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In vitro α -amylase and α -glucosidase inhibitory assay V.2

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Maira Rubí Segura-Campos¹

¹Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Mérida, Yucatán, México

Cecilia Rodríguez

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.

GUIDELINES

For α -amylase inhibitory assay:

- Starch
- Dimethyl sulfoxide
- Acetic acid

For α -glucosidase inhibitory assay:

- Sodium carbonate

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Keywords: Aqueous extract

MATERIALS

MATERIALS

Porcine pancreatic amylase Merck MilliporeSigma (Sigma-Aldrich)

Tris-HCl Merck MilliporeSigma (Sigma-Aldrich)

p-nitrophenyl glucopyranoside Merck MilliporeSigma (Sigma-Aldrich)

alpha-glucosidase enzyme Merck MilliporeSigma (Sigma-Aldrich)

STEP MATERIALS

Tris-HCl Merck MilliporeSigma (Sigma-Aldrich)

Porcine pancreatic amylase Merck MilliporeSigma (Sigma-Aldrich)

alpha-glucosidase enzyme Merck MilliporeSigma (Sigma-Aldrich)

p-nitrophenyl glucopyranoside Merck MilliporeSigma (Sigma-Aldrich)

Acarbose Merck MilliporeSigma (Sigma-Aldrich)

In vitro α -amylase inhibitory assay: Preparation of the subs...

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

Tris-HCl Merck MilliporeSigma (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 Merck MilliporeSigma (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 -am...

- 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 Merck MilliporeSigma (Sigma-Aldrich)

00:05:00

37 °C

In vitro α -glucosidase inhibitory assay: Initiation of reaction..

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

p-nitrophenyl glucopyranoside Merck MilliporeSigma (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 ...

- 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 Merck MilliporeSigma (Sigma-Aldrich)

Note

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