Inhibitory effects of an extract of fruits of *Physalis peruviana* on some intestinal carbohydrases

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**Summary**

*Physalis peruviana* is an Andean specie whose fruits are eaten as food and also has been reported in Colombian folk medicine for diabetes mellitus treatment. In addition, previous pharmacological studies on diabetic Wistar rats, an extract of *P. peruviana* fruits has been showed antidiabetic activity. In order to deepen in *P. peruviana* action mode as antidiabetic, in this investigation were determinate the Inhibitory Concentrations 50 (IC₅₀) of *P. peruviana* fruits crude extract on alpha glucosidase from *S. cerevisiae* and from intestinal rat powder, on maltase and alpha amylase enzymes. The kinetic behavior of the extract on each enzyme was also investigated, and the enzyme constant (Km) and maximum rate (Vmax) were determined. Extract of fruits of *P. peruviana* showed different IC₅₀ for alpha glucosidase from *S. cerevisiae* and intestinal rat powder, suggesting greater affinity for the enzyme of mammalian source (4114.7 and 3552.7 µg/mL, respectively). For maltase, the IC₅₀ was close to that obtained for alpha glucosidase (4191.0 µg/mL) while for alpha amylase, the extract exhibited the highest inhibition (IC₅₀: 619.9 g/mL). Regarding kinetic behavior, the extract showed competitive inhibition on alpha-glucosidase and maltase, and on the non-competitive type of alpha amylase. These suggest that inhibition of intestinal carbohydrases is one of the modes of action for the antidiabetic activity of fruits of *P. peruviana*. 
Effects of *Physalis peruviana* on intestinal carbohydrases

*Key words*: *Physalis peruviana*, intestinal carbohydrases, alpha glucosidase, alpha amylase, maltase.

**Resumen**

Evaluación in vitro del efecto de un extracto de frutos de *Physalis peruviana* sobre algunas carbohidreras intestinales

*Physalis peruviana* es una especie andina, cuyos frutos además de ser usados como alimento, son empleados en la medicina tradicional para el tratamiento de la diabetes *mellitus*. Además, estudios farmacológicos previos en ratas Wistar han demostrado actividad antidiabética de extractos de frutos de *P. peruviana*. Con el fin de profundizar en el modo de acción de la actividad antidiabética de los frutos de *P. peruviana*, en la presente investigación se determinó la concentración inhibitoria 50 (CI₅₀) del extracto crudo de frutos de *P. peruviana*, sobre las enzimas alfa glucosidasa obtenida de *S. cerevisiae* y de polvo intestinal de rata, maltasa y alfa amilasa. El comportamiento cinético del extracto sobre cada una de las enzimas también fue investigado y la constante enzimática (Km) y la velocidad máxima (Vmax) fueron determinadas. El extracto de frutos de *P. peruviana*, mostró diferentes valores de CI₅₀ para alfa glucosidasa obtenida de *S. cerevisiae* y para la obtenida de polvo intestinal de rata, sugiriendo una mayor afinidad por la enzima de origen mamífero (4114,7 and 3552,7 µg/mL, respectivamente). Para maltasa la CI₅₀ fue cercana a la obtenida para alfa glucosidasa (4191,0 µg/mL), mientras para alfa amilasa, el extracto presentó la mayor inhibición (CI₅₀: 619,9 g/mL). Respecto al comportamiento cinético, el extracto mostró inhibición de tipo competitiva sobre alfa glucosidasa y maltasa y no competitiva sobre alfa amilasa. Los resultados sugieren que la inhibición de carbohidrasas intestinales es uno de los modos de acción de los frutos de *P. peruviana* como agente antidiabético.

*Palabras clave*: *Physalis peruviana*, carbohidrasas intestinales, alfa glucosidasa, alfa amilasa, maltasa.
Diabetes Mellitus Type II is a metabolic disorder characterized by permanent hyperglycemia, which is caused by defects in the secretion or action of insulin. Chronic hyperglycemia is associated with damage, dysfunction and failure of various organs [1]. Currently, there are different treatments for diabetes mellitus type II, including changes in lifestyle, use of oral hypoglycemic drugs or biotechnology products, and pancreatic transplants [2].

However, it has recently been shown that plants play an important role in health care. The World Health Organization has estimated that approximately 80% of the population in emerging countries make regular use of traditional medicine (mostly derived from plants) and primary health care. Traditional medicine also plays an important role in the health care of the remaining 20% of the populations of the emerging economies and some developed countries [3].

The use of plants for medicinal purposes comes from our native communities, where it is passed down from generation to generation; a transmission of knowledge known as ethnomedicine. However, we cannot ignore the scientific and technological advances that have led to a better understanding of the behavior of nature, and it is essential to rigorously investigate the so-called medicinal plants, to determine their safety and effectiveness.

One of the plants used in traditional medicine for the treatment of diabetes mellitus is *P. peruviana*, commonly known as “uchuva”. In addition to its ethnopharmacological use, its fruits have shown *in vivo* antidiabetic activity, lowering blood glucose levels and some markers of oxidative stress [4].

There are different possible modes of action for antidiabetic drugs; one of them is the inhibition of enzymes necessary for polysaccharide digestion (these enzymes are also called carbohydrases). They are present in the intestinal villi, and participate in the cleavage of polysaccharides, which are converted into monosaccharides (glucose, fructose, galactose). The inhibition of this enzyme slows carbohydrate absorption and decreases postprandial blood glucose levels in both normal and diabetic subjects [2].

In recent years, many researchers have focused on the search for medicinal plants that inhibit carbohydrases [5-7], finding valuable information, particularly on plants with the presence of flavonoids, polyphenols and sugar derivatives [8]. Since fruits of *P. peruviana* have these secondary metabolites [9-11] the hypoglycemic effect may be due to inhibition of intestinal carbohydrases such as was reported by Pinto *et al.*, 2009 who...
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found that fruits of *P. peruviana* inhibited the alpha amylase and alpha glucosidase enzymes [11]. Thus, the aim of this study was to deepen in the evaluation of in vitro inhibition of the extract of fruits of *P. peruviana* on some intestinal carbohydrases and identify the type of inhibition as possible mode for its hypoglycemic action.

**Methods and materials**

**Materials**

Alpha-glucosidase derived from *Saccharomyces cerevisiae* (*S. cerevisiae*) Sigma/Aldrich G5003, p-nitrophenyl-α-D-glucopyranoside (pNPG) Sigma/Aldrich N1377, Acarbose Sigma/Aldrich A8980, Maltose Sigma/Aldrich 63418, rat intestinal powder (from acetone) Sigma/Aldrich I1630, LiquiColor a commercial kit (Human GLLQ1), porcine pancreatic alpha-amylase (PPA) Sigma/Aldrich A3170, Blue Starch Sigma/Aldrich S7776, and ethanolic extract of fruits of *Physalis peruviana*.

**Plant material**

The *P. peruviana* fruits were collected in February of 2012, directly by fruit traders from crops located in Granada-Cundinamarca, Colombia. The plant was identified and classified at the Herbario Nacional Colombiano – Universidad Nacional de Colombia and a voucher specimen was deposited in his collection under the code number (COL-574701).

**Preparation of the extract**

The ethanolic extract of fruits of *P. peruviana* used was prepared as follows: 400 g of fresh fruits were blended with 300 mL of 96% ethanol. The obtained juice was dried in a circulant air oven at 40 °C for 24 hours. The resulting dry material was milled and was placed in a 2 L percolator to which 1500 mL of 96% ethanol was subsequently added. After 24 hours, the percolator was opened and the collected extract was concentrated in a rotary evaporator.

**Total phenolic content**

The total phenolic content were determined by using the Folin-Ciocalteu assay [12]. An aliquot (100 μL) of extracts or standard solution of gallic acid (3.13, 6.25, 12.5, 25, and 50 μg/mL) was added to 2 mL tube. A reagent blank using distilled water was prepared. 100 μL of Folin-Ciocalteu phenol reagent 2N was added to the mixture and shaken. After 5 minutes 200 μL of 7.5% Na₂CO₃ solution was added to the mixture. The volume was then made up 2 mL with distilled water. After incubation for 120
minutes at room temperature, the absorbance against the reagent blank was determined at 726 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic Acid Equivalents (mg GA/g of sample dry weight).

**Inhibition of alpha glucosidase derived from *S. cerevisiae***

The alpha glucosidase inhibition activity was evaluated by measuring the release of p-nitrophenol from p-nitrophenyl-α-D-glucopyranoside (pNPG) following the method proposed by Pistia-Brueggemann, with slight modifications [13]. The assay contained 40 μL of phosphate buffer (0.5 M, pH 6.8); 10 μL of α-glucosidase (1 UI/mL, derived from *S. cerevisiae*); 10 μL of pNPG (11.3 mM) and *P. peruviana* extract or acarbose (positive control) in a range of 20 to 10240 μg/mL. The absorbance was read after incubation for 45 minutes at 37 °C in a BioRad microplate spectrophotometer reader at 550 to 415nm. Alpha-glucosidase inhibitory activity was calculated according to equation 1.

\[
\text{Inhibition} \% = 100 \times \frac{(A_{\text{Background}} - A_{\text{Blank}}) - (A_{\text{Sample}} - A_{\text{Blank}})}{(A_{\text{Background}} - A_{\text{Blank}})}
\]  

(1)

Where \( A_{\text{Background}} \), \( A_{\text{Blank}} \) and \( A_{\text{Sample}} \) are defined as the absorbance of 100% enzyme activity (only the solvent with the enzyme), the blank (the test sample without the enzyme) and the test sample (with the enzyme) respectively.

**Inhibition of alpha glucosidase extracted from rat intestinal powder**

The reaction mixture of this test was conducted under the same test conditions as those described in the previous section, changing only the source of alpha-glucosidase, which was obtained from acetone extract of rat intestine, according to previous works [14, 15]. Briefly, 100 mg of rat intestinal powder was suspended in 1 mL of NaCl (0.9%) and sonicated twelve times for 30 seconds at 4 °C. After centrifugation (15000 rpm, 30 min, 4 °C) the supernatant was used for the following tests. The alpha-glucosidase inhibitory activity was calculated according to equation 1.

**Inhibition of maltase**

Inhibition of maltase was performed by measuring the release of glucose by the Glucose Oxidase-peroxidase method [16, 17] with slight modifications. The assay contained 40 μL of phosphate buffer (0.5 M, pH 6.8); 10 μL of maltase obtained from acetone extract from rat intestine (1 UI/mL); 10 μL of maltose (7.5 mM) and 10 μL
of the extract of *Physalis peruviana* or Acarbose (positive control) in a range of 20 to 10240 μg/mL. The absorbance was read after incubation at 37 °C for 45 minutes in a spectrophotometer with a microplate reader at 540 nm (BIORAD XMark). The maltase inhibitory activity was calculated according to equation 1.

**Inhibition of alpha-amylase**

The alpha-amylase inhibition activity was performed following the methodology based on the hydrolysis of starch covalently linked to Remazol Brilliant Blue [18], described by Hansawasdi-Kawabata [19] with slight modifications. For the assay, 200μL of Remazol Brilliant Blue (10 mg/mL) was boiled for 5 min. Next, 100 μL of Tris-HCl buffer (0.05 M, pH 6.9 with calcium chloride 0.01 M), 100 μL of porcine pancreatic alpha-amylase (PPA, 2.1 IU/ml) and 200 μL of extract of *Physalis peruviana* or Acarbose (positive control) in a range of 20 to 10240 μg/mL were added. Absorbance was read after incubation at 37 °C for one hour at 595 nm, using a TECAN GENios spectrophotometer. The alpha-amylase inhibitory activity was calculated according to equation 1. All inhibitory concentrations 50 (IC50) were calculated using the GraphPad Prism version 5.00 for Windows software.

**Kinetic behavior of *Physalis peruviana* extract on the evaluated enzymes**

The kinetic behavior of the extract of each enzyme was performed following the methodology described above, except that the substrate concentrations were varied, ranging from 2.2 mM to 16.9 mM. The concentration of the extract was held constant to 5120 μg/mL. The reaction was started by adding the enzyme, and the endpoint was read at 415 nm for alpha-glucosidase, 540 nm to 595 nm for maltase and alpha-amylase at time intervals of 5 minutes for 20 min. The results were used to determine the enzyme constant (Km) and maximum rate (Vmax) [20, 21] using a linear regression according to Lineweaver-Burk model, in the GraphPad Prism version 5 for Windows Software.

**Statistics analysis**

The results were expressed as the arithmetic mean values ± standard deviation or standard error of the mean, and were analyzed statistically by the Tukey test and analyzed by two-way ANOVA, followed by the Bonferroni test for multiple comparisons. The difference between the sample and the target was considered significant (*) when p < 0.05. The statistical calculations and graphs were processed using GraphPad Prism version 5 for Windows.
Results

Total phenolic content

Previous works have been demonstrated that *P. peruviana* fruits are rich in phenolic compounds. In this investigation, it was found that employed extract content $6.3 \pm 0.8$ mg GAE/g extract, this content is less than that reported by other authors [11]. It can be explained since the solvent used in this investigation (ethanol 96%) is less polar that that the solvent used by Pinto *et al.* [11] (ethanol 12%).

Inhibitory activity of *P. peruviana* on alpha glucosidase, maltase and alpha amylase

The inhibition activities of *P. peruviana* on alpha glucosidase from *S. cerevisiae* and alpha glucosidase extracted from rat intestinal powder are showed in Figures 1 and 2 respectively. The IC$_{50}$ values found are reported in the Table 1. It is important to note that the IC$_{50}$ of acarbose and *P. peruviana* extract on alpha glucosidase differ according to the source of the enzyme; it is lower when the enzyme is extracted from the intestinal powder.

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Maltase extracted from rat intestinal powder</th>
<th>Alpha amylase from porcine pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor</td>
<td>Acarbose</td>
<td><em>P. peruviana</em> Extract</td>
</tr>
<tr>
<td>IC$_{50}$ (µg/mL)</td>
<td>$773.2 (470.0 - 1296.0)$</td>
<td>$4114.7 (2826.0 - 5990.0)$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$570.7 (457.1 - 691.4)$</td>
</tr>
</tbody>
</table>

IC$_{50}$ Inhibitory concentration $50$ – *S. cerevisiae: Saccharomyces cerevisiae* – *P. peruviana: Physalis peruviana*. The IC$_{50}$ are expressed as the mean and the confidence limits of 95%. 
Figure 1. Inhibitory activity of an extract of fruits of *P. peruviana* on alpha glucosidase from *S. cerevisiae*. Results are expressed as mean ± SEM (n = 3). Inhibitors: Acarbose and ethanolic extract of fruits *P. peruviana*.

Figure 2. Inhibitory activity of an extract of fruits of *P. peruviana* on alpha glucosidase from lyophilized rat intestinal powder. Results are expressed as mean ± SEM (n = 3). Inhibitors: Acarbose and ethanolic extract of fruits *P. peruviana*. 
Figure 1. Inhibitory activity of an extract of fruits of *P. peruviana* on alpha glucosidase from *S. cerevisiae*. Results are expressed as mean ± SEM (n = 3). Inhibitors: Acarbose and ethanolic extract of fruits *P. peruviana*. The inhibitory activity of the extract of *P. peruviana* on maltase is shown in Figure 3, and the IC$_{50}$ value found is shown in Table 1. The IC$_{50}$ of acarbose on this enzyme is lower than on alpha glucosidase (less than half) while the IC$_{50}$ of *P. peruviana* extract is very close to that found on alpha glucosidase from *S. cerevisiae*, and slightly higher than those obtained on the extracted alpha glucosidase.

![Inhibitory activity of a extract of fruits of *P. peruviana* on maltase](image)

Figure 3. Inhibitory activity of an extract of fruits of *P. peruviana* on maltase. Results are expressed as mean ± SEM (n = 3). Inhibitors: Acarbose and ethanolic extract of fruits *P. peruviana*.

For alpha amylase, the inhibitory activity of *P. peruviana* is shown in Figure 4. The IC$_{50}$ value is lower than that found on alpha glucosidase and maltase (Table 1); the lowest IC$_{50}$ obtained was for *P. peruviana*.

**Kinetic behavior of *P. peruviana* extract on the evaluated enzymes**

The reaction rates of *P. peruviana* on alpha-glucosidase, maltase and alpha amylase are shown in Figures 5 to 7, and the kinetic parameters are presented in Table 2. *P. peruviana* extract has a competitive-type behavior on inhibition of and maltase where the presence of the substrate reduces the inhibitory activity of the extract but not change the maximum rate while it was found a mixed type inhibition on alpha-glucosidase, since
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The maximum rate (Vmax) is reduced and the enzyme constant (Km) is increased. Regarding alpha amylase, *P. peruviana* extract exhibited a non-competitive behavior on this enzyme.

![Inhibitory activity of an extract of fruits of *P. peruviana* on alpha amylase. Results are expressed as mean ± SEM (n = 3). Inhibitors: Acarbose and ethanolic extract of fruits *P. peruviana.*](image)

Table 2. Kinetic parameters of the inhibition of the extract from *P. peruviana* on the different enzymes.

<table>
<thead>
<tr>
<th></th>
<th>Alpha glucosidase</th>
<th>Maltase</th>
<th>Alpha amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without inhibitor</td>
<td><em>P. peruviana</em> extract</td>
<td>Without inhibitor</td>
</tr>
<tr>
<td>Km</td>
<td>1.538</td>
<td>3.205</td>
<td>1.110</td>
</tr>
<tr>
<td>Vmax (mM/min)</td>
<td>0.011</td>
<td>0.007</td>
<td>0.009</td>
</tr>
<tr>
<td>Km/ Vmax</td>
<td>137.877</td>
<td>458.654</td>
<td>118.032</td>
</tr>
<tr>
<td>Type of inhibition</td>
<td>Mixed</td>
<td>Competitive</td>
<td>Non-Competitive</td>
</tr>
</tbody>
</table>

Vmax, Maximum rate – Km; kinetic constant.
Figure 5. Reaction rate of alpha-glucosidase on pNPG in the presence and absence of the ethanolic extract of fruits of \textit{P. peruviana}. pNPG (p-nitrophenyl-\(\alpha\)-D-glucopyranoside), \(V\); reaction rate.

Figure 6. Reaction rate of maltase on maltose in the presence and absence of ethanolic extract of fruits of \textit{P. peruviana}.
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Discussion

**Inhibitory activity of *P. peruviana* on alpha glucosidase, maltase and amylase**

The $IC_{50}$ found for *P. peruviana* on alpha glucosidase from *S. cerevisiae* (4.11mg / mL) was high but similar to the $IC_{50}$ presented by other extracts of medicinal plants traditionally used as hypoglycemicants. For these plants, $IC_{50}$ values have been reported ranging from 1.8 µg/mL for *Punica granatum* extract [22] to 17.2 mg/mL for extract of *Andrographis paniculata* Nees [23]. On the other hand, in spite of Pinto et al., not reported $IC_{50}$ values, is possible to note that inhibitions near to 50% was achieve with concentration more than 10 mg/mL of *P. peruviana* fruits extract [11]. This concentration is higher than the $IC_{50}$ finding in this investigations, beside the lower content of total phenolic compounds in our extract. These findings suggest that other compounds besides phenols could be responsible of the alpha glucosidase inhibition.

Previous works had reported that the most of the plant extracts have lower $IC_{50}$ on alpha-glucosidase extracted from *S. cerevisiae* than on the enzyme extracted from mammalian intestine [24-26]. This is not the case with *P. peruviana*, which suggests that
this extract is more specific for mammalian intestinal enzymes. This fact is important because a plant extract capable of inhibiting the digestion of complex carbohydrates in the human gastrointestinal tract should show inhibition of the enzyme extracted from the mammal, not only the enzyme obtained from microorganisms, since the values reported for inhibition of alpha glucosidase obtained from microorganisms cannot be extrapolated to enzymes from mammalian intestine.

This divergence may be due to the difference in binding site molecular recognition of enzymes. Thus, many inhibitors of alpha glucosidase from S. cerevisiae do not show inhibition on alpha glucosidase extracted from mammals [15, 27]. Some authors report that both acarbose and voglibose have high inhibitory effects of alpha glucosidase in mammals, but no inhibitory activity against alpha glucosidase form S. cerevisiae [26, 27].

For the enzyme maltase on acarbose, a lower IC50 was found than on alpha glucosidase. This is because acarbose binds to the enzyme with higher affinity than the substrate itself [28]. In the case of the ethanolic extract of fruits of P. peruviana, the IC50 was not significantly different from the IC50 on alpha glucosidase. This may be because the extract has a large number of compounds that can bind to various proteins contained in the crude enzyme solution, making its binding less specific. This fact had also been reported in other extracts, such as Stichopus japonicus [29].

For alpha amylase, the IC50 values found were lower, suggesting that the extract of P. peruviana produces a strong inhibition of this enzyme, although P. peruviana IC50 was higher than acarbose IC50 (619.9 and 229.6 µg/mL, respectively). Similar to reported for alpha glucosidase, Pinto et al. found 65% as the maximum inhibition when it was tested P. peruviana extract at concentration of 50 mg/mL, almost 100 times more than the IC50 found in this investigation [11].

The strong inhibition of acarbose on alpha amylase and lack of digestion of starch [30, 31] are related to some side effects such as flatulence and bloating, and diarrhea [15]. Thus, an extract that shows less inhibition on alpha amylase could minimize these side effects [25, 30].

**Kinetic behavior of P. peruviana extract on the evaluated enzymes**

The kinetic behavior of P. peruviana on maltose is consistent with the results obtained in the inhibition, since the extract behaved very similarly to acarbose, a drug that has a competitive type of inhibition of these enzymes. For alpha glucosidase, it was found a
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mixed type inhibition such as it was reported for some flavonoids especially quercetin [32], a flavonoid described for *Physalis peruviana* fruits [11]. However, it is important to note that non-competitive behavior is usually preferred, because this prevents the presence or absence of substrate from affecting the binding of the inhibitor to the enzyme, resulting in less interaction with meals [26, 33, 34].

Regarding to alpha amylase, the extract exhibited a non-competitive inhibition that may be related to structural differences due to the origins of the enzymes [35]. Another factor that may explain the combination of competitive and non-competitive inhibition on the evaluated enzymes presented by *P. peruviana* extract is the fact that the extract presents more than one inhibitor. Due to the presence of polyphenols, such as flavonoids, it is possible for one inhibitor to bind to the active region of alpha-glucosidase and another to bind to the active region of alpha amylase.

Flavonoids or phenolic compounds have been linked to the inhibition of alpha-glucosidase and alpha-amylase [36], and plants with phytochemical studies that have reported the presence of these secondary metabolites, or for which biological studies have demonstrated antioxidant activity, are promising in inhibiting alpha-glucosidase and amylase. For fruits of *P. peruviana*, the presence of both flavonoids and other phenols has been reported [37], including quercetin, campferol and flavonols [38, 39]. However, the findings of this investigation suggested that not only phenolics compounds but others, could be responsible for the moderate inhibition on alpha glucosidase, and the high inhibition on alpha-amylase.

Conclusions

Ethanolic extract of fruits of *P. peruviana* has shown different responses to microbial alpha glucosidase (Type I) and alpha glucosidase extracted from rat intestinal powder (Type II). Likewise, ethanol extract of fruits of *P. peruviana* type shows competitive inhibition on alpha-glucosidase, and on the non-competitive type of alpha amylase.

Based on the results of this investigation, it is suggested that inhibition of intestinal carbohydrases is one of the modes of action by which the fruits of *P. peruviana* exert hypoglycemic and antidiabetic activity.

Declaration of interest

The authors report no declarations of interest.
References


