α-Glucosidase and α-amylose inhibition potentials of ten wild Mexican species of Verbenaceae

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Abstract

Purpose: To evaluate the inhibitory activity of 10 wild Verbenaceae species from Mexico against α-glucosidase and α-amylase.

Methods: Ethanol leaf extracts of 10 Verbenaceae species from Mexico were prepared. The inhibitory activity of the extracts against α-glucosidase and α-amylase was evaluated using enzymatic protocols. At least four serial diluted concentrations of each extract was used to calculate the half-maximal inhibitory concentration (IC₅₀).

Results: The 10 evaluated Verbenaceae species showed high α-glucosidase inhibition activity, but a low inhibitory effect on α-amylase. Aloysia gratissima (IC₅₀ = 0.122 mg/mL), Verbena carolina (IC₅₀ = 0.112 mg/mL), Bouchea prismatica (IC₅₀ = 0.122 mg/mL), Verbena menthiflora (IC₅₀ = 0.071 mg/mL), Aloysia citriodora, Bouchea prismatica and Priva mexicana (IC₅₀ = 0.032 mg/mL) exhibited the strongest inhibitory activities against α-glucosidase.

Conclusion: All the Verbenaceae species studied possess α-glucosidase inhibitory effect, with P. mexicana being the one with the strongest activity. These findings demonstrate the high potential of these species as a source of natural antihyperglycemic agents for type 2 diabetes therapy.

Keywords: Hyperglycemic, Diabetes, α-Glucosidase, α-Amylase Verbenaceae, Aloysia gratissima, Bouchea prismatica, Priva mexicana

INTRODUCTION

Type 2 Diabetes mellitus (T2DM) is a chronic disease associated with a high concentration of blood glucose or hyperglycemia that can be produced by a deficient carbohydrate, lipid and protein metabolism [1]. T2DM affects an important proportion of the world population [2]. Hyperglycemia, in non-insulin dependent patients, is due to an increased starch breakdown by α-amylase and a high glucose absorption favored by the action of α-glucosidase [1-3]. The inhibition of these enzymes could reduce blood glucose levels and, therefore, decrease hyperglycemia linked to T2DM [4].
Pharmaceutical chemical compounds such as acarbose, miglitol and voglibose, are used to maintain glucose levels efficiently, but prolonged use has been associated with several side effects [5]. An alternative to avoid or minimize these side effects is to use plant extracts rich in bioactive compounds like polyphenols, which have been reported to inactivate α-amylase and α-glucosidase through non-specific enzymatic binding [6]. In Mexico, about 306 species from 235 genera and 93 families have been reported to have hypoglycemic activity, but the number is almost double considering all medicinal reports [7].

One of the most important families with medicinal properties is Verbenaceae; in Mexico, this family is represented by 26 genera and 286 species [8]. Even though many species of Verbenaceae are used in folk medicine, information about their phytochemical composition and biological activities are still scarce. The research group recently reported the phenolic composition and antioxidant properties of 10 wild Verbenaceae species from Mexico [9]. The aim of the present study was to evaluate the antihyperglycemic potentials of 10 wild Mexican species of Verbenaceae (Verbena gracilis Desf., V. carolina L., V. bipinnatifida Nutt., V. menthifolia Bentham., Lantana camara L., Phyla nodiflora (L.) Greene, Aloysia gratissima (Gill. et Hook) Tronc., Bouchea prismatica (L.) Kuntze, Priva mexicana (L.) Pers., and Lippia umbellata Cav.).

EXPERIMENTAL

Chemicals

Starch, α-glucosidase from Bacillus stearothermophilus (E.C. 3.2.1.20), α-amylase from pancreatic porcine (E.C. 3.2.1.1), acarbose, p-nitrophenyl-α-D-glucopyranoside (pNPG) and 3,5 dinitrosalicylic acid (DNS) were obtained from Sigma Aldrich (St Louis, MO). Absolute ethanol, potassium phosphate buffer (potassium phosphate monobasic (KH₂PO₄) and dibasic potassium phosphate (K₂HPO₄)), sodium carbonate (Na₂CO₃), potassium sodium tartrate (KNaC₄H₄O₆·4H₂O), sodium hydroxide (NaOH) and sodium phosphate buffer (sodium phosphate dibasic (Na₂HPO₄·2H₂O) and sodium dihydrogen phosphate monohydrate (NaH₂PO₄·H₂O)) were purchased from J.T Baker (US).

Plant material

Foliar tissues of ten species of Verbenaceae were collected from flowering plants in different locations of Durango, Mexico. Voucher specimens were collected and deposited in a Herbarium (Herbario CIIDIR). Leaves of each species were separately dried, ground, and kept in paper bags at room temperature and darkness until analysis.

Preparation of extracts

Phenolic-rich extracts were prepared according to Avila-Reyes et al [9]. Four grams of dry and ground leaves were macerated in 40 mL of 80 % ethanol (v/v) for 24 h under constant shaking (100 rpm) at room temperature. Extracts were centrifuged at 5000 rpm for 10 min. Supernatants were recovered and pellets were re-extracted with 20 mL of 20% ethanol (v/v) for 4 h, as previously described, and the supernatant was recovered. Both, 80 and 20% ethanol extracts of the same sample were combined and driven to dryness at room temperature and in darkness. Aliquots of each extract were dissolved in ethanol (80% v/v) to obtain 10 mg/mL solutions.

Evaluation of hypoglycemic activity

α-Glucosidase activity

Alpha-glucosidase assay was performed as described by Kim et al [10]. Fifty microliters of α-glucosidase from B. stearothermophilus (EC3.2.1.20) at 0.5 U/mL was prepared in 0.2 M potassium phosphate buffer (pH = 6.8) and mixed with 50 µL of 0.2 M potassium phosphate buffer (pH = 6.8) and 50 µL of extract (concentrations between 0.05 and 2 mg/mL). The mixture was pre-incubated at 37°C for 5 min, then 100 µL of 3 mM pNPG was added. The mixture was incubated for 10 min at 37°C. Then, 750 µL of 0.1 M Na₂CO₃ was added to stop the reaction, and absorbance was read at 405 nm. The inhibitory activity (H) of the extracts was calculated as in Eq 1.

\[ H(\%) = \frac{1 - (Ac - As)/Ac}{100} \]  

where Ac and As are the absorbance of the control (water instead of extract) and the extract, respectively. Values were expressed as half-maximal concentration (IC₅₀) value. Acarbose (concentration range from 10 to 100 µg/mL) was used as positive control for α-glucosidase inhibition.

α-Amylase activity

α-Amylase inhibitory activity was measured according to the method described by Kim et al [11] with some modifications. Extract (40 µL) or acarbose as positive control prepared in 20 mM sodium phosphate buffer (pH = 6.9), was left to react with 200 µL of porcine pancreatic α-
amylase solution (1 U/mL) and pre-incubated at 25°C for 10 min. After pre-incubation, 400 µL of 0.20% starch solution in 20 mM sodium phosphate buffer (pH = 6.9) was added, the mixture was again incubated for 20 min at 37°C. The reaction was stopped by adding 500 µL of 0.1% DNS reagent (1% DNS, 12% KNaC4H4O6·4H2O in 0.4 M NaOH). The tubes were boiled over a water bath for 5 min and cooled at room temperature. The reaction mixture was diluted 10 times with distilled water, centrifuged at 9500 rpm and absorbance was read at 540 nm. The control contained buffer solution instead of extract. The results are expressed as amylase inhibition (B).

B (%) = \(1 - \frac{(Ac - As)}{Ac}\)100 …………… (2)

where Ac and As are the absorbance of the control (water instead of extract) and the extract, respectively.

Statistical analysis

Three independent samples of each item were analyzed. Differences among species was determined using an analysis of variance (ANOVA) and means were separated using Duncan’s test (p < 0.05).

RESULTS

Important α-glucosidase inhibitory activity was found with the 10 Verbenaceae species, presented as IC50 values (Table 1). The ANOVA test showed statistical difference among the studied species (p < 0.0001). Four of the ten species showed no differences with the positive control (IC50 = 0.001 mg/mL). From these species, P. mexicana showed the highest antihyperglycemic activity (IC50 = 0.032 mg/mL). The samples with the lowest inhibition potential were V. gracilis (IC50 = 1.515 mg/mL) and L. umbelata (IC50 = 0.945 mg/mL). All the species showed > 50 % inhibition of α-glucosidase; A. gratissima extract (2 mg/mL) showed the highest (87.41 %) inhibition activity, while L. camara (2 mg/mL) exhibited the lowest activity (62.54 %).

All the species of Verbenaceae analyzed had dose-dependent inhibitory effects on α-amylase (Figure 1), although these effects were lower than those displayed for α-glucosidase. Because of this low activity by the studied species, IC50 calculations could not be done. Verbena menthifolia (58.45 %) showed the best inhibitory activity (58.47 %) at the highest concentration (10 mg/mL). The lowest inhibitory activity was shown by P. mexicana (26.37 %).

![Figure 1: Effect of different concentrations of ethanol extracts of 10 species of Verbenaceae on α-amylase activity](image)

Table 1: IC50 values for in vitro inhibition of α-glucosidase by 10 wild Verbenaceae species from Mexico

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inhibition (% , 2 mg/mL)</th>
<th>IC50 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lippia umbelata</td>
<td>65.999±0.98</td>
<td>0.945±0.61</td>
</tr>
<tr>
<td>Priva mexicana</td>
<td>80.707±1.87</td>
<td>0.032±0.03</td>
</tr>
<tr>
<td>Verbena bipinnatifida</td>
<td>68.800±1.22</td>
<td>0.421±0.68</td>
</tr>
<tr>
<td>Verbena gracilis</td>
<td>64.241±0.69</td>
<td>1.515±1.46</td>
</tr>
<tr>
<td>Phylla nodiflora</td>
<td>83.443±0.95</td>
<td>0.368±0.38</td>
</tr>
<tr>
<td>Verbena menthifolia</td>
<td>81.211±1.18</td>
<td>0.071±0.12</td>
</tr>
<tr>
<td>Bouchea prismatica</td>
<td>77.189±0.24</td>
<td>0.122±0.11</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>62.536±1.62</td>
<td>0.428±0.41</td>
</tr>
<tr>
<td>Verbena carolinia</td>
<td>64.304±0.45</td>
<td>0.112±0.16</td>
</tr>
<tr>
<td>Aloysia gratissima</td>
<td>87.417±0.55</td>
<td>0.122±0.12</td>
</tr>
<tr>
<td>Acarbose</td>
<td>98.223±0.00</td>
<td>0.001±0.00</td>
</tr>
</tbody>
</table>

The values represent the mean and standard deviation (n = 3). At least four serially diluted solutions of each extract were taken for IC50 calculation. Different letters in the same column indicate significant differences (p < 0.05) by Duncan’s multiple range test.
DISCUSSION

The present study was designed to evaluate the α-glucosidase and α-amylase inhibitory activity of 10 Verbenaceae species. All the studied species had higher inhibitory activity for α-glucosidase than for α-amylase, which agrees with findings in other plant species [12]. Results suggest that the hypoglycemic effect of these species of Verbenaceae is mainly through the inhibition of α-glucosidase.

According to the results, P. mexicana, V. menthifolia, and V. carolina had the highest α-glucosidase inhibitory effects. This activity may be related to their contents of total phenolics and flavonoids, as previously reported for these species [9]. These species are known to accumulate high levels of both total phenolics and flavonoids. A direct relation between total phenolics content and α-glucosidase inhibition activity was reported for the ethanol extracts of Vaccinium myrtillus L. (bilberry, Ericaceae), a plant used for diabetes treatment [13]. Biljajić et al. demonstrated that the presence of hyperoside (44.43 mg/g hydroethanolic extract), a quercetin glucoside (quercetin-3-O-galactoside), was responsible for its important antidiabetic activity. This flavonol enhances glucose utilization, increasing the activity of hexokinase enzyme and causing an increase in glycolysis, which in turn promotes the use of glucose for energy production [14]. According to a previous study [9], Verbena carolina and V. gracilis accumulate glycoside derivatives of the flavone scutellarein (five and three derivatives, respectively). However, despite their similar phenolic profiles, these two species had significantly different IC50 values.

In the present study, the ethanol extract of L. camara showed its maximum α-amylase inhibition (53.57 %) at 10 mg/mL; this result is in disagreement with those reported by Swamy et al [15], who found for different extracts of L. camara from Malaysia higher inhibition activities (acetone extracts showed 57.15% of inhibition at 0.1 mg/mL, methanol, ethyl acetate, and chloroform extracts did not reach 50% of inhibition at that same concentration). However, it is important to mention that L. camara represents a complex rather than a single species [16] and what is considered as L. camara in Mexico and in Malaysia represent different species, probably having also different phenolic profiles (affecting α-amylase inhibition activity), as a species-specific tendency of phenolic profiles has been reported [17]. Despite the important diversity of biological activities reported for L. camara, little information about its anti-diabetic properties has been reported. In the current study, L. camara showed an important α-amylase inhibitory activity, and a considerable activity against α-glucosidase activity, suggesting the potential of L. camara as a source of metabolites to treat complications related to T2DM. Several species of the genus Lippia, such as L. alba (Mill.) N.E.Br. ex Britt and P. Wilson, L. callicarpifolia Kunth, L. graveolens Kunth, L. myroicephala Cham, L. oaxacana B.L. Rob. & Greenm, L. palmeri S. Watson, L. scaberrima Sond, and Lippia sp. have been used in Mexico as herbal remedies for several illnesses, especially L. umbellata is reported for the treatment of colds and colics [8]. Previous studies on L. umbellata in Mexico [9], showed the presence of luteolin 7-O-glycoside and chrysoeriol glycoside. Some flavones (luteolin 7-O-glycoside and chrysoeriol), identified in the ethanol extract of Genista tenera (Fabaceae), diminished efficiently glucose levels in the blood [18].

Phyla nodiflora, also known as Lippia nodiflora, is a perennial herb, indigenous to the Americas, but dispersed widely by natural and human events. In Mexico, P. nodiflora has been reported for traditional use as aseptic, purgative, uterine-menstrual problems, antiparasitic, and for fever control [19]. No evidence about its antidiabetic potential has been reported in our country, while γ-sitosterol isolated from P. nodiflora collected in India showed an increasing insulin secretion effect in presence of glucose in diabetic rats [20].

Alloysia gratissima (IC50 = 0.122 mg/mL), B. prismatic (IC50 = 0.121 mg/mL) and P. mexicana (IC50 = 0.032 mg/mL) showed the best glucosidase activity, but little information is available about its biological activity and chemical composition. These species showed IC50 values similar to Clerodendrum volubile P. Beauv (Verbenaceae) (IC50 = 0.309 mg/mL) commonly used as a medicinal plant in southern Nigeria [21] Bouchea prismatic and P. mexicana showed the presence of apigenin-7-O-glycoside in ethanol extracts [9], which, as well as its derivatives, have been reported as promising drugs for the treatment of diabetes [18]. Plant extracts like Salvia cinnatana and Cephalotaxus sinensis showed antihyperglycemic action attributed to these compounds [22,23].

CONCLUSION

The ethanol extracts from the 10 analyzed Verbenaceae species showed an important and significant inhibitory activity against the α-
glucosidase enzyme but weak inhibitory activity against α-amylase. Although several Verbenaceae species are widely used for medicinal purposes in Mexico, little is known about the antidiabetic potential associated with wild species of this genus. No information about the hypoglycemic potential of these wild species has previously been reported. Thus, the evaluated species are an important source of metabolites with medicinal properties for the treatment of diabetes and its complications.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work

Authors’ contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dr. José A. Ávila-Reyes provided HPLC complementary data necessary to discuss our results and helped with the species collection. Dr. Norma Almaraz-Abarca participated writing and evaluating the manuscript. M.S. Eli A. Delgado Alvarado carried out the experiments. Dr. Rene Torres-Ricario wrote most of the manuscript. Nestor Naranjo-Jimenez helped in the collection and identification of the species. Laura S. Gonzalez-Valdez and José N. Uribe-Soto were involved in the literature review and data analysis. Andres Vasavilbazo-Saucedo and Marcela V. Gutierrez-Velazquez prepared the ethanolic extracts and helped in the experimental analysis.

REFERENCES


