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Research Article

### IN-VITRO ANTIDIABETIC ACTIVITY OF STANDARDIZED ACAIBERRY EXTRACT

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#### ABSTRACT

In the present study, the standardized Acaiberry extract was studied for in-*vitro* antidiabetic activity. The aim of this work was to evaluate the inhibitory activities of the methanolic extract of standardized *Euterpe oleracea* (Acai berry) of family Arecaceae, at different concentrations. Diabetes is a severe, metabolic disorder characterized by increased levels of glucose that circulates in the blood plasma. Alpha amylase and alpha glucosidase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus. The present study intends to screen alpha amylase and alpha glucosidase inhibitors from natural sources like plants in order to minimize the toxicity and side effects of the inhibitors currently used to control hyperglycemia. The acaiberry extract exhibited significant  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities with IC<sub>50</sub> value 6.29 mg/ml and 7.03 mg/ml respectively and well compared with standard Acarbose for alpha ( $\alpha$ )-amylase and alpha ( $\alpha$ )-glucosidase inhibitory activities respectively.

Keywords: Acaiberry, Acarbose, Alpha amylase, Alpha glucosidase.

#### 1. INTRODUCTION

Diabetes is a severe, metabolic disorder characterized by increased levels of glucose in blood that causes damage to the heart, blood vessels, eyes, kidneys and nerves overtime. Type 2 diabetes is the most common disorder which is prevalent in adults, and occurs either due to resistance to insulin or insufficient production of insulin in the body [1]. Millions of people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.5 million deaths are directly attributed to diabetes each year [2]. Among 7.7 billion total population (2019), around 463 million adult people have diabetes with a global prevalence of 9.3% and may rise to 10.9% by 2045 [3]. The conventional treatments for type 2 diabetes include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo- and disaccharides [4]. The distinct digestive enzyme in saliva is  $\alpha$ -amylase. It hydrolyses  $\alpha$ -1,4glycosidic linkages in starch to yield maltose and glucose which results in increased glucose levels. The  $\alpha$ -amylase inhibitors play a vital role in delaying the glucose absorption rate in the body thus reducing the serum blood glucose levels in diabetic individuals [5]. Inhibitors of the enzymes,  $\alpha$ - glucosidase and  $\alpha$ -amylase are recognized as novel anti diabetic drugs.  $\alpha$ -glucosidase is the key enzyme which is responsible for the hydrolysis of oligo- and/or disaccharides to simplest sugars absorbable by the body. Inhibition of this enzyme leads to prevention of conversion of disaccharides to monosaccharides, decreasing the absorption of monosaccharides through mucosal border of small intestine [6]. Some of the currently used  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors are acarbose, miglitol and voglibose etc. However, many of these synthetic hypoglycemic agents have their own limitations, which are non-specific, produce serious side effects viz., bloating, abdominal discomfort, diarrhea and flatulence leading to diabetic complications [7]. Recently, herbal medicines are gaining more importance in the treatment of obesity and diabetes as they are free from side effects, easily available and less expensive when compared to synthetic drugs.

The açai palm (*Euterpe oleracea* of family Arecaceae) is a native tree from the Amazon region, northern South America. Acai berry is used as antioxidant, antidiabetic agent, activate detoxifying enzymes, prevent cancer cell proliferation and anti-inflammatory agent [8]. The mechanism of action behind its use in the treatment of diabetes is not reported. Hence, the present study was aimed to determine the mechanism of reducing blood sugar levels using in-*vitro* studies.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals and reagents

Standardized acaiberry extract was obtained from Yucca enterprises, Mumbai, India. Porcine pancreatic  $\alpha$ amylase (EC 3.2.1.1) (PPA), Acarbose and  $\alpha$ glucosidase were obtained from Sigma Aldrich (Mumbai, India), 3,5-Dinitrosalicylic acid (DNSA color reagent), Soluble starch, p-nitrophenyl-  $\alpha$  -Dglucopyranoside (p-NPG), were obtained from SRL Laboratories (Hyderabad, India). Sodium potassium tartrate, dimethyl sulfoxide, and other chemicals were of analytical grade.

## 2.2. Preliminary phytochemical screening

Standardized acaiberry extract was subjected to various test tube reactions to detect the different classes of phytoconstituents present in it.

## 2.3. Determination of in-*vitro* α-amylase enzyme inhibitory activity of standardized Acaiberry extract

The inhibition of  $\alpha$  -amylase activity was determined according to the method described in the literature [9] with minor modifications. Stock solution of acaiberry extract was prepared by dissolving upto 100mg of each extract in 10ml of dimethyl sulfoxide. A total of 250µl of extracts of varying concentrations (1, 2, 4, 6, 8, 10 mg/ml) was placed in a tube and 250µl of 0.02M sodium phosphate buffer (pH- 6.9) containing  $\alpha$ amylase solution (0.5mg/ml) was added. Then it was incubated at 25°C for about 10min. After which 250µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at particular time intervals and further incubated at 25°C for 10min. Finally, 500µl dinitro salicylic acid (DNS) reagent was added to stop the reaction. The tubes were incubated in boiling water (5ml) and then cooled to room temperature. The reaction mixture was diluted with 5ml of distilled water and the absorbance was measured at 540nm using UV-Visible spectrophotometer (Lab India). A control was prepared using the same procedure replacing the phytochemical with distilled water. The percentage inhibition of the enzyme alpha-amylase was calculated by the formula:

% Inhibition = Absorbance (control)-Absorbance (Test) /Absorbance (control) × 100

## 2.4. Determination of α-glucosidase enzyme inhibitory activity of standardized Acaiberry extract

The inhibition of  $\alpha$  -glucosidase activity was determined according to the method described in the literature [9] with minor modifications. 1 mg of  $\alpha$  -glucosidase was dissolved in 100 ml of phosphate buffer (pH 6.8). To 100µl of phytochemical, Acaiberry extract of varying concentrations (1, 2, 4, 8, 10, mg/ml), 200  $\mu$ l  $\alpha$  glucosidase were added and the mixture was incubated at 37°C for 20 min. To the reaction mixture, 100  $\mu$ l of 3 mM p -nitrophenyl  $\alpha$  -D-glucopyranoside (p-NPG) was added and incubated at 37°C for 10 min. The reaction was terminated by the addition of 2ml sodium carbonate solution (0.1M) and the  $\alpha$  -glucosidase activity was determined spectrophotometrically at 405 nm using UV-Visile spectrophotometer (Lab India). Acarbose was used as positive control of  $\alpha$  -amylase and  $\alpha$  -glucosidase inhibitor. The concentration of the acaiberry extract required inhibiting 50% of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity under the assay conditions was defined as the  $IC_{50}$  value.

% Inhibition = Absorbance (control) – Absorbance (test) / Absorbance (control)  $\times$  100

## 3. RESULTS AND DISCUSSION

### 3.1. Preliminary phytochemical screening

Preliminary phytochemical screening of acaiberry extract has shown the presence of different classes of phytoconstituents like steroids, saponins, favonoids and phenolic compounds. favonoids and polyphenolic compounds act as antioxidants and lower the risk of various diseases like diabetes, inflammatory disorders etc.

# 3.2. In-*vitro* α-amylase enzyme inhibitory activity of acaiberry extract

The results of the study are presented in Table 1 and Fig. 1.  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors plays an important role in controlling high blood glucose levels. Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. The important function of  $\alpha$ -amylase in carbohydrate digestion is hydrolysis of 1, 4-glycosidic linkages of polysaccharides like starch, glycogen etc. into disaccharides. In the present study, Acaiberry extract inhibited the catalysis of alpha amylase at all

concentrations (1, 2, 4, 8, 10 mg/mL). Among all the test doses, Acaiberry extract has shown remarkable alpha- amylase enzyme inhibition i.e. 74.28% at 10 mg/mL concentration and it was comparable with the standard drug acarbose (85.64% percentage inhibition

at 10 mg/mL concentration). The  $IC_{50}$  value of the phytochemical, Acaiberry extract and standard (Acarbose) was found to be 6.29mg/ml and 5.23mg/ml respectively.

Table 1: In-*vitro* α-amylase enzyme inhibitory activity of methanolic extract of Acaiberry and Standard drug Acarbose

S. No	Name of sample	Concentration (mg/ml)	% Inhibition	IC <sub>50</sub> value (mg/ml)
1.	Acaiberry	1	$10.2 \pm 0.25$	- - 6.29 -
		2	22.13±0.36	
		4	34.52±0.48	
		6	49.25±0.26	
		8	62.36±0.45	
		10	$74.28 \pm 0.82$	
2.	Acarbose	1	15.31±0.96	- 5.23
		2	25.3±0.72	
		4	40.16±0.85	
		6	57.21±0.79	
		8	73.21±0.46	
		10	85.64±0.15	

All the samples are determined in triplicate and the values are expressed as means  $\pm$  SD. Acarbose is the standard  $\alpha$ -amylase inhibitor





## 3.3. In-*vitro* α-glucosidase enzyme inhibitory activity of Acaiberry extract

The results of the study are presented in Table 2 and Fig. 2. The enzyme alpha-glucosidase lies in the mucosal border of the small intestine and helps in catalysis of the last step of digestion of starch and disaccharides that are abundant in human diet.  $\alpha$ -glucosidase speeds up conversion of the disaccharides to monosaccharides, which leads to increase in glucose levels especially

postprandial hyperglycaemia. The Acaiberry extract was assessed for alpha-glucosidase enzyme inhibitory activity at different concentrations ranging from (1-10mg/ml) and it exhibited potent  $\alpha$ -glucosidase inhibitory activity in a dose dependent manner comparable with that of the standard drug, Acarbose. The IC<sub>50</sub> value of the phytochemical, Acaiberry extract and standard (Acarbose) was found to be 7.03 mg/ml and 4.87mg/ml respectively.

S. No	Name of sample	Concentration(mg/ml)	% Inhibition	IC <sub>50</sub> value (mg/ml)
1.	Acaiberry	1	6.32	7.03
		2	15.32	
		4	30.25	
		6	43.21	
		8	56.32	
		10	70.23	
2.	Acarbose	1	20.3	4.87
		2	31.25	
		4	43.28	
		6	59.35	
		8	73.26	
		10	85.21	

Table 2: In-*vitro* α-glucosidase enzyme inhibitory activity of methanolic extract of Acaiberry and Standard drug Acarbose

All the samples are determined in triplicate and the values are expressed as means  $\pm$  SD. Acarbose is the standard  $\alpha$ - glucosidase inhibitor





### 4. CONCLUSION

In the present study, the phytochemical, Acaiberry extract showed inhibition of enzymes, alpha-amylase and alpha-glucosidase which in turn decreases blood glucose levels, making the drug effective in the management of diabetes and diabetic complications. Hence, the phytochemical, Acaiberry extract can be used as an adjuvant for the management diabetes and complications associated with diabetes mellitus.

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#### **Conflicts of interest**

The authors declared no conflict of interest.

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