

In-vitro* Anti-Diabetic Activity of Ethanolic Extract of the Medicinal Plants *Desmodium triflorum*, *Allmonia nodiflora* and *Digeria muricata

**Ramkumar K.¹, Anton Smith A.², Vishwanath B. A.¹,
Venkatesan N.¹**

¹Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore – 560 064, Karnataka

²Department of Pharmacy, Annamalai University, Annamalai Nagar- 608 002, Tamil nadu,

Abstract: Objective: In the present study the ethanolic extracts of *Desmodium triflorum* (ETDT), *Allmonia nodiflora* (ETAN) and *Digeria muricata* (ETDM) were studied for Aldose reductase, alpha (α)- amylase and alpha (α)-glucosidase inhibition using an *in-vitro* antidiabetic model. 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. Result: 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. 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 & also confirm its antidiabetic property by *in vivo* studies. This study provides scientific evidence that leaves of *Desmodium triflorum*, *Allmonia nodiflora* and *Digeria muricata* have anti-diabetic efficacy. Thus, considering its relative hypoglycemic potency, they may serve as useful therapeutic agents for treating diabetes.

Keywords: *In-vitro* Antidiabetic, Aldose Reductase, α -Amylase, α -Glucosidase

Introduction:

Diabetes mellitus is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic β -cells in insulin dependent diabetes mellitus and inadequate release of insulin from the pancreatic β -cells or insensitivity of target tissues to insulin in non-insulin dependent diabetes mellitus. Aldose reductase, as a key enzyme in the polyol pathway, is reported to catalyze the reduction of glucose to sorbitol. In normal tissue, Aldose reductase has low substrate affinity for glucose, so that the conversion of glucose to sorbitol is little catalyzed. However, in diabetes mellitus, the increased availability of glucose in insulin insensitive tissues such as lens, nerve and retina leads to the increased formation of sorbitol through the polyol pathway. Sorbitol does not readily diffuse across cell membranes and the intra cellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataract, neuropathy and retinopathy. These findings suggest that Aldose reductase inhibitors prevent the

conversion of glucose to sorbitol and may have the capacity of preventing and / or treating several diabetic complications [1]. During hyperglycemia cellular levels of glucose greatly increase in tissues where glucose entry is independent of insulin. In these tissues, which include lens, retina, kidney, and peripheral nerves, this excess glucose is metabolized via an accessory pathway known as the polyol pathway. Aldose reductase is the rate-limiting enzyme of the polyol pathway. AR catalyzes glucose to sorbitol and sorbitol dehydrogenases, the second enzyme of the pathway, further converts sorbitol to fructose [2]. The digestive enzyme (α -amylase) is responsible for hydrolyzing dietary starch to maltose, which breaks down to glucose, prior to absorption. Inhibition of the α -amylase should reduce the unfavorable high postprandial blood glucose peak in diabetics. Intestinal α -Glucosidase inhibitors are reported to be powerful therapeutic agents in carbohydrate metabolic disorders, especially diabetes mellitus and obesity. Postprandial hyperglycemia and hyperinsulinemia are expected to be diminished by

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Venkatesan N. (Correspondence)

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inhibition of poly and oligosaccharide digestion in the intestinal-tract [3]. Practically, a few α -Glucosidase inhibitors of microbial origin viz., Management of diabetes without any side effect is still a challenge to the medical community. Several drugs such as biguanides, sulfonylurea and thiazolidinediones are presently available to treat the diabetes mellitus [4]. The use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects [5]. Thus searching for a new class of compounds is essential to overcome diabetic problems ultimately leading to continuous search for alternative drugs⁵. The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies [6]. Henceforth, present study was aimed to explore the *Desmodium triflorum*, *Allmonia nodiflora* and *Digeria muricata* plants are evaluating the *in-vitro* anti-diabetic activity of the different solvent extracts.

Materials and Methods:

Preparation of different plant extracts

Desmodium triflorum, *Allmonia nodiflora* and *Digeria muricata* leaves were collected from the forest of kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottai, authenticated by Chelladurai Botionist. A voucher specimen No (CCRAS-1154/2017). Fresh plant leaves were shade dried at room temperature, ground into fine powder and stored in airtight containers. Then extracted (amount 500 g) with solvents of increasing polarity such as petroleum ether, ethyl acetate, and ethanol, for 72 hours with each solvent, by continuous hot extraction using the soxhlet apparatus at a temperature of 60°C [7]. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

Rat lens Aldose reductase preparation

Crude Aldose reductase was prepared from rat lens. Eyeballs were removed from 9 week old male rats. Animal care and protocols were in accordance with and approved by Institutional Animal Ethics Committee. 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 [8].

Determination of aldose reductase activity

The method of Hayman and Kinoshita [8] was used to assay for aldose reductase (AR) activity. Enzyme specific activity was calculated as IU/mg protein and this was defined as activity of the enzyme that can produce 1 μ mol NADP⁺ from NADPH in 1 min.25. Various concentrations (50-1600 μ g/ml) of the ethanolic extract of *Desmodium triflorum* (ETDT), *Allmonia nodiflora* (ETAN) and *Digeria muricata* (ETDM) leaves were prepared in triplicate. Exactly 100 μ l of concentration prepared was then added to the assay mixture and incubated for 5-10 minutes. The assay mixture was incubated at 37°C and initiated by the addition of NADPH at 37°C. 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 following Calculations

The percentage inhibition of aldose reductase is calculated as follows:

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

Alpha-amylase inhibitory assay

The Alpha-amylase inhibitory assay of ethanol extracts of *Desmodium triflorum*, *Allmonia nodiflora* and *Digeria muricata* was evaluated according to a previously described method [9]. 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:

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

α - Glucosidase inhibition bio-assay

To 50 μ L of enzyme, add 250 μ L of buffer of test extracts (ETDT, ETAN and ETDM) and incubate at 37°C for 30 minutes. Add 500 μ L 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 [10].

Glucose estimation (Glucose oxidase method)

Mix 100 μ L of test extracts with 500 μ L 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:

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

Results and Discussion:

Aldose Reductase Inhibition Assay

The results of *in-vitro* antidiabetic effect using Aldose reductase inhibitory assay of the ethanol extracts of *Desmodium triflorum*, *Allmonia nodiflora*, *Digeria muricata* and acarbose were shown in Table 1. The ethanol extracts revealed a significant inhibitory action of Aldose reductase enzyme. The ETDT showed inhibitory activity of IC₅₀ value is 581.7 μ g/ml, ETAN showed inhibitory activity of IC₅₀ value is 458.8 μ g/ml and ETDM showed inhibitory activity of IC₅₀ value is 286.2 μ g/ml. Acarbose is a standard drug used for Aldose reductase inhibitor assay. Acarbose showed inhibitory activity of IC₅₀ value is 2.5 μ g/ml. Aldose reductase is present in all target tissues that develop diabetic complications [11], In our study result revealed that plants extract prevents diabetic complications especially diabetic retinopathy and nephropathy. A comparison of Aldose reductase inhibitory activity between the standard drug and plant extracts has been depicted in Figure 1 and 2.

Table 1: Aldose Reductase Inhibition Assay

	Conc (μ g/ml)	OD @405 nm	% Inhibition	IC 50(μ g/ml)
Control	0	0.669	0.00	0
Acarbose	0.3125	0.515	23.02	2.5
	0.625	0.457	31.69	
	1.25	0.373	44.25	
	2.5	0.299	55.31	
	5	0.169	74.74	
	10	0.109	83.71	
ETDT	50	0.656	2.00	581.7
	100	0.637	4.72	
	200	0.512	9.45	
	400	0.494	21.17	

ETAN	800	0.409	47.89	458.8
	1600	0.295	55.89	
	50	0.619	7.41	
	100	0.579	13.39	
	200	0.445	33.48	
	400	0.370	44.69	
ETDM	800	0.319	52.32	286.2
	1600	0.153	77.20	
	50	0.602	10.06	
	100	0.507	24.26	
	200	0.456	31.78	
	400	0.441	34.05	
	800	0.395	40.90	
	1600	0.295	55.88	

Aldose reductase inhibition assay for Acarbose

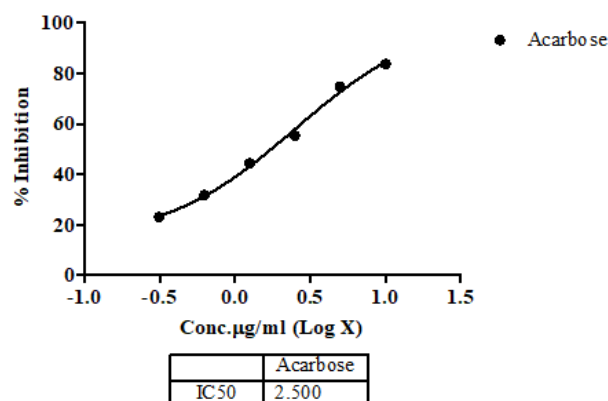


Figure 2: Aldose Reductase Inhibition Assay for Acarbose

Aldose reductase inhibitory activity of Ethanolic extracts

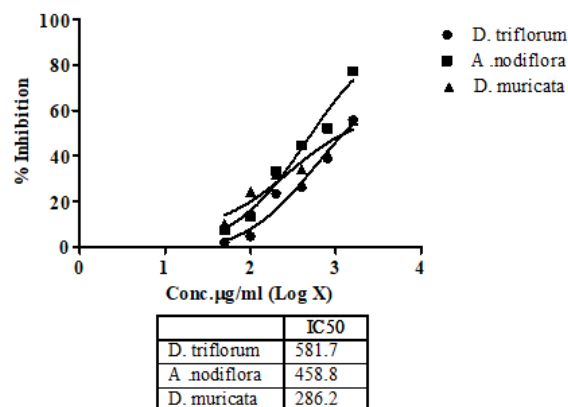


Figure 3: Aldose Reductase Inhibition Assay for ETDT, ETAN and ETDM

Alpha amylase inhibitory activity

In this study the *in-vitro* alpha amylase inhibitory activities of the ETDT, ETAN and ETDM were investigated. The result of experiment showed that ethanolic extract increased inhibitory activity against Alpha amylase enzyme shown in Table 2. The ETDT showed inhibitory activity of IC₅₀ value is 537.7 μ g/ml, ETAN showed inhibitory activity of IC₅₀

value is 629.0 $\mu\text{g/ml}$ and ETDM showed inhibitory activity of IC 50 value is 276.8 $\mu\text{g/ml}$. Acarbose is a standard drug used for alpha amylase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 3.29 $\mu\text{g/ml}$. A comparison of alpha amylase inhibitory activity between the standard drug and plant extracts has been depicted in Figure 3 and 4. So the plant extracts might be used as starch blockers since it prevents or slows the absorption of starch in to the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltotriose 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 assay

	Conc. $\mu\text{g/ml}$	OD at 590nm	% Inhibition	IC50 $\mu\text{g/ml}$
Control	0	0.71	0.00	0
Acarbose	0.3125	0.65	8.99	3.29
	0.625	0.58	18.82	
	1.25	0.50	30.48	
	2.5	0.44	37.64	
	5	0.31	55.90	
ETDT	10	0.27	62.08	537.7
	50	0.66	7.23	
	100	0.62	12.54	
	200	0.55	22.29	
	400	0.48	33.12	
ETAN	800	0.36	49.09	629
	1600	0.31	59.37	
	50	0.66	6.65	
	100	0.64	10.54	
	200	0.58	18.99	
ETDM	400	0.48	31.94	276.8
	800	0.40	43.63	
	1600	0.31	56.95	
	50	0.65	15.28	
	100	0.63	24.68	
	200	0.56	41.25	
	400	0.46	51.25	
	800	0.35	58.95	
	1600	0.22	69.37	

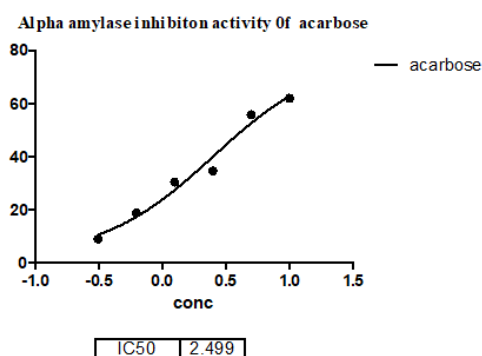


Figure 3: Alpha amylase inhibition assay of acarbose

Alpha amylase inhibitory activity of Ethanolic extract

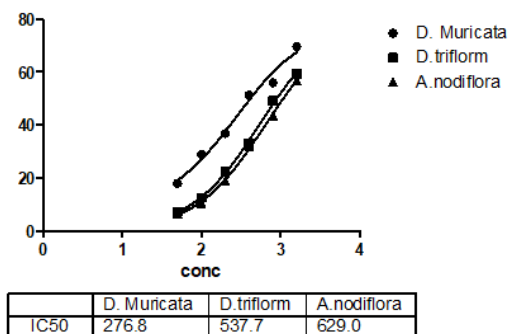


Figure 3: Alpha amylase inhibition assay of ETDT, ETAN and ETDM

Alpha Glucosidase Inhibition Assay

The results of *in-vitro* antidiabetic activity using alpha glucosidase inhibitory assay of ETDT, ETAN, ETDM and acarbose were shown in Table 3. The extract revealed a significant inhibitory action of α -glucosidase enzyme. The ETDT showed inhibitory activity of IC50 value is 525.1 $\mu\text{g/ml}$, ETAN showed inhibitory activity of IC 50 value is 457.5 $\mu\text{g/ml}$ and ETDM showed inhibitory activity of IC 50 value is 305.9 $\mu\text{g/ml}$. Acarbose is a standard drug used for alpha glucosidase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 4.546 $\mu\text{g/ml}$. Thus the inhibition of the activity of alpha glucosidase by ETDT, ETAN and ETDM 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]. A comparison of alpha glucosidase inhibitory activity between the standard drug and plant extracts has been depicted in Figure 5 and 6.

Table 3: Alpha Glucosidase Inhibition Assay

	Conc ($\mu\text{g/ml}$)	OD @ 405nm	% Inhibition	IC50 ($\mu\text{g/ml}$)
Control	0	0.996	0.00	0.00
Acarbose	0.3125	0.864	13.25	4.546
	0.625	0.748	24.90	
	1.25	0.665	33.23	
	2.5	0.504	49.40	
	5	0.474	52.41	
ETDT	10	0.197	80.22	525.1
	50	0.83	16.67	
	100	0.747	25.00	
	200	0.705	29.22	
	400	0.667	33.03	
ETAN	800	0.4752	52.29	457.5
	1600	0.456	54.22	
	50	0.853	14.36	
	100	0.775	22.19	
	200	0.7235	27.36	
ETDM	400	0.654	34.34	305.9
	800	0.6229	37.46	
	1600	0.4957	50.23	
ETDM	50	0.78	21.69	

	100	0.746	25.10	305.9
	200	0.6898	30.74	
	400	0.5897	40.79	
	800	0.5195	47.84	
	1600	0.4894	50.86	

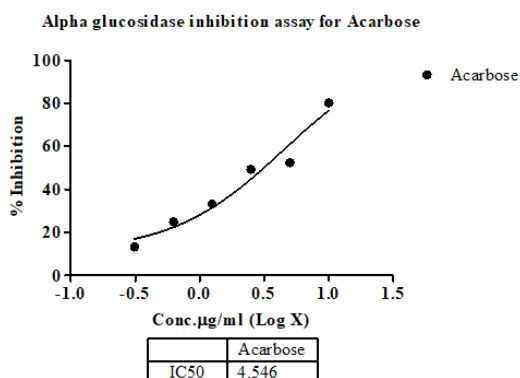


Figure 5: Alpha Glucosidase Inhibition Assay for Acarbose

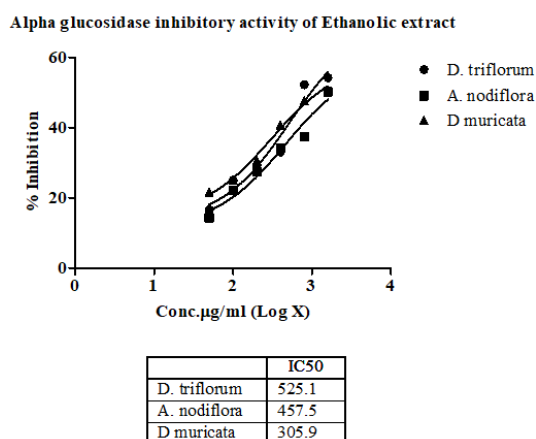


Figure 6: Alpha Glucosidase Inhibition Assay for ETDT, ETAN and ETDM

Conclusion:

To investigate the biological activities of ETDT, ETAN and ETDM of their antidiabetic activities of the ethanol extract of the plants has been analysed. As a result, we found that the extract of ETDT, ETAN and ETDM 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. Although the *in-vitro* antidiabetic effects of ETDT, ETAN and ETDM results indicate that has potential as a crude drug and a dietary health supplement. The plants showed significant enzyme inhibitory activity, so the compound isolation, purification and characterization which are responsible for inhibiting activity, have to be done for the usage of antidiabetic agent. Further studies are also required to elucidate whether the

plant have antidiabetic potential by *in-vivo* for corroborating the traditional claim of the plant.

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