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Working

In vitro α -amylase and α -glucosidase inhibitory assay [↗](#)

Version 2

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ABSTRACT

Diabetes mellitus is an endocrine disease characterized by hyperglycemia that affects the metabolism of carbohydrates, proteins, and fats. The unique approach to decreasing postprandial hyperglycemia is to reduce or slow down dietary carbohydrate digestion. The inhibition of α -amylase and α -glucosidase enzymes is a strong therapeutic goal of controlling the postprandial glycemic reaction. This protocol describes the anti-hyperglycemic activity of aqueous extracts measuring the *in vitro* inhibition of these enzymes.

EXTERNAL LINK

[doi: 10.5138/ijaps.2010.0976.1055.01009](https://doi.org/10.5138/ijaps.2010.0976.1055.01009)

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Rodríguez-García CM, Ruiz-Ruiz JC, Peraza-Echeverría L, Peraza-Sánchez SR, Torres-Tapia LW, Pérez-Brito D, Tapia-Tussell R, Herrera-Chalé FG, Segura-Campos MR, Quijano-Ramayo A, Ramón-Sierra JM, Ortiz-Vázquez E (2019) Antioxidant, antihypertensive, anti-hyperglycemic, and antimicrobial activity of aqueous extracts from twelve native plants of the Yucatan coast. PLoS ONE 14(3): e0213493. doi: [10.1371/journal.pone.0213493](https://doi.org/10.1371/journal.pone.0213493)

PROTOCOL STATUS

Working

GUIDELINES

For α -amylase inhibitory assay:

- Starch
- Dimethyl sulfoxide
- Acetic acid

For α -glucosidase inhibitory assay:


- Sodium carbonate

MATERIALS

NAME	CATALOG #	VENDOR
Porcine pancreatic amylase		Sigma Aldrich
Tris-HCl		Sigma Aldrich
p-nitrophenyl glucopyranoside		Sigma Aldrich
alpha-glucosidase enzyme		Sigma Aldrich

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Tris-HCl		Sigma Aldrich
Porcine pancreatic amylase		Sigma Aldrich
alpha-glucosidase enzyme		Sigma Aldrich

NAME 	CATALOG # 	VENDOR 
p-nitrophenyl glucopyranoside		Sigma Aldrich
Acarbose		Sigma Aldrich

SAFETY WARNINGS

In vitro α -amylase inhibitory assay: Preparation of the substrate solution

- 1 Starch suspended in 0.2 mL of 0.5 M Tris-HCl pH 6.9 (0.01M CaCl₂)



Tris-HCl
by Sigma Aldrich

 **2 mg Starch**

In vitro α -amylase inhibitory assay: Pre-incubation

- 2 Boil (5 min) and pre-incubate the substrate solution

 **00:05:00**

 **37 °C**

In vitro α -amylase inhibitory assay

- 3 Aqueous extract dissolution

 **1 mg dissolved with 1 mL of 0.1% DMSO**

In vitro α -amylase inhibitory assay: Mix and incubation

- 4 Mix 0.2mL of the aqueous extract with the substrate solution, and 0.1 mL of porcine pancreatic amylase in Tris-HCl buffer (2 U/mL)

 **00:10:00**



Porcine pancreatic amylase
by Sigma Aldrich

 **37 °C**

In vitro α -amylase inhibitory assay: Stop reaction

- 5 Add 0.5 mL of acetic acid (50% v/v) and centrifuge

 **00:05:00 at 1811 g**

 **4 °C**

In vitro α -amylase inhibitory assay: Calculation of the α -amylase inhibition

- 6 The inhibitory activity is calculated using the formula $(Ac^+ - (Ac^- - (As - Ab)) / (Ac^+ - (Ac^-)) \times 100$, where Ac⁺, Ac⁻, As, Ab are defined as the absorbance, at 595 nm, of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), the test sample (with enzyme), and a blank (a test sample without enzyme), respectively

In vitro α -glucosidase inhibitory assay: Mix and incubation

- 7 Mix 2 U/mL of the α -glucosidase enzyme with 20 μ L of aqueous extract (1mg/mL)



alpha-glucosidase enzyme

by [Sigma Aldrich](#)

00:05:00

37 °C

In vitro α -glucosidase inhibitory assay: Initiation of reaction and incubation

- 8 Add 1 mM of pNPG (pH 6.8; buffered with 50mM of phosphate) and incubate



p-nitrophenyl glucopyranoside

by [Sigma Aldrich](#)

20 μ l

00:20:00

37 °C

In vitro α -glucosidase inhibitory assay: Stop reaction

- 9 Add 1 mM of sodium carbonate

50 μ l

In vitro α -glucosidase inhibitory assay: Calculation of the α -glucosidase inhibition

- 10 The inhibitory activity is calculated using the formula (Ac

+

) – (Ac

-

) – (As - Ab)/(Ac

+

) – (Ac

-

) \times 100, where Ac

+

, Ac

-

, As, and Ab are defined as the absorbance, at 405 nm, of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), the test sample (with enzyme), and a blank (a test sample without enzyme), respectively.



Acarbose

by [Sigma Aldrich](#)



Acarbose is an anti-hyperglycemic drug that is used as a control



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