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First Approach to Unveiling the Antidiabetic Potential of *Agave potatorum*: α-Glucosidase Inhibition and Phytochemistry

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Abstract. *Agave potatorum* Zucc. leaves infusion is used in traditional medicine to treat type II diabetes mellitus, however, there are no studies that validate this use. Therefore, this work aims to evaluate the potential antidiabetic activity of *A. potatorum* leaves infusion from one to eight years old by assessing the α-glucosidase inhibitory activity. Thin-layer chromatography (TLC)–bioautography and spectrophotometry using acarbose as a positive control were used to assay the α-glucosidase inhibitory activity. The phytochemical constituents responsible for the inhibition detected by bioautography were assayed with qualitative phytochemical screening methods. Regardless of the age of the agave, all leaves possess secondary metabolites (SM) capable of inhibiting α-glucosidase, according to the bioautography assay and the inhibition determined by UV-vis. The inhibition values of infusions at 2 mg/mL showed that for agave aged 2, 4, 5, 7, and 8 years old the inhibition was higher than 50 %, in the same range as acarbose at the same concentration. Phytochemical analysis revealed the presence of terpenes and phenolic compounds like tannins, flavonoids, anthraquinones, phenol carboxylic acids, and coumarins in the inhibition zones of the bioautography assay. Therefore, the antidiabetic activity of *A. potatorum* reported in traditional medicine is achieved through the inhibition of the enzyme α-glucosidase by the diverse secondary metabolites in leaves.
Keywords: TLC-bioautography; ethnomedicine; phytochemical analysis; type 2 diabetes mellitus.

Resumen. La infusión de hojas de *Agave potatorum* Zucc. se utiliza en la medicina tradicional para tratar la diabetes mellitus de tipo II, sin embargo, no existen trabajos que validen dicho uso. El objetivo de este trabajo fue evaluar la potencial actividad antidiabética de las infusiones de hojas de *A. potatorum* de uno a ocho años de edad mediante la evaluación de la actividad inhibitoria de la α-glucosidasa. Para ensayar la actividad inhibitoria de la α-glucosidasa se utilizó cromatografía en capa fina (TLC)-bioautografía y espectrofotometría utilizando acarbosa como control positivo. Los constituyentes fitoquímicos responsables de la inhibición detectada por bioautografía se analizaron con métodos cualitativos de cribado fitoquímico. Independientemente de la edad del agave, todas las hojas poseen metabolitos secundarios capaces de inhibir la α-glucosidasa, según el ensayo de bioautografía y la inhibición determinada por UV-vis. Los valores de inhibición de las infusiones a 2 mg/mL mostraron que, para las infusiones de hojas de agave de 2, 4, 5, 7 y 8 años la inhibición fue superior al 50 %, en el mismo rango que la acarbosa a la misma concentración. El análisis fitoquímico reveló la presencia de terpenos y compuestos fenólicos como taninos, flavonoides, antraquinonas, ácidos carboxílicos fenólicos y cumarinas en las zonas de inhibición del ensayo de bioautografía. Por lo tanto, la actividad antidiabética de *A. potatorum* descrita en la medicina tradicional se

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lleva a cabo a través de la inhibición de la enzima α-glucosidasa por los diversos metabolitos secundarios presentes en las hojas y casi de manera independiente de la edad de la planta.
Palabras clave: TLC-bioautografía; etnomedicina; análisis fitoquímico; diabetes mellitus tipo 2.

Introduction

Type II Diabetes Mellitus (T2DM) is a severe and chronic metabolic disease characterized by high blood glucose levels. The International Diabetes Federation 2022 [1] estimated that by 2021, 537 million people were affected by diabetes and that 6.7 million people will die from diabetes and its complications worldwide. The management of patients with T2DM generates high social and economic costs, so mitigating this condition is a global challenge [2].

The α-glucosidase is the enzyme that hydrolyses oligosaccharides and heterosides of α-glucose, providing an aglycone and a glucose unit. The inhibition of this enzyme [3-5] is considered one of the best strategies to decrease the postprandial rise in blood glucose levels and avoid late diabetic complications. Inhibitors of α-glucosidase work by inhibiting this enzyme [6], thereby hindering glucose absorption in the gastrointestinal tract and minimizing postprandial hyperglycemia, a key component of diabetic pathogenesis. These inhibitors reduce the synthesis of very low-density lipoprotein (VLDL), i.e., they do not have lipogenic potentials [5] like insulin or sulfonylureas. However, the commercial α-glucosidase inhibitors currently used as hypoglycaemic agents, acarbose, voglibose, and miglitol [2,6] cause side effects such as stomach upset, diarrhea, and flatulence that patients do not tolerate and drop out of treatment. The search for therapeutic alternatives with fewer side effects for the control of T2DM has led to the identification of secondary metabolites (alkaloids, flavonoids, phenols, and terpenes) from commonly used vegetables [7] and in other natural products from plants and microorganisms [8,9] with potential α-glucosidase inhibitory activity.

Agave potatorum Zucc. is an endemic plant from Oaxaca, Mexico, used to produce tobalá mezcal. The leaves, more than 50 % of the plant, are considered an agro-industrial waste during agave harvesting. In traditional medicine, the infusion or juice of *A. potatorum* leaves is used to treat the symptoms of diabetes mellitus and to prevent blood clots; the roasted leaves are used to treat inflammatory conditions and wounds [10,11]. However, no scientific studies validate the medicinal uses of *A. potatorum.*

In this study, we evaluate the antidiabetic activity by the ability of α-glucosidase inhibition due to secondary metabolites present in the infusions of one-year-old to eight-year-old leaves of *A. potatorum* by thin-layer chromatography (TLC)–bioautography and spectrophotometry. The diversity of secondary metabolites present in the leaves of *A. potatorum* is poorly understood because they are in low concentration in the infusions and can be very complex. Therefore, to increase the concentration and to identify the families of secondary metabolites responsible of the inhibitory activity of α-glucosidase, the phytochemical profile of extracts obtained by sequential extraction of ascending polarity of 7 and 8-year-old leaves was determined, to valorize the agave leaves that turn into agro-industrial waste, since at this age the plant is harvested for the production of mezcal.

Material and methods

Apparatus and chemicals used

Ultrasonic bath Branson Ultrasonics© 3800 (serial number BGJ101625780B, Danbury CT, USA), rotary evaporator R-100 Büchi© (Flawil, Switzerland), analytical balance Vel-lab© (China), silica gel coated with fluorescent indicator F254, size 20 x 20 cm, (Merck, Darmstadt, Germany), micropipettes of 0.5-10, 10-100 and 100-1000 µL (Jf Lhabo), 30-300 µL multichannel micropipette (AHN Biotechnologie GmbH), a microplate reader photometer (Thermo Fisher Scientific MultiSkan FC (China)) and a Pulverisette-19 mill of Fritsch (Germany) were used. All chemicals and reagents used were of analytical grade. 2-naphthyl-α-D-glucopyranoside was purchased from Goldbio© (St. Louis, MO, USA); the enzyme α-glucosidase from *Saccharomyces cerevisiae*, p-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium tosylchloramide,

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diphenylboryloxyethylamine, polyethylene glycol 4000, trichloroacetic acid, Fast Blue B salt, and silica gel for column chromatography (60-230 mesh) were purchased from Sigma-Aldrich© (Toluca, Mexico). Acarbose (pharmaceutical use) was extracted from acarbose tablets Laboratorios Pisa©.

Plant material

Healthy *A. potatorum* leaves were collected in Infiernillo, Zaachila, Oaxaca, Mexico (16° 89′ N; 97° 19′ W; 1969 meters above sea level) from three independent plants in September 2021. From the samples of each specimen one basal, one middle, and one apical leaf were taken and mixed to have only three samples of each year. The plants were identified according to morphological descriptors [10,12] and was corroborated by comparison with the voucher OAX 21200 deposited in the herbarium of CIIDIR Oaxaca by Abisai García Mendoza. Leaves were washed with abundant water and cut into 0.5-0.7 cm longitudinal strips, then dried at room temperature (18-28 °C) isolated from sunlight and under an air current until reaching constant weight (8 days). The dried material was ground in an electric mill to diminish the particle size and stored in sealed polyethylene bags at room temperature in a dry place.

Preparation of *A. potatorum* leaves extracts

First, infusion of *A. potatorum* leaves were prepared with boiling water since that is used in traditional medicine to treat T2DM. 0.5 g of *A. potatorum* leaves from each year (1 to 8 years) were placed in beakers and 2.5 mL of distilled water at boiling point was added and stood for 30 minutes. The infusion was centrifugated at 6000 rpm for 10 minutes and a springer filter of 0.4 µm was used to filter the supernatant, which was immediately used for the determinations. An aliquot of each infusion was used to determine the solid percentage by the oven technique [13].

Since the secondary metabolites are in low concentration in the infusion extract, a sequential extraction process with ultrasound-assisted extraction using the increasing polarity of solvents was achieved. 10 g of 7- and 8-year-old *A. potatorum* dried leaves were subjected to sequential US-assisted extraction in an ultrasonic bath at 40 kHz for 120 min at 22 °C (temperature was controlled with small ice bags) in hexane (Hex), ethyl acetate (EtOAc), ethanol (EtOH), and water (H2O). The extraction process with each of the solvents was exhaustive (3×20 mL). The successive extracts were concentrated in a rotary evaporator under reduced pressure at 40 °C. The extract obtained with each solvent was weighed, and its yield extraction percentage was calculated. The process was performed two times with three replicates. The average and standard deviation (SD) are reported. The yield was calculated using equation 1.

$$yield\ (\%) = \frac{w_i}{w_{agave}} x100 \qquad \text{Eq. 1}$$

Where w_i corresponds to the total mass obtained from three successive extractions with each solvent i (Hex, EtOAc, EtOH, H2O) and w_{agave} is the mass of dried agave leaves. The samples were transferred to an amber vial and stored at 4 °C until their use.

Bioautography screening for α-glucosidase inhibitory activity of *A. potatorum* leaves extracts

The detection of inhibitory activity of α-glucosidase was performed according to Simões-Pires *et al*. [14] with slight modifications. Ten units of α-glucosidase were dissolved in 4.0 mL of sodium acetate buffer solution. The buffer solution was prepared by dissolving 2.05 g of sodium acetate in 50 mL of distilled water, brought to pH 7.5 with the addition of 0.1 M acetic acid. Serial dilutions were prepared from fresh infusions to obtain 50, 100, 200 and 400 µg of solids at each spot for the bioautographic assay. The acarbose as positive control was assayed at the same quantities. Neither infusions nor acarbose were eluted. Each extract (100 µg) obtained with a sequential extraction process was loaded on the plate and the TLC was developed. The mobile phase was evaporated under a stream of cold air. Afterwards, the plate was sprayed with the enzyme solution and preincubated in a humid atmosphere in a plastic chamber at 37 °C for 30 min. Subsequently, the substrate solution, a 1:1 mixture of 2-naphthyl-α-D-glucopyranoside (2.0 mg/mL in absolute ethanol) and an aqueous solution of Fast Blue B salt (2.5 mg/mL) was sprayed. The enzyme cleaved the substrate producing α-naphthol, which reacts with Fast Blue B salt. After 5 min, the inhibitory spots appeared as white spots

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corresponding to a positive enzyme inhibition. Glucosidase inhibition was detected under white light, and the Rf values of the spots were determined. All experiments were performed in triplicate.

Assay of α-glucosidase inhibitory activity of *A. potatorum* leaves infusion

The percentage of inhibition was determined according to Yang et al. [15] with slight modifications. In a 96-well microplate, 50 µL of α-glucosidase (0.065 U/mL) solution with 50 µL of agave infusion at 2 mg/mL or acarbose (4, 2, 1, 0.5, 0.25 mg/mL) were preincubated for 15 min at 37 °C. Subsequently, 50 µL of 5 mM p-NPG substrate was added and then incubated to react for 60 min at 37 °C. The reaction was stopped with 50 µL of Na2CO3 solution (0.2 M). The absorbance was measured at 405 nm, the wavelength at which the formed p-nitrophenol absorbs, in a microplate reader. Enzyme and substrate solutions were diluted in phosphate buffer pH 7.0, Na2CO3 in water, acarbose, and infusions in buffer pH 7.0 with 2 % DMSO (dimethyl sulfoxide). The percentage of inhibition was determined using Equation 2.

$$\%\, inhibition = \left[1 - \frac{Abs_{sample} - Abs_{sample\ blank}}{Abs_{control} - Abs_{blank}}\right] * 100 \qquad \text{Eq. 2}$$

Where Abs corresponds to the absorbance in the wells, *Abs sample* to the well containing test sample, enzyme solution, substrate solution and stop solution, *Abs sample blank* to the sample blank, where buffer solution was added in place of enzyme solution; *Abs control* to the control, where buffer solution with 2 % DMSO without extract or positive control was added in place of the sample; and *Abs blank* to the blank, which contains buffer solution and buffer solution with 2 % DMSO instead of enzyme and sample solution. Each concentration was
assayed in triplicate and the results are expressed as mean value and its SD.

Phytochemical screening of crude extracts obtained by increasing polarity of *A. potatorum* leaves

The presence of phytoconstituents groups was tried to be detected in *A. potatorum* leaves infusion, however, due to the low concentration they were not detected. Therefore, the crude extracts, obtained by increasing polarity of *A. potatorum* leaves, were assayed using the methods described by Wagner and Bladt [16] and Bailey and Bourne [17]. All crude extracts were eluted on TLC plates with the specified solvents (Hex and EtOAc extracts were eluted in 85 Hex:15 EtOA; and EtOH and H2O extracts in 50 EtOAc:50 EtOH). On the eluted plates, the presence of triterpenes/steroids [Liebermann-Burchard reagents (LB), vanillin-1 % phosphoric acid (VP), vanillin-sulfuric acid (VS)], coumarins, anthrones and anthraquinones [10 % potassium hydroxide (KOH)], flavonoids, coumarins and phenolcarboxylic acids [Natural Products-Polyethylene glycol 4000 (NP-PEG)], tannins [iron III chloride (FeCl3)], phenolcarboxylic acids [Fast Blue B salt (FBB)] and sugars and fructans of short degree of polymerization (SDP) [(aniline-diphenylamine (AD)] were tested. Visualization was performed according to the methodology for each reagent. Results are reported as weak (+), moderate (++) and strong (+++), depending on the concentration of metabolites in each test.

Statistical analysis

All experiments were performed in triplicate. Means, standard errors, and standard deviations were calculated from replicates within the experiments. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test using InfoStat software. Statistical significance of differences was accepted at $p<0.05$.

Results

Agave potatorum leaves extracts and TLC-Bioautography assay

A. potatorum leaves contained 11.1 ± 2.6 % of dry material, that is 88.9 % of water. The extraction percentage yields of infusion ranged from 24.5 to 42.3 %, the value for each year is in the supplementary

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material. In Table 1 the yields of sequential extraction are presented, and they are higher in EtOH and H_2O, suggesting that the secondary metabolites of polar structure are present in higher concentrations.

Table 1. Extraction yield and secondary metabolites in the extracts of *A. potatorum* leaves from plants of 7- and 8- year-old in the inhibition zone of α-glucosidase.

Extraction solvent	**Extraction yield (%) ± S.D.**		**Identified secondary metabolites**
	7 years	**8 years**	
Hex	2.49 ± 0.02	1.23 ± 0.03	Terpenes, saponins, steroids, phenylpropanoids (++), tannins (+), flavonoids, coumarins phenolcarboxilic acids, anthrones and anthraquinones (not conclusive*[1]).
EtOAc	1.39 ± 0.01	0.89 ± 0.03	Terpenes, saponins, steroids, phenylpropanoids (+++), tannins (++), coumarins, phenolcarboxilic acids (+), anthraquinones (not conclusive*[1]), D-fructose, D-glucose (++)*[2].
EtOH	1.47 ± 0.01	3.84 ± 0.04	Terpenes, saponins, steroids, phenylpropanoids (++), tannins (+), flavonoids, coumarins, glicosilated coumarins, phenolcarboxilic acids, (+), anthrones, anthraquinones (not conclusive*[1]), D-fructose, SDP-fructans (+++).
H_2O	11.78 ± 0.34	22.02 ± 0.35	Terpenes, saponins, steroids, phenylpropanoids (+), anthrones, anthraquinones (not conclusive*), D-fructose, SDP-fructans (+++).

*[1]The results were not conclusive since the eluted TLC plates showed the same color under UV light in these areas.
*[2]The sugar assay was positive for D-fructose and D-glucose because there are glycosylated secondary metabolites.

The TLC-bioassays of all infusion extracts showed α-glucosidase inhibitory activity (Fig. 1**(a)**). No inhibitory activity zones of the infusions were observed at the doses evaluated when these were eluted in the same system as the extract in H_2O, which could be due to the low concentration of active compounds in the infusion in comparison to the crude extracts (see Table 1). This behavior is similar to the inhibition by conduritol B epoxide [14], which presents inhibition of α-glucosidase at 0.1 µg, but a dose of 10 µg is necessary to observe the white zone on the TLC plate. Therefore, the inhibition of the infusion was evaluated without eluting the extract in the TLC plate (Fig. 1**(a)**).

The inhibitory activity assays of the crude extracts of 7- and 8-year-old *A. potatorum* leaves showed at least one active spot in each one (Fig. 1**(b)**). The extracts of 7- and 8-year-old leaves in Hex as well as in EtOAc showed similar enzyme inhibition zones, the highest at Rf=0.14-0.41 (Fig. 1**(b)**). Additionally, they showed other small inhibition zones, in Hex7 at Rf= 0.59, 0.80, and 0.97, in Hex8 at Rf= 0.51, in EtOAc7 and in EtOAc8 at Rf= 0.59-0.67, but the inhibition zone was more intense in the EtOAc8 than in EtOAc7. As for the EtOH (Fig. 1**(c)**) and H_2O extracts (Fig. 1**(d)**), in the EtOH one, there are two inhibition zones at Rf= 0.00-0.60 and 0.81-0.99, and in the H_2O extract, only one inhibition spot at Rf= 0.00-0.49 is clear. The main difference between 7- and 8-year-old leaf extracts is that the former showed higher inhibitory activity concerning that of the 8-year-old one.

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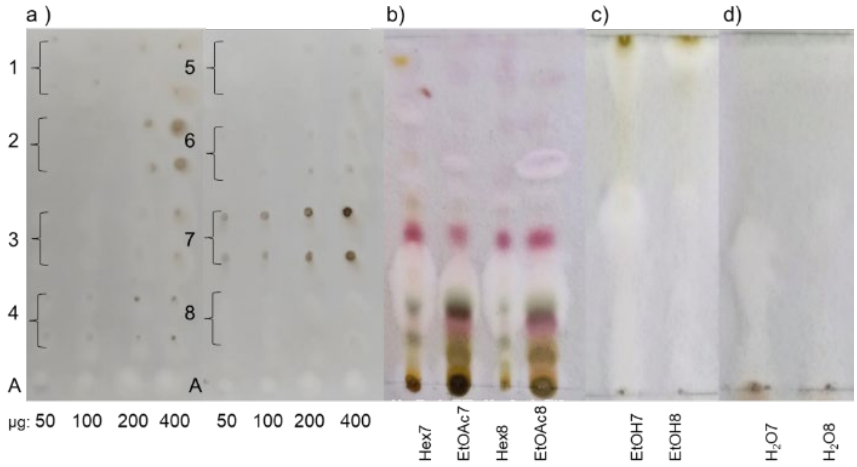

Fig. 1. Bioautographic inhibition of α-glucosidase by: **(a)** acarbose (A) and infusions of 1- to 8- year-old *A. potatorum* leaves at 50 to 400 µg, not eluted, **(b)** Hex and EtOAc (eluted in 85 Hex : 15 EtOAc), **(c)** EtOH and **(d)** H_2O (eluted in 50 EtOAc : 50 EtOH) crude extracts of 7- and 8- year-old *A. potatorum* leaves at 100 µg.

Phytochemical profile

To identify the families of secondary metabolites responsible of α-glucosidase inhibition, a phytochemical profiling was performed on the crude extracts in Hex, EtOAc, EtOH, and H_2O from the *A. potatorum* leaves 7 and 8 years old on TLC plates, following the methodology described by Wagner and Bladt [16].

The presence of terpenic compounds according to Liebermann-Buchard, the vanillin-sulfuric acid and the vanillin-phosphoric acid test (Supplementary Information) were detected as inhibitors in the crude extracts of Hex, EtOAc, EtOH, and H_2O. Additionally, the inhibition regions tested by Fast blue B salt, $FeCl_3$, NP-PEG, and KOH can be associated with phenolic compounds (Supplementary Information). The assays of anthrones and anthraquinones were not conclusive. The red-brown color obtained with fast blue B test in Hex and EtOAc at Rf=0.16-0.25 (active inhibition zone) and in the elution front of EtOH 7- and 8-year leaves extracts indicate that phenolcarboxylic acids, coumarins, tannins, or flavonoids could be responsible for α-glucosidase inhibition (Supplementary Material). The presence of tannins was confirmed by the $FeCl_3$ test, mainly in the Hex, EtOAc, and EtOH (only in the elution front) extracts of 7-year-old leaves (Supplementary Information). In the EtOH extract of 7- and 8-year-old leaves, the presence of coumarins or phenolcarboxylic acids was observed in phosphorescent blue (Supplementary Information), and a slight presence of flavonoids, glycosylated coumarins or anthrones in the elution front in the 7-year-old extract, revealed by the natural products test. This same test indicates that coumarins or phenolcarboxylic acids are found in the 7- and 8- year-old leaves EtOAc extract (Rf=0.15). Anthraquinones, which have a characteristic red fluorescence at 365 nm by the KOH test, were overlapped in the inhibition zone of the Hex, EtOAc, and EtOH extracts with secondary metabolites that are seen red at 365 nm. In the 7- and 8-year leaves EtOH extract, a slight presence of coumarins was observed in blue by the KOH test, confirming the result obtained with the natural products reagent.

The results of the sugar test (diphenylamine-aniline) were positive for D-fructose in the EtOH and H_2O extracts with greater red-brown intensity compared to the Hex and EtOAc extracts, which were also positive, but with less intensity. The positive areas in the EtOH and H_2O extracts could be associated with the presence of D-fructose and D-glucose, previously reported in the *A. potatorum* leaves extracts [18]. The presence of sucrose is not observed with this test, which has not been reported in the agave genus.

Inhibition percentage of α-glucosidase by active extracts

To compare the inhibitory activity on α-glucosidase of *A. potatorum* leaves infusions of different ages concerning acarbose, the inhibitory activity was determined, and the results are shown in Fig. 2. Fig.2**(b)**

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shows that there is no significant difference in the inhibition capacity of leaf infusions of 2, 4, 5, 7, and 8 years old, which present an inhibition percentage similar to that of acarbose (65.08 ± 6.80 %, Fig. 2**(a)**) at the same concentration. However, it should be noted that acarbose is a competitive inhibitor, while in the infusions there may be a complex mixture of competitive and non-competitive inhibitors.

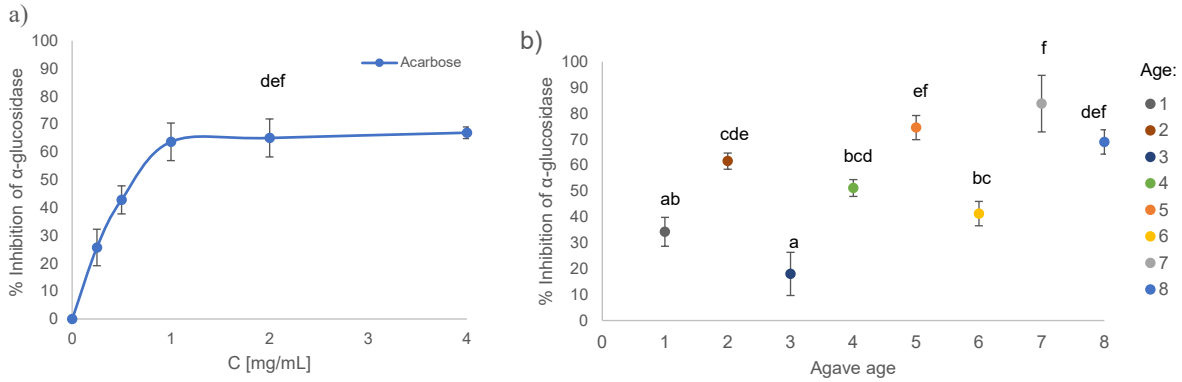

Fig. 2. Graphs of α-glucosidase inhibition, **(a)** acarbose at 4, 2, 1, 0.5, 0.25 mg/mL and **(b)** *A. potatorum* leaves infusions of different ages at 2 mg/mL. Different letters in the graphic indicate a significant difference ($p<0.05$, Tukey).

Discussion

The *A. potatorum* leaves can be a source of secondary metabolites, however, to increase the yield extraction in a non-polar solvent and the stability of these metabolites is recommended to remove water, since it represents more than 80 % of weight, and specifically the values of water percentage in the *A. potatorum* leaves agree with previously reported [18]. These results of extraction percentages suggest a higher content of polar metabolites which were extracted with EtOH and H_2O solvents and identified in the phytochemical profile.

The TLC-bioautography assays evidenced that *A. potatorum* leaves regardless of age have secondary metabolites with the potential to act as α-glucosidase inhibitors. All infusion extracts from *Agave* leaves showed α-glucosidase inhibitory spots from 100 µg onwards, with no age-dependent tendency. Although the inhibition zones of the 7-year-old leaves infusions were not well observed due to the oxidation and overlapping of compounds, when they were separated in the Hex, AcOEt, EtOH, and H_2O crude extracts and they were eluted, a higher inhibitory activity concerning the 8-year-old leaves was observed. These results showed that indeed, as reported about its ethnomedicinal use [10,11], secondary metabolites capable of inhibiting α-glucosidase in a direct dose-response relationship are present in the infusion of *A. potatorum* of all ages evaluated.

To unveiling the secondary metabolites responsible of the antidiabetic potential of *A. potatorum*, a phytochemical profiling was performed on the crude extracts in Hex, EtOAc, EtOH, and H_2O from the *A. potatorum* leaves 7 and 8 years old on TLC plates, following the methodology described by Wagner and Bladt [16] and contrasted with the α-glucosidase inhibitory zones. The compounds inhibiting the α-glucosidase enzyme, present in the zones of inhibition, correspond to terpenic compounds and may be triterpenic (saponins, steroids) or short-chain (phenylpropanoids) in nature [16], phenolic compounds as phenolcarboxylic acids, coumarins, tannins, or flavonoids. These results agree with previous reports of antidiabetic activity of secondary metabolites present in *Agave*. For example, the inhibitory activity of α-amylase [19,20] has been evaluated by the compounds apigenin, puerarin and p-coumaric acid, present in *A. americana*; those compounds are classified as a flavonoid, an isoflavonoid, and a phenolcarboxylic acid, respectively. Likewise, extracts of *A. cupreata* [21], a species morphologically and genetically similar to *A. potatorum*, have demonstrated anti-inflammatory and antibacterial activity.

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Considering the capacity of the infusions of *A. potatorum* leaves to inhibit α-glucosidase at a concentration of 2 mg/mL; their ingestion could minimize postprandial hyperglycemia by decreasing the absorption of sugars in the intestine. The inhibitory activity would be independent of age since there is no correlation between the percentage of inhibition of the enzyme and the age of the agave plant, and due to no significant difference in the inhibition capacity of leaves infusions of 2, 4, 5, 7 and 8 years-old that of acarbose at the same concentration, except for plants of one and three years old. Therefore, the leaves, an industrial waste in the mezcal production, could be revalued as a source of inhibitors of α-glucosidase.

In general, the wide range of secondary metabolite families that inhibit α-glucosidase from *A. potatorum* extracts explains that such inhibition can occur competitively and non-competitively, as has been described for other natural compounds such as flavonoids [22]. However, it is of interest to evaluate whether the secondary metabolites present in the leaves also can act as stimulators for insulin secretion, which would result in a dual system for the treatment of T2DM.

Conclusions

The results of this first approach to unveiling the antidiabetic potential confirmed the α-glucosidase inhibitory potential of *A. potatorum* leaves infusions, validating its traditional medicinal use in T2DM treatment. The results of bioautography showed a dose-dependent α-glucosidase inhibition reaction of the leaves infusions regardless of the age of the plant. The TLC-phytochemical screening of the active spots in the extracts indicates that the responsible secondary metabolites of the α-glucosidase inhibition correspond to terpenes, steroids, flavonoids, coumarins, tannins and phenol carboxylic acids. Although the bioautographic inhibition results are encouraging, it is still necessary to isolate and deepen the structure of the metabolite, the mechanism, percentage and concentration of inhibition, stability of the bioactive compounds, *in vivo* and *in vitro* toxicity tests to determine their toxicity.

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