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IN-VITRO STUDY ON ALPHA AMYLASE AND ALPHA GLUCOSIDASE INHIBITIORY ACTIVITY OF ETHANOLIC STEM EXTRACT - PARTHENIUM HYSTEROPHORUS LINN

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ABSTRACT

The present study deals with evaluation of inhibitory activity on ethanolic extract of stem, Partheniumhysterophorus Linn. On alpha amylase and alpha glucosidase enzymes at varying concentrations. The IC 50 values of ethanolic stem extract Partheniumhysterophorus was 27.98 µg /ml and 108.94µg/ml for alpha amylase and alpha glucosidase inhibition. This study suggest that ethanolic stem extract P.hysterophorus indicates the potential of extract to manage diabetes with excellent anti – diabetic activity.

Key words: In-Vitro Glucoside, Parthenium Hysterophorus Linn , Ethanolic extract, Amylase Activity Inhibitiory.

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INTRODUCTION

Diabetes mellitus (DM) is one of the non-communicable life threatening diseases. These are non-infectious and non-transmissible. This is largely due to physical inactivity, unhealthy diets, obesity, raised blood cholesterol and glucose (Singh SP, *et al.* 2015). DM is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin (West IC, *et al.* 2000*)*. Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes (Unuofin, *et al.* 2018).

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MATERIALS AND METHODS

Collection and identification of plant material :

The plant material (Stem of Partheniumhysterophorus) was collected locally from Chennai, Tamil Nadu , India. The collected plant was authenticated by Prof. P. Jayaraman, M.Sc.,Ph.D., Director at PARC , Chennai .(Reg . No. of the Certificate: PARC / 2021 / 4504)

Preparation of the plant extracts :

The stems of Partheniumhysterophorus were obtained locally and washed with distilled water, shade-dried and grinded as coarse powder. The ethanolic extract of crude drug material was obtained by extracting 100 grams of Powdered Dried plant in 500 ml of Ethanol on a soxhlet apparatus for 72 hours. Contents are filtered , then evaporated , air dried and stored in air tight container

Assay for alpha - amylase inhibition :

(Apostolidis, *et al. 2007*).The α-amylase inhibitory activity of the test sample (EE) was carried out according to the standard method with minor modification (Unuofin et al., 2018). 100 µl of α-amylase solution (0.1 mg/ml) was mixed with different concentrations (10, 20, 40, 80, 160, and 320µg/ml) of test sample, reference standard (Acarbose) and control (without standard/test sample) and pre- incubated at 37 °C for 15 min. Then, 100 µl of starch solution was added to initiate reaction and incubation was done at 37 °C for 60 min., then 10 µl of 1 M HCl and 100 µl of iodine reagent were added to the test tubes. The absorbance of the mixture was measured at 565 nm. α-amylase

Inhibitory activity was measured using the formula :

$$\% \text{ of Inhibition} = \left[\frac{(\text{OD of test} - \text{OD of control})}{\text{OD of test}} \right] \times 100$$

Assay for alpha - glucosidaseinhibition :

The effect of the test sample (EE) on α-glucosidase activity was determined according to the method described by Apostolidis et al. 2007, using α-glucosidase enzyme. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 100 mM phosphate buffer, and pH 6.8. 200 µl of α-glucosidase was pre-incubated with different concentrations (10, 20, 40, 80,160 and 320µg/ml) of the extract for 10 min. Then 400 µl of 5.0 mM (pNPG) as a substrate dissolved in 100 mM phosphate buffer (pH 6.8) was then added to start the reaction. The reaction mixture was incubated at 37 °C for 20 min and stopped by adding 1 ml of Na2CO3 (0.1 M). The yellow-colored reaction mixture, 4-nitrophenol, released from pNPG was measured at 405 nm using UV - VIS spectrophotometer. Voglibose was used as a positive control and the inhibitory activity of α-glucosidase was calculated using the following formula :

$$\% \text{ of Inhibition} = \left[\frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \right] \times 100$$

RESULT AND DISCUSSION

In - vitro anti – diabetic activity was carried out ethanolic stem extract of Parthenium hysterophorous by using amylase and – glucosidase inhibitory assay. Parthenium hysterophorous showed inhibitory activity on – amylase and –glucosidase. Acarbose, a synthetic drug which has the activity to inhibit – amylase act as standard for -amylase inhibitory assay, whereas for – glucosidase inhibitory assay – voglibose act as standard drug. Different concentrations (10, 20, 40, 80, 160, 320) of ethanolic extract of stem Parthenium hysterophorous were subjected to – amylase inhibitory assay and – glucosidase inhibitory assay. Percent –amylase and – glucosidase inhibition of ethanolic extracts was plotted as concentration in comparision with percentage of inhibiton as shown in the figure 1 and 2. For Alpha – amylase inhibition assay the maximum percentage of inhibition was 98.14814 obtained at the concentration of 320 µg/ml, where for the standard drug shows 97.82022 at the concentration of 320µg/ml . The IC50 values of (stem extract) and the reference standard (Acarbose) was found to be 27.98 and 10.44 respectively.

For Alpha –glucosidae inhibition assay the maximum percentage of inhibition was 89.56814 obtained at the concentration of 320 µg/ml, where for the standard drug shows 98.59787 at the concentration of 320µg/ml. The IC50 value of the given test sample (EE) and positive control (Voglibose) was found to be 108.94 µg/ml and the standard drug (Voglibose) was 64.16 µg/ml, respectively.

Inhibition Of Alpha - Amylase:

The inhibitory activity of the ethanolic extract of stem Parthenium hysterophorous results are shown in the below table.

For Alpha –glucosidae inhibition assay the maximum percentage of inhibition was 89.56814 obtained at the concentration of 320 µg/ml, where for the standard drug shows 98.59787 at the concentration of 320µg/ml. The IC50 values of (stem extract) and the reference standard (Acarbose) was found to be 27.98 µg/ml and 10.44 µg/ml respectively.

Inhibition Of Alpha - Glucosidase:

The inhibitory effect of alpha glucosidase was tested for reference standard (voglibose) and ethanolic stem extract of *Parthenium hysterophorous* and results are shown below in table.

Table 1: Inhibition Of Alpha-Amylase:

	Singlet	Duplicate	Triplicate	Mean
Control	0.032	0.03	0.035	**0.032333**

OD at 565 nm					% of inhibition					
Sample	**Conc. (µg)**	**Singlet**	**Duplicate**	**Triplicate**	**Singlet**	**Duplicate**	**Triplicate**	**Mean**	**SD**	**IC50 Value**
Acarbose	10	0.054	0.048	0.05	40.1235	32.638889	35.3333	36.0319	3.79087	**10.44**
	20	0.101	0.097	0.104	67.9868	66.666667	68.9103	67.8546	1.12762	

	40	0.16	0.156	0.162	79.7917	79.273504	80.0412	79.7021	0.39158	
	80	0.383	0.384	0.387	91.5799	91.579861	91.6451	91.6016	0.03769	
	160	0.643	0.64	0.638	94.9715	94.947917	94.9321	94.9505	0.01983	
	320	1.481	1.483	1.486	97.8168	97.819735	97.8241	97.8202	0.0037	

		OD at 565 nm			% of inhibition					
Sample	**Conc. (µg)**	**Singlet**	**Duplicate**	**Triplicate**	**Singlet**	**Duplicate**	**Triplicate**	**Mean**	**SD**	**IC50 Value**
EE	10	0.036	0.039	0.044	10.1852	17.094017	26.5152	17.9315	8.19713	**27.98**
	20	0.055	0.059	0.053	41.2121	45.19774	38.9937	41.8012	3.114368	
	40	0.082	0.085	0.08	60.5691	61.960784	59.5833	60.7044	1.19449	
	80	0.267	0.263	0.263	87.8901	87.705957	87.706	87.7674	0.10634	
	160	1.09	1.089	1.092	97.0336	97.030915	97.0391	97.0345	0.00415	
	320	1.746	1.743	1.749	98.1481	98.144961	98.1513	98.1481	0.00318	

Concentration (µg/ml)	% of inhibition for sample	% of inhibition for standard
10	17.93145	36.03189
20	41.80119	67.85457
40	60.70441	79.70211
80	87.76735	91.60162
160	97.03454	94.95049
320	98.14814	97.82022

Table 2: Inhibition Of Alpha - Glucosidase:

				Mean
Control	**0.597**	**0.592**	**0.594**	**0.59433**

Samples	**Conc. (µg)**	**Singlet**	**Duplicate**	**Triplicate**	**Singlet**	**Duplicate**	**Triplicate**	**Mean**	**SD**	**IC50 Value**
Voglibose	10	0.471	0.478	0.475	20.7515	19.573752	20.0785	20.1346	0.5908948	**29.92**
	20	0.359	0.362	0.355	39.5962	39.091419	40.2692	39.65227	0.5908948	
	40	0.228	0.234	0.23	61.6377	60.628155	61.3012	61.18901	0.5140298	
	80	0.135	0.131	0.133	77.2855	77.958497	77.622	77.62199	0.3365115	
	160	0.051	0.046	0.049	91.419	92.260236	91.7555	91.81155	0.4234343	
	320	0.006	0.011	0.008	98.9905	98.149187	98.654	98.59787	0.4234343	

Sample	Conc. (µg)	Singlet	Duplicate	Triplicate	Singlet	Duplicate	Triplicate	Mean	SD	IC50 Value
SJ	10	0.577	0.57	0.575	2.91643	4.0942232	3.25294	3.4212	0.6066547	
	20	0.504	0.511	0.506	15.1991	14.021312	14.8626	14.69434	0.6066547	**64.16**
	40	0.436	0.437	0.433	26.6405	26.472238	27.1453	26.75266	0.3502523	
	80	0.215	0.212	0.214	63.825	64.329781	63.9933	64.04936	0.2570149	
	160	0.11	0.118	0.115	81.4919	80.145822	80.6506	80.76276	0.6799975	
	320	0.061	0.065	0.06	89.7364	89.063376	89.9047	89.56814	0.4451629	

Conc.(µg)	VOGLIBOSE	SJ
10	20.1346	3.4212
20	39.65227	14.69434
40	61.18901	26.75266
80	77.62199	64.04936
160	91.81155	80.76276
320	98.59787	89.56814

Figure :1 Standard and sample test for inhibition for alpha amylase

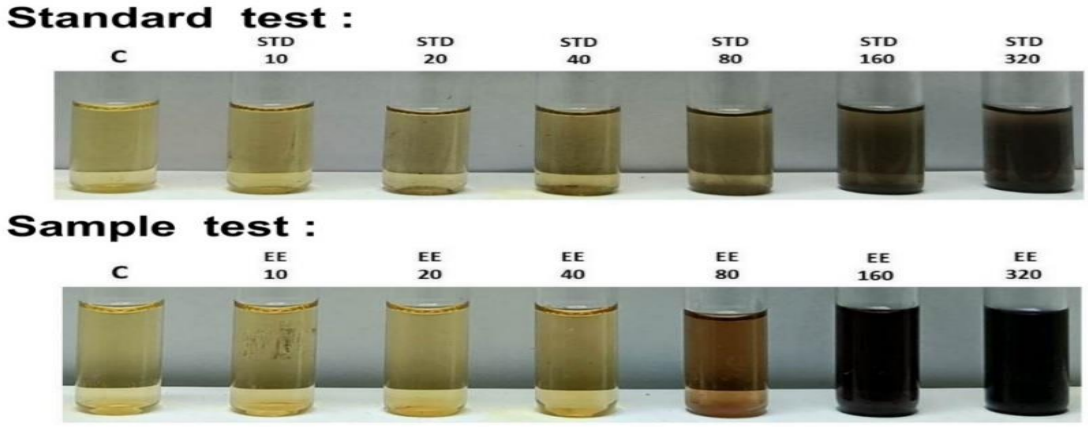


Figure 2: Standard test and sample

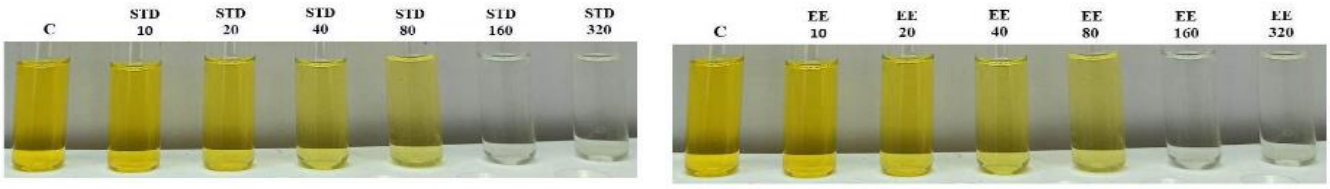


Figure 3: Standard Sample Test :

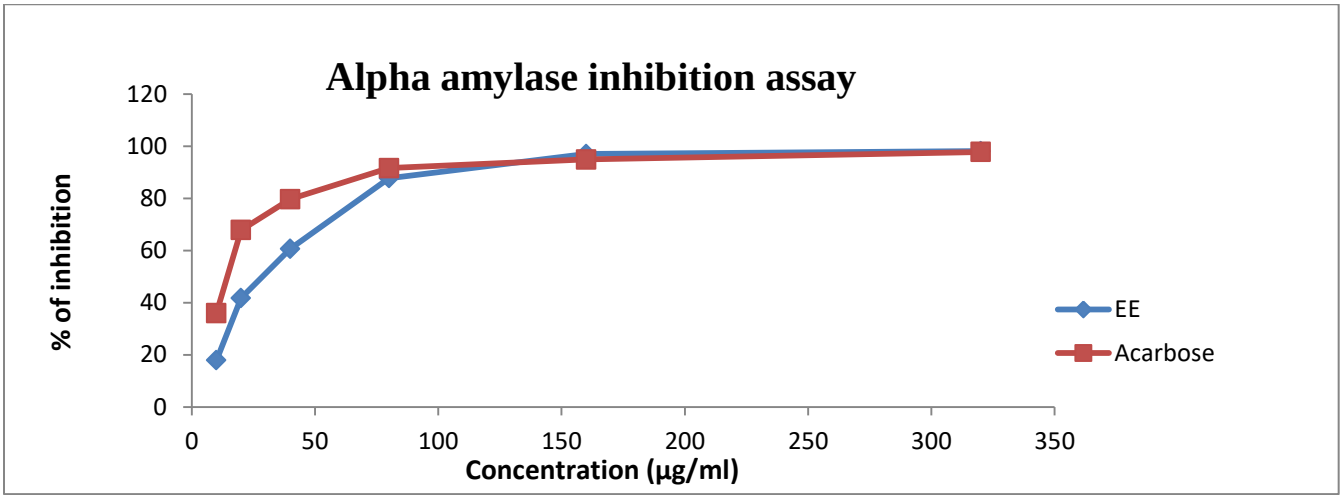
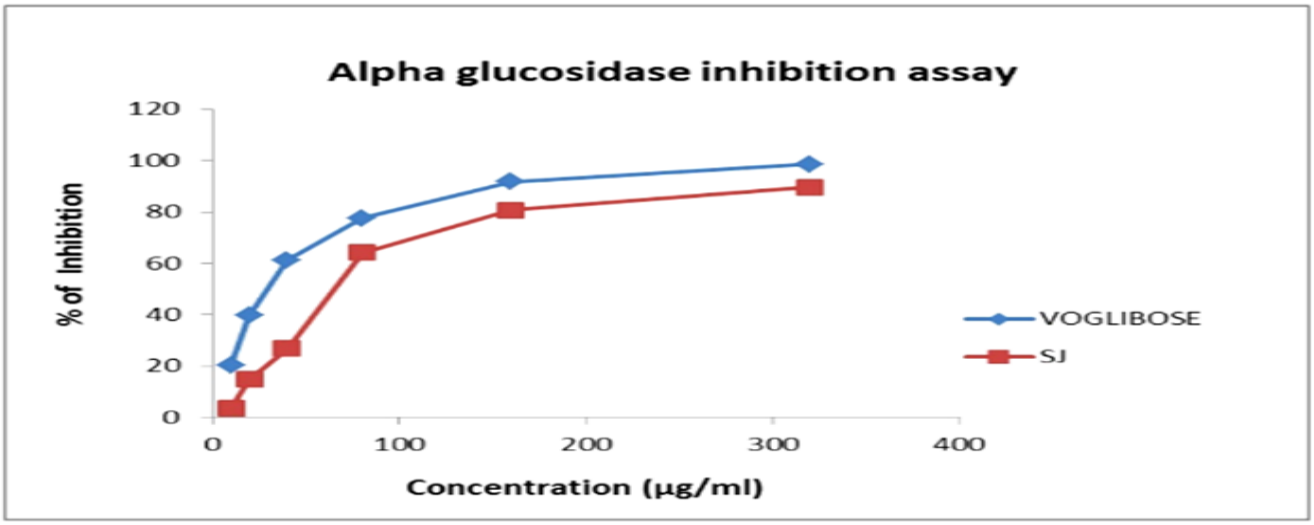


Figure 4: percentage of alpha –glucosidase inhibition assay



CONCLUSION:

The ethanolic extract of stem P.hysterphorus showed potential percentage of inhibition for alpha amylase and alpha glucosidase enzyme. Inhibitory effect of stem increases with increase in concentration. By the natural presence of chemical constituents in the ethanolicstem extract of P.hysterphorus , they are effective in inhibitory effect of α- amylase and α- glucosidase . Hence, from this study it is concluded that the ethanolic stem extract of P.hysterphorus exhibit potential anti- diabetic activity.

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