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Anti-diabetic activity of *Erythrina subumbrans (Hassk.) Merr.*

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ABSTRACT

α-amylase inhibitors present in pancreatic region has an operative strategy by controlling the breakdown of starch and helps to minimize the post-prandial hyperglycemia levels. In this study, vegetative (leaf) part of herbal plant *Erythrina subumbrans (Hassk.)* was assessed for anti-diabetic activity. Aqueous ethanol (80 %) extract was prepared in the different concentration (10, 20, 40, 80, 160, and 320 μg/ml). Acarbose was used as a standard and treated in similar way as that of sample. Control samples were also prepared without standard and sample solutions. A known volume of α-amylase solution was added (0.1mg/mL) was added to standard, sample, control solutions which were preincubated at 37 °C for 15 minutes. Further, known volume of starch solution was added and incubated for 60 min to initiate the reaction. Hydrochloric acid (HCl) and iodine reagent was added to the test tubes and absorbance was measured at 580 nm in UV-Vis spectrophotometer. A strong pancreatic amylase inhibitory activity (>50 %) was obtained from aqueous ethanolic extract with IC_{50} (half maximal inhibitory concentration) value of 23 μg/ml against standard acarbose with IC_{50} value of 27 μg/ml. The values endorse Erythrina subumbrans (Hassk.) for further experiments on their potential for managing Diabetes.

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INTRODUCTION

Medicinal plants and their products are important therapeutic agents for treating various disease in most of the developing countries (Devkota *et al.*, 2017). Since the Vedic period, the extract obtained from various vegetative parts of plants were used and practice were in existence till date in countries of south Asia, including India (Pandey *et al.*, 2013). India is a diversified country in both cultural and naturally. Biodiversity in India is inimitable due to its isolated topographical locations and climatic conditions. More than 8000 species were reported in India for traditional medicinal purpose. However, these resources have been under exploited and there is an enormous potential to perform the scientific activity to support traditional use of medicine.

Diabetes mellitus (DM) is one of the metabolic disorder due to high level of blood glucose (Hyperglycemia) with instable metabolism of proteins, fats and carbohydradrates resulting in low secretion of insulin in body (WHO, 1999). This incidence of metabolic changes in body is increasing globally and expected to reach 300 million by the year of 2025 with India expected with high reporting of cases on diabetes (Gupta and Phatak, 2003). One of the primary threats to human body is Type 2

diabetes due to intensification in occurrence, disabling problems and its is chronic (Leena and Jill, 2010). Many wide-ranging medicinal strategies are available to treat diabetes. However, conventional treatments include increasing the secretion of endogenous insulin, increasing the insulin action at target cell, intake of hypoglycemic drugs like sulfonylureas, biguanids and inhibiting degradation of starch by glycosidases like α-glucosidase and α-amylase (Rang *et al.*, 2003).

In the digestive system, one of the key enzymes is Pancreatic a-amylase (E.C. 3.2.1.1) which is responsible to catalyze the initial stage in starch hydrolysis to convert to smaller oligosaccharides inclusive of maltose and other form of oligoglucans. By reacting with α-glucosidase, these compounds were converted to from glucose which on absorption enters the blood circulatory system. Post-prandial hyperglycemia (PPHG) will be observed in elevated levels due to fast degradation of starch. It has been proven that Human pancreatic α-amylase (HPA) in small intestine relates to rise in post-prandial level of glucose. It is therefore considered as significant features in diagnosis of Type 2 Diabetes (Eichler *et al.*, 1984).

Hence, α-amylase plays a vital role in controlling diabetes by inhibiting the enzyme secretion resulted in delay in digestion of starch. Pancreatic inhibitor α-amylase delays digestion of carbohydrates resulting in significant decrease in glucose absorption rate and reduce the level of glucose in serum (Post-prandial) (Tarling *et al.*, 2008). Traditional medicines are getting more attention for the diagnosis of Diabetes due to less cost and side effects when compared to Hypoglycemic synthetic analogs (Grover *et al.*, 2002).

The exploration for new compounds that are pharmacologically active is by evaluating the sources naturally such as therapeutic plants either crude extracts obtained from plants can leads to effective and more definite inhibitors of α-amylase (Tarling *et al.*, 2008). The kinetic properties of acarbose a potent α-glucosidase inhibitor helps in reducing the pancreatic α-amylase helps preventing other neurological and renal changes. (Creutzfeldt, 1999). As a lifetime management in controlling diabetes using acarbose is well borne, that improves by controlling the glucose level if taken as monotherapy or as a combination therapy (Mertes, 2001).

This research experiment was carried out to initiate the search for pharmacological activity specifically towards pancreatic α-amylase and to evaluate the traditional use in treatment of diabetes.

MATERIALS AND METHODS

Material

Chemical such as α-amylase solution, Hydrochloric acid, Acarbose and Iodine reagent were purchased from SISCO research laboratories Ltd, Maharashtra, India. Other chemicals procured from local manufactures (Analytical reagent grade).

Plant material

The leaves of Erythrina subumbrans (Hassk.) Merr were collected from western Ghats of India and authenticated at Siddha central research institute (Ministry of AYUSH, Government of India), Chennai by Research officer and Head of pharmacognosy department Dr K.N. Sunil Kumar and confirmed by Assistant Director in-charge Dr P. Sathiyarajeswaran (Authentication certificate 112.04011901 dated 04 Apr 2019). The harvested vegetative part was dried in a nominal condition and stored in container made of Kraft paper.

Preparation of plant Extract

The plant material was washed with distilled water for several times and was subjected to air drying under the shade. After drying they were ground by an electrical mixer until they became a powder. Then the powdered samples were stored in a dark place and subjected to extraction method (**?**). Extraction of powdered samples was done using aqueous ethanol (80%). Aliquots of 50 g of the powdered samples were soaked in 250 ml of the solvent for 72 hrs. Later the samples were filtered, and concentrated under reduced pressure using a rotary evaporator and keep stored at room temperature (25° C).

In Vitro α-Amylase Inhibitory Activity

The α-amylase inhibitory activity of the aqueous ethanolic extract of Erythrina subumbrans (Hassk.) was carried out according to the method with minor modification (Unuofin *et al.*, 2018). In details, α-amylase solution was prepared in concentration of 0.1 mg/mL. 100 μl of the prepared solution were mixed with different concentration of test sample (i.e., 10, 20, 40, 80, 160, and 320 μg/ml), standard (acarbose), and control (without standard/test samples) which were pre-incubated at 37 °C for 15 minutes. 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 Hydrochloric acid (HCl) and 100 μl of iodine reagent were added to the test tubes. The absorbance of the mixture was measured at 580 nm by using UV-visible spectrophotometer. The standard, sample and control sample final solutions were shown in Figures 1 and 2.

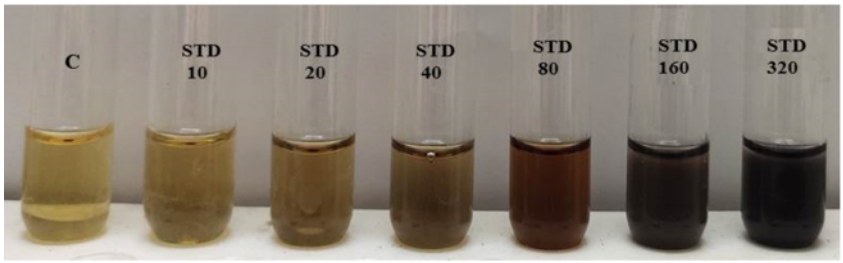

Figure 1: Standard solution

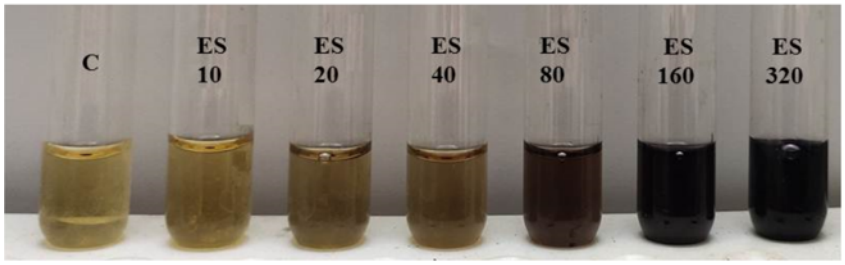

Figure 2: Sample solution

Table 1: Extract Yield values

Solvent system	Powder weight (g)	Extract weight (g)	Extract Yield (%)
Aqueous ethanol (80%)	50 g	2.62 g	5.24

Table 2: α-Amylase Inhibitory Activity-Standard (Absorbance)

Type	Control Absorbance	Conc. (μg)	Absorbance		
			Prp-1	Prp-2	Prp-3
Acarbose	0.155	10	0.218	0.205	0.212
		20	0.298	0.293	0.286
		40	0.374	0.366	0.379
		80	0.637	0.671	0.688
		160	0.825	0.834	0.836
		320	1.632	1.635	1.638

Prp.: Preparation, Conc.:Concentration

Table 3: α-Amylase Inhibitory Activity-Standard (%Inhibition)

Type	Conc. (μg)	% Inhibition			Mean	Std.Dev	IC 50 value
		Prp-1	Prp-2	Prp-3			
Acarbose	10	28.7462	24.2276	26.7296	26.5678	2.2636	27.13
	20	47.8747	46.9852	45.6876	46.8492	1.0999	
	40	58.4670	57.5592	59.0150	58.3471	0.7353	
	80	75.6149	76.8505	77.4225	76.6293	0.9239	
	160	81.1717	81.3749	81.4195	81.3220	0.1321	
	320	90.4820	90.4995	90.5169	90.4995	0.0174	

Prp: Preparation, Conc.:Concentration

Table 4: α-Amylase Inhibitory Activity-Sample (Absorbance)

Type	Control Absorbance	Conc. (μg)	Absorbance		
			Prp-1	Prp-2	Prp-3
Erythrina subum-brans (ES)	0.155	10	0.226	0.219	0.224
		20	0.312	0.316	0.308
		40	0.389	0.385	0.396
		80	0.694	0.691	0.698
		160	0.942	0.949	0.952
		320	1.981	1.975	1.984

Prp: Preparation, Conc.:Concentration

Table 5: α-Amylase Inhibitory Activity-Sample (%Inhibition)

Type	Conc. (μg)	% Inhibition			Mean	Std.Dev	IC 50 value
		Prp-1	Prp-2	Prp-3			
Erythrina subumbrans (ES)	10	31.2684	29.0715	30.6548	30.3316	1.1335	23.21
	20	50.2137	50.8439	49.5671	50.2082	0.6384	
	40	60.0686	59.6537	60.7744	60.1655	0.5666	
	80	77.6177	77.5205	77.7459	77.6280	0.1131	
	160	83.5103	83.6319	83.6835	83.6085	0.0889	
	320	92.1588	92.1350	92.1707	92.1549	0.0182	

Prp: Preparation, Conc.:Concentration

The percentage α-amylase inhibitory activity was measured using the following expression : % of Inhibition = [(OD of test - OD of control)/OD of test] x 100 Where, OD of Test is the absorbance of the test and OD of control is the absorbance of the control. All experiments were performed in triplicate. From the data obtained, a curve to be plotted and the IC_{50} value will be calculated

RESULTS

Plant Extract

The percentage yield of the ethanolic extracts was calculated by using the following equation: yield (%) = (weight of extract / weight of dried plant material) x 100 and the obtained yield was presented in Table 1.

α-Amylase Inhibitory Activity

The average control (i.e., without standard/test samples) absorbance value is 0.155 AU. The absorbance value and % inhibition of standard Acarbose was calculated using the formula and details of calculation was summarized in Tables 2 and 3. Similarly, absorbance value and % inhibition of aqueous ethanolic extract of Erythrina subumbrans (Hassk.) Merr sample preparation was calculated using the formula and the details of calculation was summarized in tTables 4 and 5. From the data, a curve was plotted, and the inhibitory concentration (IC_{50}) value was calculated. IC_{50} is defined as the concentration of the samples required for a 50% inhibition of enzyme. IC_{50} value was calculated from the graph by plotting concentration on X-Axis and % inhibition on Y-axis and the statistical curve was displayed in Figure 3 . The IC_{50} value of the ethanolic extract of *Erythrina subumbrans (Hassk.) Merr* was found to be 23.21 μg/ml and the standard drug (Acarbose) was 27.13 μg/ml, respectively.

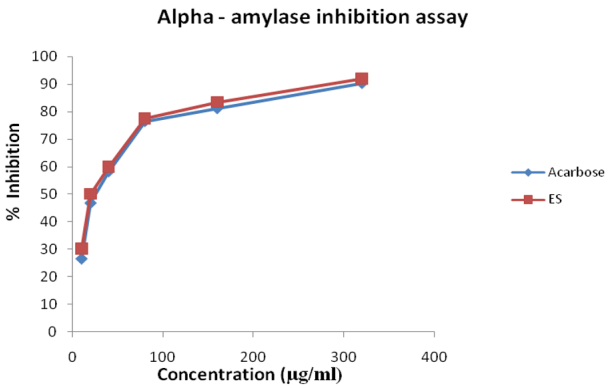

Figure 3: Statistical curve

DISCUSSION

Medicinal plants contain several primary and secondary metabolites, known to be a bioactive compound, exhibits health encouraging activity in Human system, including several activities like antidiabetic and antioxidant activity (Altemimi *et al.*, 2017). Phenolic and flavanoids are the known compounds that are commonly dispersed in medicinal species and are owing a prominent antioxidant and other medical role in diet followed by humans (Balasundram *et al.*, 2006). In phenolic compounds, the largely occurring group is Flavonoids and possess biological activity like Anti-ulcer, anti-diabetic, anti-oxidant, hepatoprotective and anti-carcinogen properties (John *et al.*, 2014).

α-amylase inhibitors such as acarbose works by inhibiting the hydrolase enzyme that's helps to reduce the glucose level in postprandial, thus glucose absorption is delayed (Kang *et al.*, 2012). In this study, aqueous ethanol extract of *Erythrina subumbrans (Hassk.) Merr* have a potential antidiabetic activity with IC_{50} values of 23.21 μg/ml as compared to the positive control, acarbose (IC_{50}: 27.13mg/ml). Literature article reported that the enzyme inhibition of plant containing the phenolic compounds, possess a significant inhibitory action on α-amylase and suggested to diabetes treatment (Panda, 2009). The inhibitory and stoppage of action of metabolism in carbohydrate enzymes conversion, such as α-amylase are discussed as possible therapeutic sites in treatment of diabetes (Barrett and Udani, 2011).

CONCLUSIONS

In this study, leaves of *Erythrina subumbrans (Hassk.) Merr* was collected from western Ghats of India and authenticated at Siddha central research institute, Chennai. Extraction was carried out using aqueous Ethanol (80%) and the obtained extract was subjected for α-amylase inhibitory assay. A promising α-Amylase inhibitory activity was observed from aqueous ethanolic extract of E. *subumbrans* (IC_{50}: 23.21μg/ml) compared against standard Acarbose (IC_{50}: 27.13mg/ml). Other studies to be focused on α-glucosidase; in-vivo study valuations and chemical analysis-based Bioassays.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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