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Short Article

Confirmation of Best Method for Detection of Alpha Amylase Activity of *Cucumis Melo Var Agrestis* (Wild Musk Melon)

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A B S T R A C T

Background: Alpha amylase inhibition study important in the study of antidiabetic potential. Various methods involved in detection of alpha amylase inhibition detection of best method are important in research. But there is no paper regarding these criteria therefore current research is focused on detection of best method for alpha amylase activity.

Aim and Objective: Aim of this research is to identify the best method for detection of alpha amylase activity. Objectives of this research are to extract the plant material Cucumis melo var agrestis and evaluate alpha amylase inhibitory activity.

Methods: alpha amylase inhibition activity by iodine-starch calorimetric method and DNSA calorimetric method. Results: inhibitory alpha amylase activity by DNSA method IC_{50} of standard acarbose was 372.04µg/ ml and HALEC was 445.30µg/ ml. inhibitory alpha amylase activity by DNSA method IC_{50} of standard acarbose was 129.46 µg/ ml and HALEC was 153.31 µg/ ml.

Conclusion: From the result concluded that HALEC has anti-diabetic potential and starch-iodine calorimetric method was best method for detection of alpha amylase inhibitory activity.

Keywords: Iodine-starch Color Assay, DNSA Color Assay, In Vitro Antidiabetic Activity

Introduction

Starch was a glucose polymers composed by the alpha-1, 4 and alpha-1, 6 glucosidic bonds. Alpha-1, 4 linkage and the alpha-1, 6 linkage were possible for starch molecules of different structures. An unbranched single chain polymer of glucose units with alpha-1,4 glucosidic bonds is amylose and branched glucose polymer with alpha-1,6 glucosidic linkages is amylopectin.[1] Human saliva and pancreatic secretion contains alpha-amylase for digestion of starch. Alpha amylase is an enzyme which hydrolysis the starch and glycogen. The a-amylases which cleave 1, 4-α -D-glucosidic bonds and can bypass but cannot hydrolyse 1, 6-α-D-glucosidic branch points. Cleavage of 1, 4-α-D-glucosidic produces glucose units from starch. Alpha amylase can increases blood glucose level by cleave 1, 4- α -D-glucosidic

bonds of starch. So that inhibition of alpha amylase activities is important for control of blood glucose level.[2]

Material Methods

Material

All chemicals and reagents were analytical grade (AR). α-Amylase was obtained from Sigma-Aldrich (USA). 3, 5-Dinitrosalicylic acid and potato starch were from Fluka (China). Potassium sodium tartrate, Iodine and potassium iodide were from Ajax Finechem (New Zealand). Leaves of Cucumis melo var agrestis was collected from village of Pungavarnatham, Thoothukudi district, Tamil Nadu, India month of December 2017 and authenticated from Government Siddha Medical College Chennai, voucher specimen no. GSMC/ MB-87/ 18.

Method

Extraction of plant material

Hydro-Alcoholic Extract of *Cucumis melo var agrestis* was prepared by 10g of finely powdered leaf powder was extracted with 100ml of 60% methanol in soxhlet apparatus for 48 hours. The extract was concentrated under distillation and dried at room temperature until get solid mass.[3]

Determination of α-Amylase inhibition assay by DNSA method[4,5]

The α-amylase inhibition was determined by an assay described from Worthington Biochemical Corp. (1978). Different concentrations (0.1-1mg/ ml) of plant extracts and standard acarbose were prepared with phosphate saline buffer. 500 µl of the extracts or standard and 500 µl of α-amylase were incubated at 25°C for 10 min. After pre-incubation, 500 µl of 0.5% starch solution was added and incubated at 25°C for 10 min. The reaction was stopped by adding 1.0 ml of DNSA reagent. The mixture was heated in a water bath for 5, cooled in cold water for 10 min and measured at 540 nm with a spectrophotometer. And percentage inhibition was determined by using following formula.

$$\text{Percentage inhibition} = \frac{Control - Test}{control} \times 100$$

Control: Absorbance without sample only of enzyme activity.

Test: Absorbance of standard or extract with enzyme activity.

Determination of α-Amylase inhibition by starch-iodine Color Assay[6,7]

The α-amylase inhibition was determined by assay described from Xiao et al. with slight modification based on the starch-iodine test. 500µl of extracts or standard acarbose at different concentration (0.1-1mg/ ml) were added to 500 µL of α-amylase solution and were incubated at 37 °C for 10 min followed by added 500 µL soluble starch (1%, w/ v) and incubated at 37 °C for 15 min. 1 M HCl (20 µL) was added for stops the enzymatic reaction. After stop the reaction added of 100 µL of iodine reagent. The color change was noted and the absorbance was read at 620 nm.

$$\text{Percentage inhibition} = \frac{A - C}{B - C} \times 100$$

A=absorbance of the sample, B=absorbance of blank (without α-amylase) and C=absorbance of control (without starch).

Result and Discussion

Percentage inhibition of alpha amylase enzyme activities for HALEC and Acarbose by DNSA method was shown in Figure 1. IC_{50} values of standard and HALEC was found to be 372.04µg/ ml and 445.30µg/ ml respectively. There is no significant difference between acarbose and HALEC which confirms the plant extract having potent antidiabetic activity. Percentage inhibition of alpha amylase enzyme activities for HALEC and Acarbose by Iodine method was shown in Figure 2. IC_{50} values of standard and HALEC was found to be 129.46µg/ ml and 153.31µg/ ml respectively. There is no significant difference between acarbose and HALEC which confirms the plant extract having potent antidiabetic activity. Both DNSA assay and starch-iodine color assay were used for the study of inhibition of alpha amylase acticity. Both methods starch was used for substrate for assay method and alpha amylase hydrolyses starch and release of glucose and maltose. DNSA reagent produces color change with glucose and Iodine reagent produces color change with starch. From DNSA method only reducing sugar was produces orange red color and DNSA method but not color produced with non-reacted substrate of starch. But in Iodine method starch produces blue color with iodine which measures the alpha amylase activity by unreacted starch. If the alpha amylase produces amlylose there is no color change by DNSA method but other than starch (amylose, amylopectin, glucose) iodine does not produce blue color there by decreased the color by increased alpha amylase activity. So that starch-iodine color assay is the best method for detection of inhibition of alpha amylase enzyme.

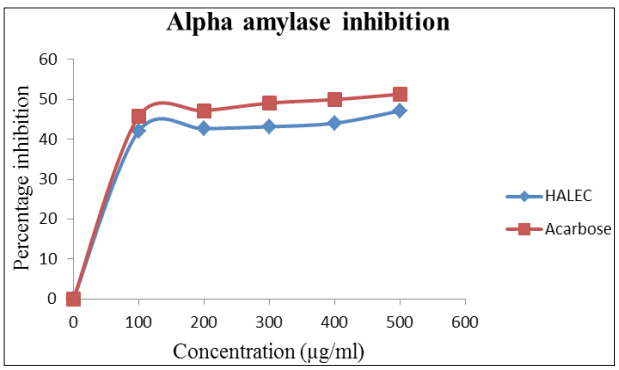

Figure 1.Percentage inhibition of alpha amylase for HALEC and acarbose by DNSA method

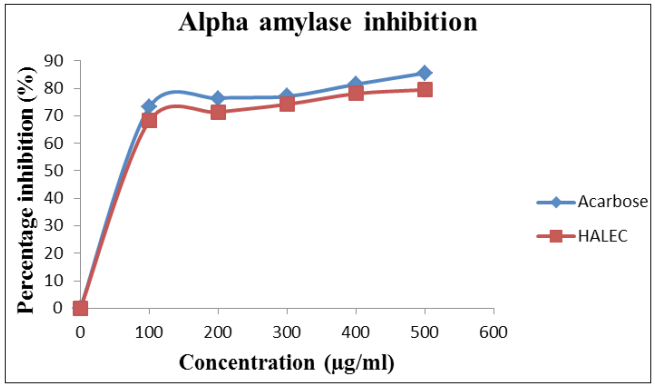

Figure 2.Percentage inhibition of alpha amylase for HALEC and acarbose by starch-iodine color assay method

Conclusion

From the results of this research concluded that HALEC having potent antidiabetic activity and starch iodine method was best method for detection of alpha amylase inhibition activity.

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Conflict of Interest: None

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