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Working

Angiotensin-converting enzyme inhibitory assay

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ABSTRACT

The use of extracts obtained from various plants is an activity developed for years with the purpose of taking advantage of resources from the regions to prevent or cure a specific disease. On the other hand, the application of in vitro techniques, as angiotensin-converting enzyme inhibitory assay have allowed finding the relationship of organic compounds with biological activities and thus be able to determine which type of plants or their extracts are suitable to be used as a treatment for hypertension. In this protocol is described a method based on the colorimetric reaction of the hippuric acid with trichloro-triazine (TT).

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Francisco Gilberto Herrera Chalé, Jorge Carlos Ruiz Ruiz, Juan Jose Acevedo Fernández, David Abram Betancurt Ancona, Maira Rubi Segura Campos. ACE inhibitory, hypotensive and antioxidant peptide fractions from *Mucuna pruriens* Revista Process Biochemistry. Octubre 2014. Vol. 49(10). pp. 1691 – 1698.

PROTOCOL STATUS

Working

GUIDELINES

1. Test tubes
2. Micropipettes
3. Water bath
4. Distilled water

SAFETY WARNINGS

Take care of temperatures and reaction time.

BEFORE STARTING

1. HHL prepared at a concentration of 0.3% in a mixture of 40 µmol potassium phosphate buffer.
2. TT dioxane (Sigma) solution (3% w/v)

1 Aqueous extract (40 µL) was added to a 20 µL solution of ACE (Sigma) (100 mU/mL), and incubated at 37 °C for 5 min.

2 Subsequently, 100 µL of HHL prepared at a concentration of 0.3% in a mixture of 40 µmol potassium phosphate buffer (JT Baker, Mexico)

3 Later 300 µM sodium chloride buffer (JT Baker, Center Valley, Pennsylvania, USA), previously adjusted to pH 8.3 either with 1 M HCl (JT Baker) or 1 M NaOH (JT Baker), and incubated at 37 °C for 45 min.

4 The reaction was stopped by adding 360 mL of TT dioxane (Sigma) solution (3% w/v) and 720 mL of 0.2 M phosphate buffer (JT Baker) (pH 8.3).

5 After centrifuging the reaction mixture at 10,000 × g for 10 min, enzymatic activity was determined in the supernatant by measuring absorbance at 382 nm (Perkin-Elmer, Lambda XLS, UV/VIS spectrophotometer).

6 The tests were performed in triplicate and antihypertensive activity was quantified by a regression analysis of ACE inhibitory activity (%) versus phenolic compounds concentration in aqueous extract and defined as an IC_{50} value, that is, the concentration of phenolic compounds required to produce 50% ACE inhibition under the described conditions.

7 For angiotensin-converting enzyme inhibitory assay lisinopril (antihypertensive drug) was used as control.