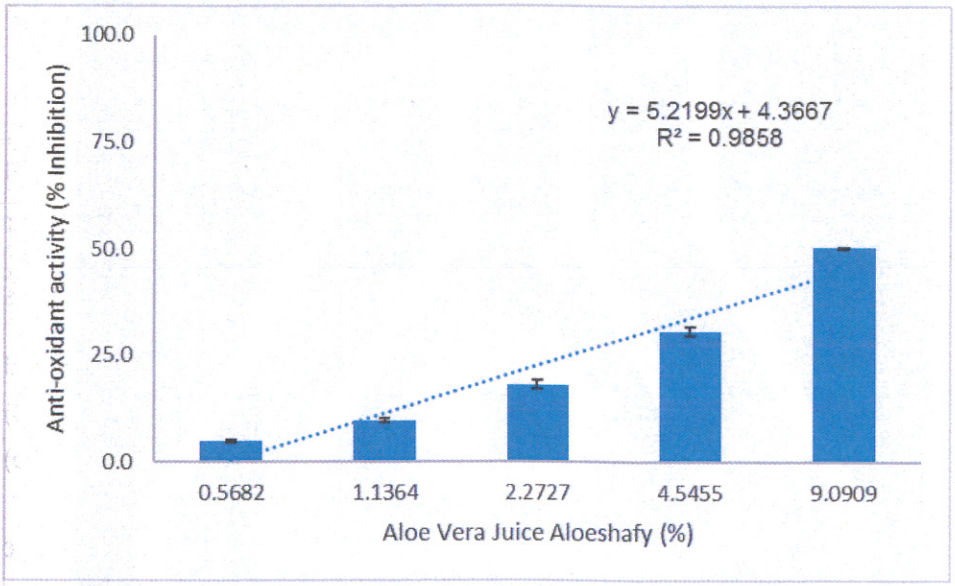


Figure 2: Antioxidant activity of Aloe Vera Juice Aloeshafy

Table 1: Data on Antioxidant activity of Aloe Vera Juice Aloeshafy

TESTED CONENTRATION %	REPLICATE			INHIBITION %			AVE	SEM
	R1	R2	R3	R1	R2	R3		
0.568	0.905	0.902	0.906	4.84	5.15	4.73	4.91	0.22
1.136	0.856	0.862	0.855	9.99	9.36	10.09	9.81	0.40
2.272	0.789	0.779	0.769	17.03	18.09	19.14	18.09	1.05
4.545	0.648	0.669	0.66	31.86	29.65	30.60	30.70	1.11
9.090	0.471	0.475	0.473	50.47	50.05	50.26	50.26	0.21

Negative Control (Absorbance) = 1.03, where absorbance of the sample less than absorbance of the negative control (Refer 2.2.1.6)

R1, R2, R3 = replicates

* Data are the mean ± SEM of three separate experiments.
** SEM (standard error mean) is the standard deviation of the sampling distribution
*** The SEM shall be less than 5.0

Assay integrity: Acceptable (R^2>0.9)

The data verify the integrity of the assay

4.0 Discussion

Based on figure 2, Aloe Vera Juice Aloeshafy promote anti-oxidant activity at the concentration range of 0.568 % to a maximum of 9.09 %. The sample showed dose dependence anti-oxidant activity with IC_{50} value 8.74 ± 0.095 %.

5.0 Conclusion

Based on the result, Aloe Vera Juice Aloeshafy possess anti-oxidant properties.

6.0 References

1. SIRIM/MOA 3:2017 (Annex G) – Determination of antioxidant free radical scavenging assay (ABTS).

2. Standardized Mangifera indica extract is an ideal antioxidant. Lai Teng Ling, Su-Ann Yap et.al. (2009). Food Chemistry, 113, 1154-1159.

3. Antioxidant activity applying an improved ABTS radical action decolorization assay. Roberta Re, Nicoletta Pellegrini, et.al. (1999). Free Radical Biology & Medicine, Vol. 26,1231-1237.

SIRIM Berhad
Industrial Biotechnology Research Centre, Building 19
Tel : 03-5544 6953/6960
Fax: 03-5544 6988

TEST REPORT

REPORT NO: R111/18/B19/33	PAGE: 1 of 5

Applicant	:	Mohd Salehuddin Bin Abdul Razak
Manufacturer / Company	:	PIJ Manufacturing Sdn Bhd, Unit F4, POIC SME Factory, PLO 76, Jalan Nibong 4, Tanjung Langsat Industrial Complex, 81700 Skudai, Johor.
Sample	:	One (1) Aloe Vera Juice Aloeshafy
Reference standard / Method of Test	:	Biochemical Trypsin Assay
Description of Sample	:	Received one sample with the following identification: The sample is yellow colour liquid
Date Received	:	28th February 2018
Job No.	:	J111/18
Issue Date	:	5th April 2018

Approved signatories,

Prepared by :

(HARMAYUMI WAHID)
Researcher
Industrial Biotechnology Research Centre
SIRIM Berhad

Approved by :

(DR. NURUL IZZA NORDIN)
MJMM0579
Senior Researcher
Industrial Biotechnology Research Centre
SIRIM Berhad

SIRIM Berhad
(No. Syarikat 367474 - V)
1, Persiaran Dato' Menteri
Seksyen 2, Peti Surat 7035
40700 Shah Alam
MALAYSIA
Tel: 60-3-55446000
Hotline: 60-3-55103535
Faks: 60-3-55108095
Website: www.sirim.my

Biochemical Trypsin Assay

1. Principle of the assay

The anti-inflammatory effect of the sample (Aloe Vera Juice Aloeshafy) was evaluated by measuring the inhibition activity against trypsin, a serine proteinase that strongly implicated in acute pancreatitis. The assay was designed to screen the ability of sample to inhibit trypsin activity, which is associated with the conversion of N-α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) into *p*-nitroaniline. Thus, the outcome of this study shall substantiate whether the sample has anti-inflammatory property.

2. Method

2.1 Sample preparation

Sample of Aloe Vera Juice Aloeshafy was weighed and dissolved in aqueous. Tested concentration ranged from 0.078 mg/ml to a maximum of 5.0 mg/ml was studied.

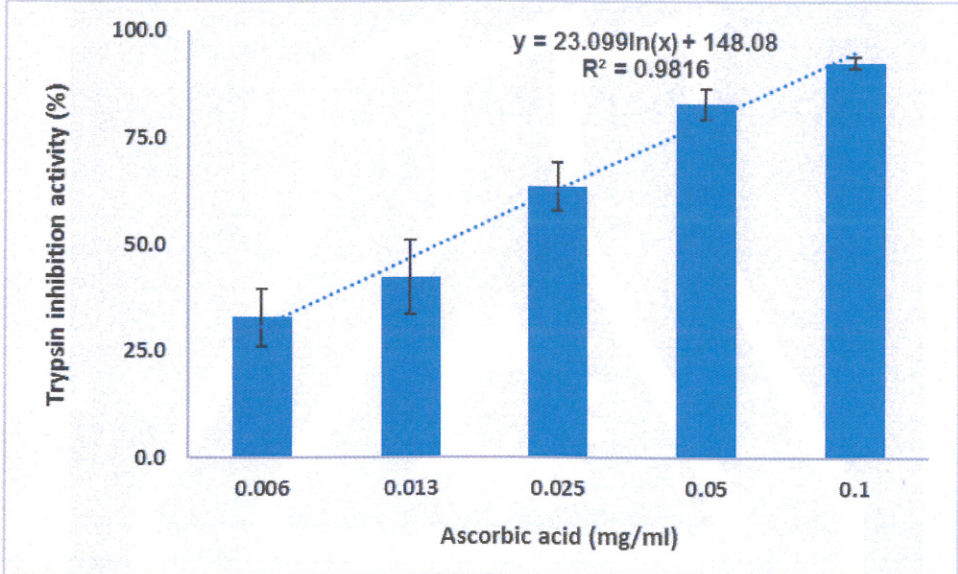
2.2 Trypsin Inhibition

The assay consists of 20 mM BAPNA, which was dissolved in 0.2 M TEA-HCL buffer, pH 7.8 with 65 % DMSO (for enhanced solubility) and 0.3 ml of sample. 5450 units of enzyme dissolved in TEA-HCL buffer were added into the assay, made up 1 mL of reaction mixture. The assay, with or without the sample was pre-incubated at 37 °C for 10 minutes. The enzyme kinetics began when substrate was added into the assay. The amount of *p*-nitroaniline (end product) released through the enzymatic reaction were measured via spectrophotometer at λ=405 nm for 2 minutes. The degree of trypsin inhibition was calculated as below;

$$\% \text{ Inhibition} = \frac{\text{Absorbance of reference} - \text{Absorbance of test}}{\text{Absorbance of reference}} \times 100$$

3. Results

3.1 Positive control: Ascorbic acid



Concentration (mg/ml)	Trypsin inhibition activity (%)				
	R1	R2	R3	Average	StDev
0.006	38.3	35.1	25.5	32.9	6.6
0.013	32.8	44.3	49.8	42.3	8.7
0.025	58.5	62.1	69.9	63.5	5.8
0.05	84.7	85.6	78.9	83.0	3.6
0.1	91.0	93.3	93.6	92.6	1.4

*Data are the mean±StDev of three separate experiments.
**StDev (standard deviation) is the amount of variation or dispersion of a set of data values.

Assay integrity: Acceptable (R^2>0.9)
The data verify the integrity of the assay
Ascorbic acid was used as a standard. The IC_{50} value of ascorbic acid (0.014 mg/ml) shall indicate at least 50% inhibition of trypsin activity.

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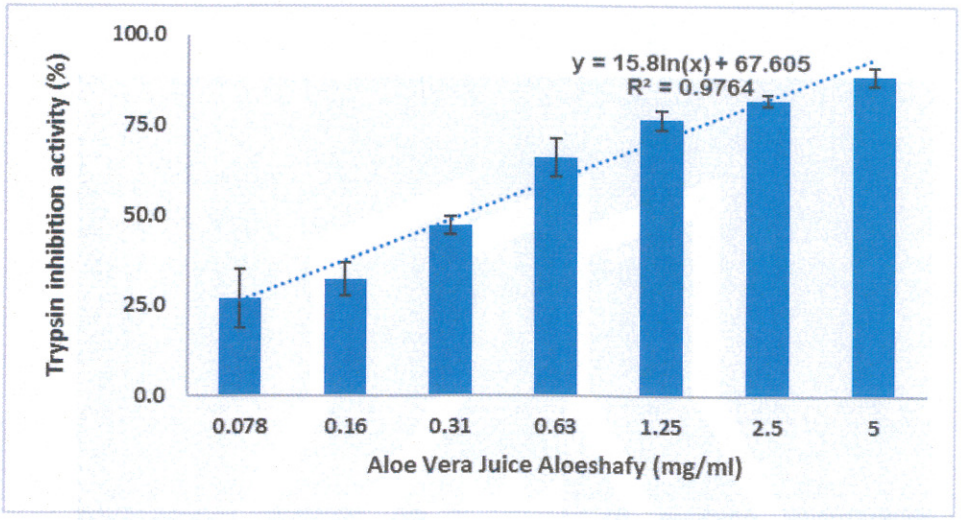
3.2 Sample



Concentration (mg/ml)	Trypsin inhibition activity (%)				
	R1	R2	R3	Average	StDev
0.078	34.3	28.6	18.2	27.0	8.1
0.16	33.8	36.1	27.2	32.3	4.6
0.31	44.3	48.8	48.2	47.1	2.4
0.63	60.5	71.1	65.8	65.8	5.3
1.25	79.5	74.1	75.6	76.4	2.8
2.5	80.5	82.3	83.4	82.1	1.4
5	86.0	90.8	89.4	88.7	2.5

*Data are the mean±StDev of three separate experiments.
**StDev (standard deviation) is the amount of variation or dispersion of a set of data values.

Discussion:

The data demonstrate that the Aloe Vera Juice Aloeshafy promote trypsin inhibition activity at the concentration range of 0.078 mg/ml to a maximum of 5.0 mg/ml. The sample showed dose dependence activity in inhibiting trypsin with IC_{50} value 0.33mg/ml.

4.0 Conclusion

Based on the result, the Aloe Vera Juice Aloeshafy possess anti-inflammatory properties.

5.0 References

1. Parellada, J, (1995). Flavonoid inhibitors of trypsin and leucine aminopeptidase: a proposed mathematical model for IC_{50} estimation. *Journal of natural products,* 58 (6): 823-829.
2. Ceppa EP, Lyo V, Grady EF, (2011). Serine proteases mediate inflammatory pain in acute pancreatitis. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 300(6):G1033-G1042.

SIRIM Berhad
Industrial Biotechnology Research Centre, Building 19
Tel: 03-55446953/6960
Fax: 03-55446988

TEST REPORT

REPORT NO: R352/18/B19/33	PAGE: 1 of 10

Applicant	:	Mohd. Salehuddin bin Abdul Razak
Company	:	PIJ MANUFACTURING SDN BHD Unit F4, POIC SME Factory (Halal Park), PLO 76, Jalan Nibong 4, Tanjung Langsat Industrial Complex, 81700 Pasir Gudang, Johor Darul Ta'zim, MALAYSIA.
Sample	:	One (1) bottle of 500ml Aloe Vera Juice : : Batch No F20170207
Reference standard / Method of Test	:	In-house Alpha-Amylase, Alpha Glucosidase and Lipase Inhibition Assay
Description of Sample	:	Received one sample with the following identification: The sample is light yellow color
Date Received	:	29th May 2018
Job No.	:	J352/18
Issue Date	:	17th August 2018

Approved signatories,

(Dr. THEANMALAR MASILAMANI)
Senior Researcher
Industrial Biotechnology Research Centre
SIRIM Berhad

(DR. NURUL IZZA NORDIN)
MJMM0579
Senior Researcher
Industrial Biotechnology Research Centre
SIRIM Berhad

SIRIM Berhad
(No. Syarikat 367474 - V)
1, Persiaran Dato' Menteri
Seksyen 2, Peti Surat 7035
40700 Shah Alam
MALAYSIA
Tel : 60-3-55446000
Hotline : 60-3-55103535
Fax : 60-3-55108095
Website : www.sirim.my

Inhibition of carbohydrate digestion (Alpha-Amylase and Alpha-glucosidase Inhibition Assay) and Inhibition of fat digestion (Lipase Inhibition Assay) test of Aloe Vera Juice

1. Background

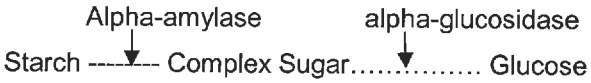
Diabetes mellitus (DM) is a metabolic disorder that is characterized by high levels of blood glucose with disturbances of carbohydrate, lipid, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Between two types of diabetes, type 2 is more prevalent than type 1, with more than 90% of the total diabetic patients suffering from it. Type 2 diabetes (T2D) is a disease caused by an imbalance between blood sugar absorption and insulin secretion. Postprandial hyperglycemia plays an important role in the development of T2D. Regulating plasma glucose level is vital for delaying or preventing T2D. The ability of a drug or diet to delay the production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase is one of the therapeutic approaches for decreasing postprandial hyperglycemia. At present, the use of insulin secretagogues and sensitizers constitute the predominant line of therapy, however, the use of carbohydrate digesting enzyme inhibitors play a vital role in controlling hyperglycemia by reducing the intestinal absorption of glucose. Thus, these inhibitors slows the digestion of carbohydrate keeping blood glucose from rising too high after meals.

Obesity is becoming a worldwide epidemic, resulting in a major risk factor for coronary heart disease including diabetes mellitus and metabolic syndrome. The major source of unwanted calories are the dietary lipids, therefore lipid metabolism play a major role in maintaining energy homeostatis. Pancreatic lipase is the primary enzyme responsible for breakdown of fat molecules, specifically triglycerides to monoglycerides, aiding absorption of fats. Inhibition of lipase activity and hence calorific intake is one medical approach to treat obesity. Orlistat, a specific drug for inhibiting pancreatic lipase that reduces dietary fat absorption by 30%, has been used for clinical use.

1.1 Principle of the assay

Alpha-amylase is enzyme that hydrolyzes the starches and glycogen. These enzymes are contained in the saliva and pancreatic fluid of animals, and transform starches and the like into maltose or sucrose (complex sugar). Subsequently, the disaccharides, such as maltose

and sucrose, are then transformed into glucose by the enzyme alpha-glucosidase, which exists in the cell membrane of the small intestine mucous membrane. The glucose is then absorbed from the small intestine and carried into the blood and thus raising the blood glucose level. Therefore, to inhibit a superfluous energy supply or control blood glucose levels, in other words, to prevent or treat obesity and diabetes, it is very important to control the activity of these enzymes such as alpha-amylase and alpha-glucosidase.



Lipase inhibitors are substances used to reduce the activity of lipases found in the intestine. Lipases are secreted by the pancreas when fat is present. The primary role of lipase inhibitors is to decrease the gastrointestinal absorption of fats. Fats then tend to be excreted in feces rather than being absorbed to be used as a source of caloric energy, and this can result in weight loss in individuals. These inhibitors could be used for the treatment of obesity, which can subsequently lead to Type II diabetes and cardiovascular diseases if not managed. Orlistat is an example of a commercial lipase inhibitor. The lipase inhibition assay was performed using Porcine Pancreatic Lipase (PPL) and *para*-nitrophenyl palmitate (*p*NPP) as substrate. The basis of this assay protocol is the determination of *para*-nitrophenol (*p*NP) released as a result of enzymatic hydrolysis of *p*NPP.

2.0 Acceptance Criteria (Acceptable (R^2>0.9)

Acarbose was used as a standard. The IC_{50} value of acarbose for Alpha-amylase and Alpha-glucosidase inhibition are 4.55 µg/ml and 0.276 mg/ml respectively, shall indicate at least 50% inhibition of those two enzyme activity. The IC_{50} value for the positive control Orlistat in the lipase inhibition assay is 2.125 µg/ml. IC_{50} indicates the concentration of sample which inhibits 50% of alpha-amylase and alpha glucosidase activity.This indicates integrity of the assay, and the IC_{50} of the positive control. The R^2 value of the dose response curve is more than 0.9.

3.0 Method

3.1 Sample preparation

The Aloe vera sample was tested from the stock solution of 100% juice. The tested concentration indicates the final concentration of the test sample in the assay system which takes account of the dilution factor. The highest tested concentration of the sample for alpha amylase and alpha glucosidase inhibition assay were 25% and 40% respectively and 44% for lipase inhibition assay. Five dilutions of the sample were carried out.

3.2 Inhibition of Alpha-amylase Activity

Alpha amylase activity using starch as the substrate and porcine pancreatic alpha-amylase as enzyme were assayed spectrophotometrically. Aloe vera juice sample was added into the reaction mixture with 2 units porcine pancreatic alpha-amylase and 20mM potassium phosphate buffer with 6mM sodium chloride at pH 6.9. The mixture was incubated for 15 minutes at 37°C. After 15 minutes, 1% starch (substrate) was added to the mixture and further incubated for 15min. The reaction was then stopped with 3,5-dinitrosalicyclic (DNS) color reagent and heated for 5 minutes in 95°C water bath. The DNS solution reacts with the reducing sugar produced and will become 3-amino-5-nitrosalicyclic forming a brick-red colour upon heating at 95°C. The absorbance value was measured at 540nm. Acarbose was used as positive control.

$$\% \text{ inhibition} = \frac{(A - A2) - (B - B2)}{(A - A2)} \times 100\%$$

A : Absorbance of reference (without sample)

A2 : Absorbance of reference blank (without enzyme and without sample)

B : Absorbance of test (with sample and enzyme)

B2 : Absorbance of test blank (with sample and without enzyme)

3.3 Inhibition of Alpha-glucosidase Activity

Alpha-glucosidase activity using *para*-nitrophenyl-α-d-glucosidase as the substrate and alpha-glucosidase (from *Saccaharomyces cerevisiae*) enzyme were assayed spectrophotometrically. Aloe vera juice sample was added into the reaction mixture with 1 unit alpha-glucosidase and 100 mM potassium phosphate buffer with 3.2 mM Magnesium chloride at pH 6.8. The mixture was pre-incubated for 15 minutes at 37°C. After 15 minutes, 5mM *para*-nitrophenyl-α-d-glucosidase (substrate) was added to the mixture and further incubated for 15min. The reaction was then stopped with 0.2 M sodium carbonate. The substrate *para*-Nitrophenyl-α-d-glucosidase is hydrolysed by enzyme alpha-glucosidase and produces *para*-Nitrophenol. *para*-Nitrophenol is a yellowish product, absorbance was measured at 405nm. Acarbose was used as positive control

$$\% \text{ inhibition} = \frac{(A - A2) - (B\text{-}B2)}{(A - A2)} \times 100\%$$

A : Absorbance of reference (without sample)

A2 : Absorbance of reference blank (without enzyme and without sample)

B : Absorbance of test (with sample and enzyme)

B2 : Absorbance of test blank (with sample and without enzyme)

3.4 Lipase Inhibition Assay (Anti-obesity)

Lipase activity using *para*-nitrophenyl-palmitate (*p*NPP) as the substrate and porcine pancreatic lipase (PPL) enzyme were assayed spectrophotometrically. Aloe vera juice sample was added into the reaction mixture with 5 mg/ml porcine pancreatic lipase and 100 mM Tris-HCL buffer with 5 mM Calcium chloride at pH 8.0. The mixture was pre-incubated for 15 minutes at 40°C. After 15 minutes, 10 mM *para*-nitrophenyl-palmitate (substrate) which is dissolved in isopropanol was added to the mixture and further incubated for 5 minutes. At the end of the reaction a turbid solution was obtained and 0.4ml of DMSO is added to clear the solution. The substrate *para*-nitrophenyl-palmitate is hydrolysed by the porcine pancreatic lipase and produces *para*-Nitrophenol. *para*-Nitrophenol is a yellowish product, absorbance was measured at 410nm. Orlistat was used as positive control.

$$\% \text{ inhibition} = \frac{(A - A2) - (B\text{-}B2)}{(A - A2)} \times 100\%$$

A : Absorbance of reference (without sample)

A2 : Absorbance of reference blank (without enzyme and without sample)

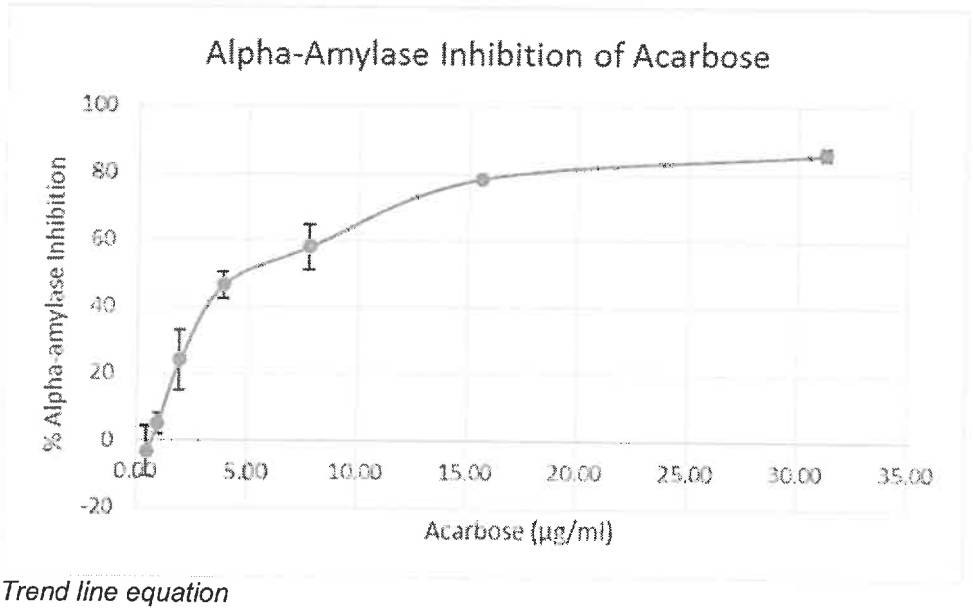
B : Absorbance of test (with sample and enzyme)

B2 : Absorbance of test blank (with sample and without enzyme)

4.0 Results

4.1 Alpha-amylase Inhibition

Positive Control Acarbose (IC_{50} = 4.55 ± 0.11 µg/ml)



Trend line equation
y = 23.08ln(x) + 10.659
R^2 = 0.9872

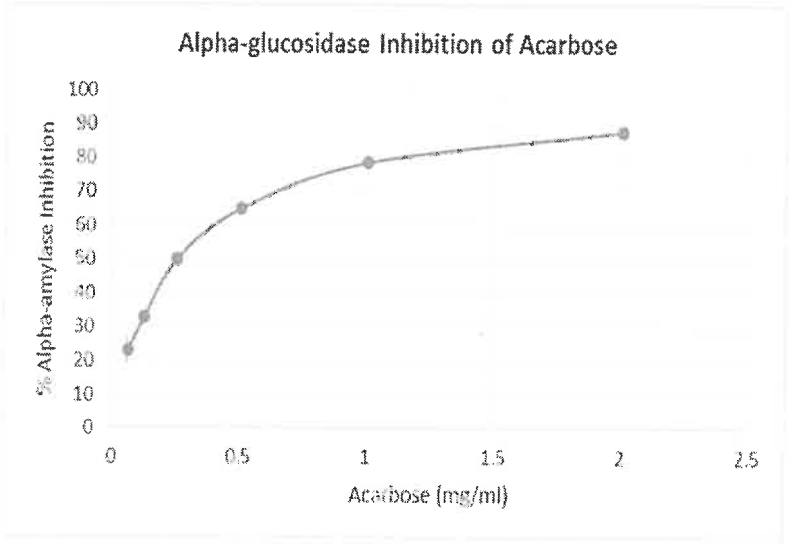
Acarbose Tested Concentration (µg/ml)	% Alpha-amylase Inhibition			
	R1	R2	Average	Stdev
0.49	-8	2	-3	7.5
0.98	7	3	5	2.9
1.95	18	30	24	8.8
3.91	49	44	47	4.0
7.81	53	63	58	6.8
15.63	79	78	78	0.6
31.25	84	87	86	2.0

4.2 Aloe Vera Juice Alpha-amylase Inhibition

Aloe Vera Tested concentration (%)	Alpha-amylase Inhibition (%)			
	R1	R2	Average	Stdev
3.125	-19	-15	-17	2.7
6.25	-30	-19	-24	7.6
12.5	-27	-26	-26	0.4
25	-10	-18	-14	5.6

4.3 Alpha Glucosidase Inhibition

Positive control : Acarbose (IC_{50} = 0.276 ± 0.007 mg/ml)



Trend line equation
y = 19.66ln(x) + 76.551
R^2 = 0.993

Acarbose Tested concentration (mg/ml)	**% Alpha-glucosidase Inhibition**			
	R1	**R2**	**Average**	**Stdev**
0.0625	25	20	23	3.7
0.125	33	33	33	0.1
0.25	51	49	50	1.8
0.5	65	65	65	0.3
1	79	78	79	0.9
2	89	87	88	1.7

Data are the average±StDev of two separate experiments.
** StDev (standard deviation) is the amount of variation or dispersion of a set of data values.

4.4 Aloe Vera Juice Alpha-glucosidase Inhibition

Aloe VeraTested concentration (%)	% Alpha-glucosidase Inhibition			Stdev
	R1	R2	ave	
2.5	-44	-28	-36	11.8
5	-43	-25	-34	12.9
10	-36	-17	-26	13.4
20	-20	-1	-10	13.4
40	31	21	26	7.5

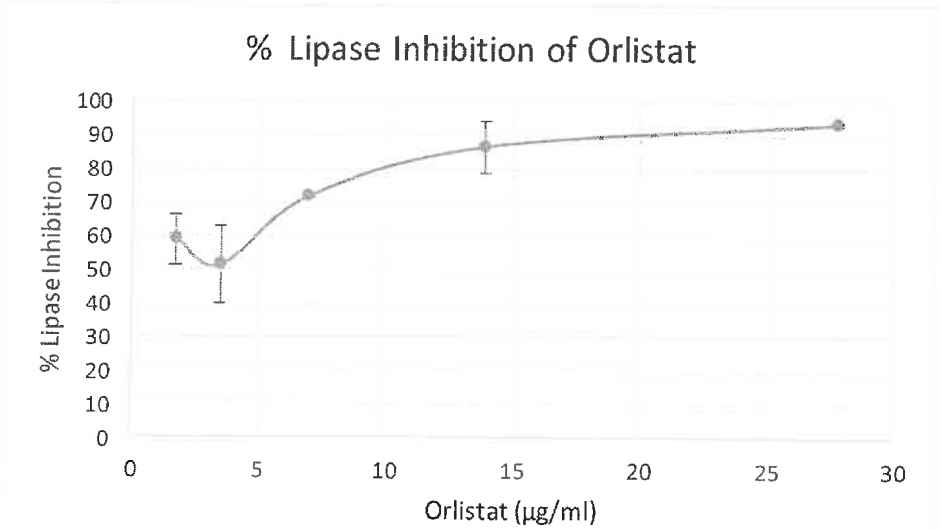
Data are the average±StDev of two separate experiments.
StDev (standard deviation) is the amount of variation or dispersion of a set of data values

TEST REPORT

4.5 Anti-obesity using Lipase Inhibition Assay

Positive Control Orlistat

Lipase Inhibition IC_{50} = 2.125 µg/ml ± 2.7



Trend line equation
y = -0.08x2 + 3.9195x + 46.869
R^2 = 0.922

Orlistat Tested Concentration (µg/ml)	% Lipase Inhibition			
	R1	R2	Average	Stdev
2	54	64	59	7.3
3	60	43	52	11.4
7	72	72	72	0.2
14	81	92	87	7.7
28	94	93	94	0.5

Data are the average±StDev of two separate experiments.
StDev (standard deviation) is the amount of variation or dispersion of a set of data values

4.6 Aloe Vera Juice lipase Inhibition

Aloe vera Tested Concentration (%)	% Lipase Inhibition			
	R1	R2	Average	Stdev
1	-28	5	-11	23.1
3	23	-8	7	21.9
6	-14	-17	-15	2.4
11	-13	-36	-25	16.3
22	-33	-15	-24	12.5
44	30	36	33	4.0

Data are the average±StDev of two separate experiments.
StDev (standard deviation) is the amount of variation or dispersion of a set of data values

5.0 Discussion

The results indicate that Aloe vera juice did not exhibit alpha-amylase inhibition activity. Aloe Vera juice showed alpha-glucosidase inhibition activity of 26 ± 7.5% at the highest tested concentration of 40% Aloe Vera. Aloe vera juice showed lipase inhibition activity of 33% ± 4.0 at the highest tested concentration of 44%.

6.0 Conclusion

Based on the results, Aloe Vera juice possess anti-hyperglycemic namely the alpha-glucosidase inhibition and lipase inhibition activity.

7.0 References

1. Oliveira G.R.B et al. 2017. Stem bark of *Endopleura uchi (Huber) Cuatrec* : Inhibition of pancreatic lipase and anti-oxidant activity. *Journal of Medicinal Plants Research.* **11** (30): 472- 479.
2. Telagari M & Hullatti K. 2015 . In vitro α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. Extracts and fractions. *Indian Journal of Phamacology* Jul-Aug **47** (4) : 425-429.
3. Taukoorah U & Mahomoodally M.F 2016. Crude Aloe vera Gel Shows Antioxidant Propensities and Inhibits Pancreatic Lipase and Glucose Movement *In Vitro*. Advances in Pharmacological Sciences. **2016** :1-9