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GC-MS analysis and *In-vitro* anti-diabetic activity of ethanolic extract of the*Atrocarpus Heterophylus* (Unriped Jack Fruit)

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Abstract

Objective: In the present study the ethanolic extracts of *Atrocarpus Heterophylus* (ETAH) were studied for Aldose reductase, alpha (α)- amylase and alpha (α)-glucosidase inhibition using an *in-vitro* anti diabetic and evaluate the GC-MS analysis of active compounds of ETAH**.**
Methods: The serial extraction was carried out with a series of solvents: Petroleum ether, ethyl acetate and ethanol with increasing polarity using Soxhlet apparatus. The concentrated and dried extracts were subjected to the antidiabetic activity was assessed by employing standard *in-vitro* techniques.
Results and discussion: The result showed ethanolic extract exhibited significant aldose reductase, α-amylase and α-glucosidase inhibitory activities with an all plant extracts respectively and well compared with standard acarbose drug. GC-MS analysis results revealed that ETAH contain the compounds are Cyclohexanol and Nonadecene. This knowledge will be useful in finding more potent antidiabetic principle from the natural resources for the clinical development of antidiabetic therapeutics.
Conclusion: The investigation confirms that ethanolic extract exhibited highest antidiabetic activity among all extracts, Additional studies on needed for purification, characterization and structural elucidation of bioactive compounds from ethanolic extract.

Keywords: *In-vitro* antidiabetic, Aldose reductase, α-amylase, α-glucosidase, GC-MS analysis.

1. INTRODUCTION

Diabetes mellitus is an endocrine disorder in which glucose metabolism is compromised due to total loss of insulin following pancreatic -cell destruction in insulin dependent diabetes mellitus and insufficient release of insulin from the pancreatic -cells or insulin sensitivity of the target tissues in non-insulin dependent diabetes mellitus. According to reports, the polyol pathway's essential enzyme aldose reductase catalyses the conversion of glucose to sorbitol. Aldose reductase has a low substrate affinity for glucose in normal tissue, which results

in little catalysis of the conversion of glucose to sorbitol. Since sorbitol does not easily diffuse across cell membranes, it has been suggested that intracellular sorbitol accumulation contributes to the development of chronic diabetes problems such cataract, neuropathy, and retinopathy. These results imply that Aldose reductase inhibitors reduce the conversion of glucose to sorbitol and may be able to prevent and/or treat a number of problems associated with diabetes [1]. Prior to absorption, the digestive enzyme (-amylase) hydrolyzes dietary starch to maltose, which then breaks down to glucose. Diabetes patients' unfavourable high postprandial blood glucose peak should be decreased by -amylase inhibition. It is believed that intestinal -Glucosidase inhibitors are potent therapeutic agents for diseases of the metabolism of carbohydrates, particularly diabetes mellitus and obesity. The suppression of poly- and oligosaccharide digestion in the digestive tract is anticipated to reduce postprandial hyperglycemia and hyperinsulinemia [2].

Nonadecane molecule has potential as an antidiabetic.Because the -amylase enzyme is inhibited by nonadecane in vitro, it has potential as an antioxidant and anti-diabetic agent. Currently, a number of medications, including biguanides, sulfonylurea, and thiazolidenediones, are available to treat diabetes mellitus. These medications' pharmacokinetic characteristics, subsequent failure rates, and associated side effects limit their use [3]. As a result, finding a new class of chemicals is crucial for treating diabetes complications, which necessitates ongoing research for substitute medications [4]. Therefore, the goal of the current study was to investigate Atrocarpus Heterophylus (Family: Moraceae) and assess the *in- vitro* anti-diabetic effectiveness of the various solvent extracts.

2. MATERIALS AND METHODS

2.1. Preparation of different plant extracts

Collections of *Atrocarpus Heterophylus* from Cuddalore in Tamil Nadu, India. The botanical survey of cuddalore therapeutic plants served as the basis for taxonomic identification. Small chunks of freshly picked, unripe jackfruit were shade-dried at room temperature, pounded into a fine powder, and kept in airtight containers. Then, using the Soxhlet equipment and ethanol as the solvent, 500 g of the sample was continuously extracted over the course of 72 hours [6]. Using a rotating evaporator and lowered pressure, the extracts were concentrated to a constant weight. The extracts were gathered and stored in a desiccator until they were needed for additional research.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis of ETAH was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused with a capillary column (30 × 0.25 µm × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 mL·min^{-1}, and an injection volume of 2 µL was employed (a split ratio of 10 : 1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C·min^{-1} to 200°C, then 5 °C·min^{-1} to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectrum was taken at 70 eV; a scan interval of 0.5 sec and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass version-5.2.

2.2. Identification of the compounds

Interpretation of the GC-MS was conducted using the database of the National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

2.3. *In-vitro* anti diabetic study

2.3.1. Rat lens Aldose reductase preparation

Aldose reductase was prepared from rat lens. Eyeballs were removed from 9-week-old male rats. Lenses were dissected by posterior approach and homogenized in 10 volumes of 100 mM potassium phosphate buffer pH

6.2. The homogenate was centrifuged at 15,000 Xg for 30 min at 4°C and the resulting supernatant was used as the source of Aldose reductase.

Determination of aldose reductase activity

The method of Hayman and Kinoshita [7] was used to assay for aldose reductase activity. Enzyme specific activity was calculated as IU/mg protein, and this was defined as activity of the enzyme that can produce 1µmol NADP+ from NADPH in 1 min.25. Various concentrations (50-1600 µg/ml) of the ETAH were prepared in triplicate. Exactly 100 µl of concentration prepared was then added to the assay mixture and incubated for 5-10 minutes. The assay mixture was incubated at 37ºC and initiated by the addition of NADPH at 37ºC.The change in the absorbance at 340 nm due to NADPH oxidation was measured spectrophotometrically.Acarbose was used as standard drug. The inhibition of aldose reductase was calculated using the followingCalculations.

The percentage inhibition of aldose reductase is calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

2.3.2. Alpha-amylase inhibitory assay

The Alpha-amylase inhibitory assay of ETAH was evaluated according to a previously described method [8]. In brief, 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 ml of dinitrosalicylic acid color reagent. At this time, the test tubes were placed in a water bath (100 °C for 5 min) and cooled until room temperature was attained. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following Calculations.

The percentage inhibition of α-amylase is calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

2.3.3. α- Glucosidase inhibition bioassay

To 50 µL of enzyme, add 250 µL of buffer of test extracts ETAH and incubate at 37°C for 30 minutes. Add 500 µL of sucrose solution and incubate at 37°C for 20 minutes, heat on boiling water bath for 2 minutes to arrest the reaction and cool. Measure glucose concentration by Glucose Oxidase method [9].

Glucose estimation (Glucose oxidase method)

Mix 100 µL of test extracts with 500 µL of glucose reagent (Glucose reagent kit) then incubate at room temperature for 10 minutes. Measure the absorbance at 510 nm.

Calculation

The percentage inhibition of α - glucosidase is calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

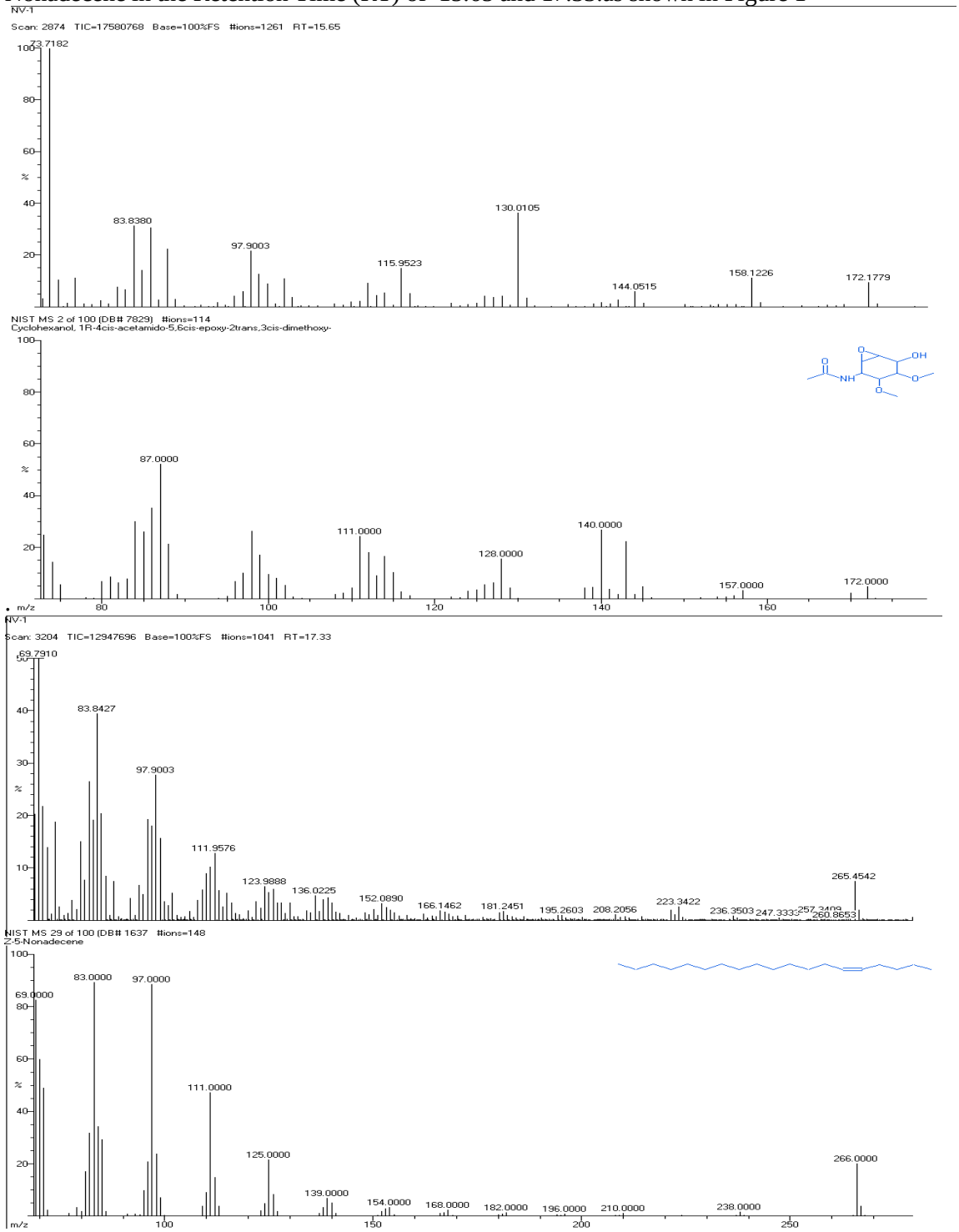
2.4. Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA). The least significant difference test was used for mean comparisons and ($p < 0.05$) was considered to be statistically significant.

3. RESULTS AND DISCUSSION:

3.1. Identification of Ethanolic Extract of *Atrocarpus Heterophylus*

GC-MS confirmed the complete structure of ETAH which was identified to be a mixture of Cyclohexanol and Nonadecene in the Retention Time (RT) of 15.65 and 17.33.as shown in Figure 1



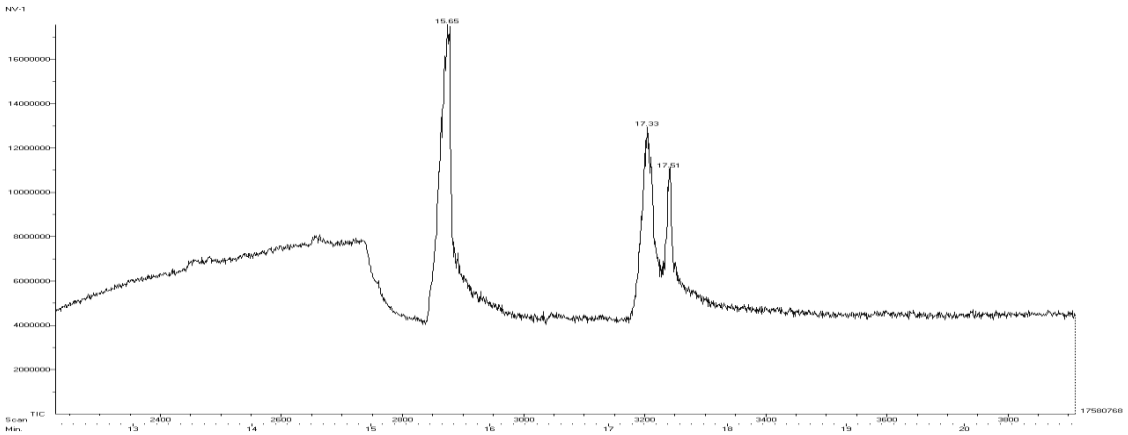


Figure -1: GC-MS of ETAH

3.2. Aldose Reductase Inhibition Assay

The results of *in-vitro* antidiabetic effect using Aldose reductase inhibitory assay of the ethanolic extracts of *Atrocarpus Heterophylus* and acarbose were shown in Table 1. The ethanol extracts revealed a significant inhibitory action of Aldose reductase enzyme. Ethanolic extract showed inhibitory activity of IC 50 value is 258.7µg/ml. Acarbose is a standard drug used for Aldose reductase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 3.1µg/ml. Aldose reductase is present in all target tissues that develop diabetic complications [11], In our study result revealed that ethanolic extract prevents diabetic complications especially diabetic retinopathy and nephropathy.

Table- 1: Aldose Reductase Inhibition Assay

	Conc (µg/ml)	OD @405 nm	% Inhibition	IC 50(µg/ml)
Control	0	0.659	0.00	0
Acarbose	0.3125	0.524	22.02	4.0
	0.635	0.467	32.69	
	1.15	0.343	42.25	
	2.4	0.289	53.31	
	6	0.179	71.74	
	10	0.119	82.71	
Ethanol Extract	50	0.516	12.02	238.7
	100	0.415	35.09	
	200	0.512	22.53	
	400	0.515	23.07	
	800	0.436	36.20	
	1600	0.321	41.74	

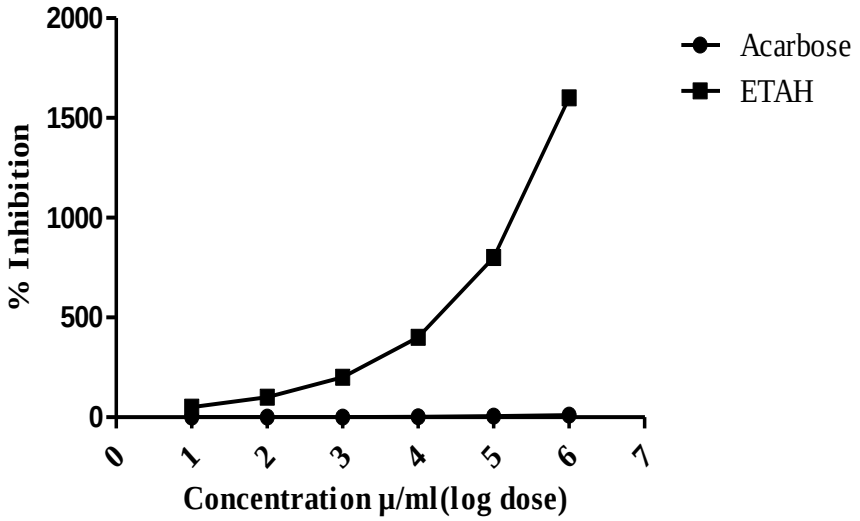


Figure - 2: Aldose Reductase Inhibition Assay

3.3. Alpha amylase inhibitory activity

In this study the *in- vitro* alpha amylase inhibitory activities of the ETAH were investigated. The result of the experiment showed that ethanolic extract increased inhibitory activity against Alpha amylase enzyme shown in Table 2. Ethanolic extract showed inhibitory activity of IC 50 value is 239.6µg/ml. Acarbose is a standard drug used for alpha amylase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 2.15µg/ml. So, the plant extracts might be used as starch blockers since it prevents or slows the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars.

In our study, the ethanolic extract showed maximum α- amylase inhibitory activity, which could be attributed to the presence of polyphenols and flavonoids. Because polyphenols are not only capable of reducing oxidative stress but also of inhibiting carbohydrate hydrolyzing enzymes because of their ability to bind with proteins [12].

Table- 2: Alpha amylase inhibitory activity

	Conc. µg/ml	OD at 590nm	% Inhibition	IC50 µg/ml
Control	0	0.81	0.00	0
Acarbose	0.3135	0.49	8.97	2.32
	0.615	0.48	17.92	
	1.35	0.40	31.58	
	2.4	0.24	36.64	
	4	0.28	54.90	
	10	0.37	67.08	
Ethanol Extract	50	0.84	15.36	228.6
	100	0.91	33.54	
	200	0.76	51.18	
	400	0.61	63.34	
	800	0.50	63.83	
	1600	0.31	74.54	

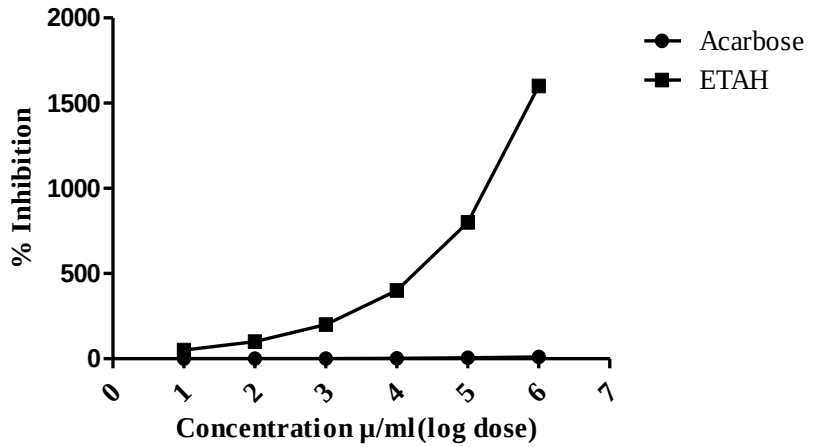


Figure-3: Alpha amylase inhibitory assay

3.4. Alpha Glucosidase Inhibition Assay

The results of *in-vitro* antidiabetic activity using alpha glucosidase inhibitory assay of ETAH and acarbose was shown in Table 3. The extract revealed a significant inhibitory action of -glucosidase enzyme. Ethanolic extract showed inhibitory activity of IC 50 value is 328.2µg/ml. Acarbose is a standard drug used for alpha glucosidase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 3.978µg/ml. Thus, the inhibition of the activity of alpha glucosidase by ETAH would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation [13].

Table - 3: Alpha Glucosidase Inhibition Assay

	Conc (µg/ml)	OD @ 405nm	% Inhibition	IC50 (µg/ml)
Control	0	0.865	0.00	0.00
Acarbose	0.3235	0.865	12.24	3.878
	0.654	0.743	23.90	
	1.32	0.654	31.24	
	2.6	0.523	44.20	
	5	0.454	51.31	
	10	0.186	81.21	
Ethanol Extract	50	0.875	31.27	383.2
	100	0.954	35.18	
	200	0.850	29.73	
	400	0.592	38.71	
	800	0.954	56.04	
	1600	0.869	59.98	

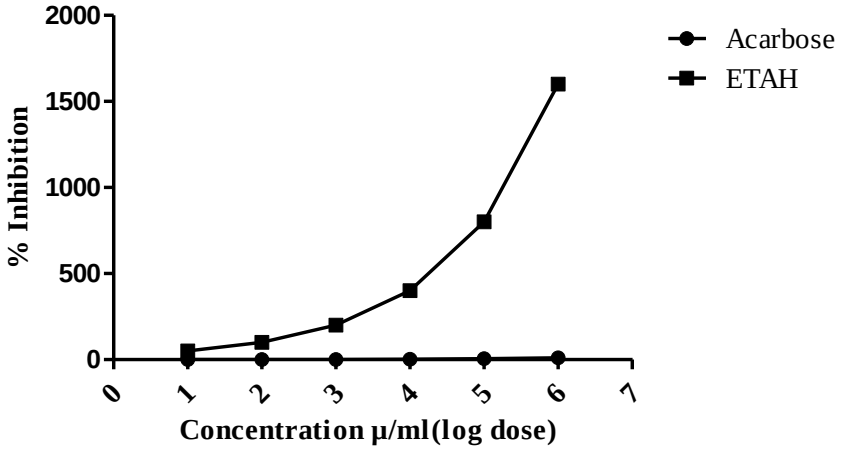


Figure - 4: Alpha Glucosidase Inhibition Assay

CONCLUSION

To investigate the *in-vitro* antidiabetic activities of the ethanol extract of *Atrocarpus Heterophylus* has been analysed. As a result, we found that the extract of ETAH inhibitory activity against aldose reductase, α-amylase and α- glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus. Cyclohexanol and Nonadecene compounds possesses significant blood glucose-lowering activities. At present, the exact mechanism of action of the Cyclohexanol and Nonadecene is not yet known and will be the subject of further studies

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