Antidiabetic potential of extracts from Cylindropuntia imbricata (Haw.) F.M. Knuth, Opuntia engelmannii Salm-Dyck ex Engelm., Ibervillea sonorae (S. Wats.) Greene and Theobroma cacao L.

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ABSTRACT
Diabetes mellitus is a disease that affects more than 537 million people in the world without decreasing. When diabetes becomes complicated, it damages several organs until it causes death. The drugs in use to counteract the disease produce side effects; a circumstance that has led to research on plants with anti-diabetic properties. The objective of this study was to evaluate the antidiabetic potential of extracts obtained by maceration of the following parts of the plant: Cylindropuntia imbricata (Cactaceae) cladode and seed, Opuntia engelmannii (Cactaceae) cladode and seed, Ibervillea sonorae (Cucurbitaceae) root and Theobroma cacao (Malvaceae) seed mixed with solvents of different polarity (hexane, ethyl acetate, dichloromethane and methanol). A total of 24 extracts were obtained and subjected to the following analyses: 1) phytochemical screening to determine their composition, 2) toxicity in B16F10 cells using the alamar blue test, 3) antioxidant capacity through DPPH inhibition, and 4) in vitro evaluation to determine their antihyperglycemic capacity (inhibition of alpha-glucosidase). The results obtained from methanolic extracts with O. engelmannii and T. cacao seeds, as well as ethyl acetate extracts with T. cacao and C. imbricata seeds showed antioxidant and antihyperglycemic activity. No toxicity in B16F10 cells, and antidiabetic potential in vitro.

Keywords: diabetes, plant extracts, α-glucosidase, DPPH, alamar blue, phytochemistry.

Potencial antidiabético de extractos de Cylindropuntia imbricata (Haw.) F. M. Knuth, Opuntia engelmannii Salm-Dyck ex Engelm., Ibervillea sonorae (S. Wats.) Greene y Theobroma cacao L.

RESUMEN
La diabetes mellitus es una enfermedad que no decrece y afecta a más de 537 millones de personas en el mundo. La diabetes al complicarse daña varios órganos hasta causar la muerte. Los fármacos en uso para contrarrestar la enfermedad producen efectos secundarios; circunstancia que ha propiciado la investigación en plantas con propiedades antidiabéticas. El objetivo de este estudio fue evaluar el potencial antidiabético de los extractos obtenidos por maceración de las siguientes partes de la planta: del cladodio y semilla de Cylindropuntia imbricata (Cactaceae), del cladodio y semilla de Opuntia engelmannii (Cactaceae), de la raíz de Ibervillea sonorae (Cucurbitaceae) y de la semilla de Theobroma cacao (Malvaceae) mezcladas con solventes de diferente polaridad (hexano, acetato de etilo, dicrorometano y metanol). Se obtuvieron un total de 24 extractos sometidos a los análisis: 1) de cribado fitoquímico para determinar su composición, 2) de toxicidad en células B16F10 mediante la prueba de azul alamar, 3) de capacidad antioxidante a través de la inhibición de DPPH, y 4) de evaluación in vitro para conocer su capacidad antihiperglucémica (inhibición de la alfa glucosidasa). Los resultados obtenidos, de los extractos metánolos con las semillas de O. engelmannii y T. cacao, así como los de acetato de etilo con las semillas de T. cacao y C. imbricata mostraron una actividad antioxidante y antihiperglucémica. Sin toxicidad en las células B16F10, y con potencial antidiabético in vitro.

Palabras clave: diabetes, extractos vegetales, α-glucosidasa, DPPH, azul alamar, fitoquímica.
INTRODUCTION

Diabetes mellitus (DM) is a non-communicable disease that affects more than 537 million people in the world (International Diabetes Federation, 2021). This number is increasing in rural and poor populations worldwide and it is estimated that it will become one of the main causes of disability in the next two decades, in addition to being considered one of the main causes of death in the world (Dogan, Celik & Kaya, 2015). This metabolic disease is characterized by persistent hyperglycemia due to the lack of insulin production by the pancreas or the inability of secreted insulin to control blood glucose levels (Bustos-Brito, Andrade-Cetto, Giraldo-Aguirre, Moreno-Vargas & Quijano, 2016; Sulyman, Akolade, Sabiu, Aladodo & Muritala, 2016).

There are two main types of diabetes, insulin-dependent diabetes mellitus (IDDM, type 1 diabetes) and non-insulin dependent diabetes mellitus (NIDDM, type 2 diabetes). More than 90% of patients suffer NIDDM, in which insulin resistance plays a key role in the appearance and development of the disease (He, Deng, Shi, Zhang & Lü, 2014).

Long-term complications of diabetes include retinopathy with the possible loss of sight; nephropathy that leads to renal insufficiency; peripheral neuropathy with the risk of ulceration, amputation, and Charcot foot; and autonomic neuropathy that causes gastrointestinal, genitourinary, and cardiovascular problems (Bustos-Brito et al., 2016). Prolonged hyperglycemia can lead to organ failure, and finally death (Dogan et al., 2015). For this reason, early diagnosis is important for adequate and effective treatment of patients with diabetes mellitus.

There is also increasing evidence that excessive production of highly reactive free radicals, mostly generated by hyperglycemia, cause oxidative stress, which increases the progression of diabetes and its long-term complications (He et al., 2014).

Strict control of postprandial glycemia can prevent and delay the development of long-term complications. The enzyme, α-glucosidase, is responsible for the final stage of digestion of starch and glycogen in the diet; therefore, α-glucosidase inhibitors as well as components of functional foods are possible pharmacological candidates for delaying the postprandial absorption of glucose (Liu, Kongstad, Wiese, Jäger & Staerk, 2016).

There are currently diverse drugs on the market for the treatment of diabetes mellitus, such as glycoside hydrolase inhibitors, biguanides, sulfonylureas and thiazolidinediones. However, these synthetic drugs produce undesirable side effects such as nausea, flatulence, vomiting, anorexia, diarrhea, hepatotoxicity and weight gain, among others (Fowler, 2007; Sulyman et al., 2016). An alternative or coadjuvant of these pharmacological treatments that have shown effectiveness are medicinal plants with antidiabetic activity. These are more frequently used by patients with diabetes and are recommended by health professionals (He et al., 2014).

In this sense, in recent years, research has been carried out that focuses on the identification of plants that have molecules with antidiabetic activity. Some of the novel bioactive compounds isolated from plants have been shown to have similar or greater antidiabetic activity than drugs used in traditional medicine; for example, roseoside, epigallocatechin gallate or beta-pyrazol-1-ylalanine (Patel, Prasad, Kumar & Hemalatha, 2012).

Mexican flora includes species used in traditional medicine as antidiabetics. For example, C. imbricata and O. engelmannii are plants that are used to treat diabetes in Mexican traditional medicine; however, there are no studies that support their use as antidiabetic plants. The literature reports studies in other species of the genus Opuntia, where molecules with antidiabetic effects have been found, as in the case of O. dillenii, which presents antihyperglycemic and antioxidant activity in rats with diabetes induced by streptozotocin (STZ) (Zhao, Lan, Huang, Ouyang & Zeng, 2011). Another study, of Opuntia streptacantha reported an antihyperglycemic effect similar to acarbose in rats with diabetes induced by STZ, although its mechanism of action was due α-glucosidase inhibition (Becerra-Jiménez & Andrade-Cetto, 2012).

Another plant used in traditional medicine is the wereke (I. sonorae), whose aqueous and dichloromethane extracts have a hypoglycemic effect when injected in rats with diabetes induced with alloxan (Alarcón-Aguilar, Calzada-Bermejo, Hernandez-Galicia, Ruiz-Angeles & Roman-Ramos, 2005; Sinagawa-García, Gutiérrez-Diez, Mora-Olivo, Juárez-Aragón & Torres-Castillo, 2015). Another study reported that the aqueous extract of I. sonorae induces the consumption of glucose in 3T3 L1 adipose cells (Zapata-Bustos, Alonso-Castro, Gómez-Sánchez & Salazar-Olivo, 2014). Research carried out with Theobroma cacao has shown that the extract of toasted and fermented seeds has a hypoglycemic and hypocholesterolemic effect in rats with diabetes induced by STZ, although its mechanism of action was due α-glucosidase inhibition (Becerra-Jiménez & Andrade-Cetto, 2012).
The basis for the search for natural products and their separation using different solvents with different polarity is important for the extraction of simple compounds from plant extracts (Su, Zeng, Chen, Chen, Guo & Huang, 2012), thus, by gradually increasing the polarity of the solvent in a sequential extraction, adequate separation of the compounds can be achieved. There are studies where the biological activity of medicinal plant extracts has been found by separating the groups of compounds extracted using different types of solvents (Ncube, Finnie & Van Staden, 2012).

Taking into consideration the aforementioned, this work aims to provide information on the in vitro antidiabetic potential of hexanic, dichloromethane, ethyl acetate and methanolic extracts of C. imbricata, I. sonorae, T. cacao and O. engelmannii, under the presumption that extracts obtained with an increasing polarity gradient will have a greater wealth of molecules that are potentially useful for the treatment of diabetes.

MATERIALS AND METHODS

Acquisition of plant extracts

Wild plants were collected in populations of Mexico; these were then dried, pulverized, and packaged for storage. To obtain the extracts, 1 kg of plant material was macerated consecutively for one week in each of the following solvents of increasing polarity, hexane (H), dichloromethane (D), ethyl acetate (A) and methanol (M). During this time, the samples were maintained in constant mixing at room temperature. Finally, the extract was filtered, dried in a rotavapor (Buchii), and weighed; its yield was determined with the following equation:

\[
\% \text{ yield} = \frac{\text{weight obtained}}{\text{initial weight}} \times 100
\]

Where:

Weight obtained = weight of plant material obtained after extraction
Initial weight = weight of plant material before extraction

Phytochemical screening

Samples of crude plant extracts were dissolved in different solvents (DMSO= dimethyl sulfoxide, ethanol or methanol). The qualitative phytochemical analysis of alkaloids, carbonyls, flavonoids, phenolic compounds, saponins, steroids, sesquiterpene lactones, tannins and carbohydrates was performed according to Verde-Star, García-González & Rivas-Morales (2016) and Kuppusamy, Yusoff, Parine & Govindan (2015).

Viability of B16F10 cells

Cell culture B16F10 rat melanoma cells were used to study the toxic effect of the plant extracts. Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum in cell culture flasks. Cells were subcultured in fresh medium three times a week and incubated at 37 °C in an atmosphere of 95% air and 5% CO₂.

Evaluation of cell viability

The analysis was carried out according to the methods of Arunachalam et al. (2016) and Kakde et al. (2016). The lyophilized compounds were resuspended in 1% DMSO and in saline phosphate buffer using a sonicator. B16F10 cells were cultured in 96-well plates (approximately 10,000 cells per well) and incubated for 24 hours at 37 °C in 5% CO₂. Treatments and the control (1% DMSO and saline phosphate buffer) were added, and the plates were incubated for 24 hours. Alamar blue was added, and the plates incubated for 4.5 h; plates were read with an ELISA reader with an excitation/emission wavelength of 530 nm and 590 nm, respectively. The experiment was performed in triplicate.

Determination of antioxidant activity using the DPPH method

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a radical that has an unpaired electron that has a blue-violet color that turns to pale yellow when it reacts with an antioxidant (Ramos-Llica, Castañeda-Castañeda & Ibáñez-Vásquez, 2008). The assay was performed according to Mokrani & Madani (2016); for this, the compounds were dissolved in their corresponding vehicles (DMSO, ethanol o methanol). DPPH was prepared in the dark at a concentration of 28 mg/L of methanol. Two milliliters were placed in a test tube and was read in a spectrophotometer at 517 nm with an absorbance range of 0.730 to 0.770. An aliquot of 50 µL of each of the extracts was taken and 1950 µL of DPPH solution was added and mixed in a vortex. Twenty milliliters were incubated in the dark and read in a spectrophotometer at 517 nm. Vitamin E was used as a positive control.

The percentage of radical-scavenging activity of DPPH or the percentage of inhibition was obtained with the following equation:

\[
\text{Percentage inhibition} = \frac{A0 - A1}{A0} \times 100
\]

α-glucosidase inhibition

The assay was performed according to the method proposed by Gu et al. (2011) and Ortíz-Martínez, Cordero-Pérez & Leos-Rivas (2016). The lyophilized compounds were resuspended in 1% DMSO and in saline phosphate buffer using a sonicator. In a 96-well plate, 25 µL of treatments were placed with 25 µL of the enzyme (0.1 unit per mL) and this was incubated for 15 min at 37 °C (treatment time). A total of 50 µL of substrate
(5 mM) was added to the treatments and this was incubated for 10 minutes (reaction time). The reaction was stopped with 50 µl of Na₂CO₃ in blanks, 25 µl of treatments, 50 µl of Na₂CO₃, 25 µl of enzyme and 50 µl of substrate were added. Plates were read in an ELISA reader at 405 nm. Acarbose was used as a positive control. The inhibitory effect of α-glucosidase was calculated with the following equation:

\[
\text{% inhibition} = \left(\frac{\text{Abs Control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100
\]

IC₅₀ levels of the samples and acarbose were calculated using the results of the α-glucosidase inhibition assay.

**Statistical Analysis**

Data analysis for cell viability was performed by IBM SPSS Statistics 20.0. The results were analyzed statistically with a prior arcsine transformation of data using an ANOVA test (p ≤ 0.05) and Tukey’s mean comparison test (p ≤ 0.05) to determine the differences between treatments.

**Results**

**Phytochemical screening**

The results of the phytochemical characterization of the study plants were positive for triterpenes (H, D, A, M), saponins (M), coumarins (H, D, A, M), alkaloids (D, A, M), for *I. sonorae* root; flavonoids (D, M), triterpenes (H, D, A), saponins (M), coumarins (D, A, M) and quinones (M) for the cladode of *C. imbricata*; tannins (D, A, M) flavonoids (M), triterpenes (H) coumarins (H, D, A, M), for *C. imbricata* seeds, and tannins (A, M), flavonoids (D, M), triterpenes (H, A, M), saponins (M), quinones (D, A, M) for *T. cacao* seed.

**Viability in B16F10 cells**

The evaluation of the 24 extracts at a concentration of 200 µg/mL on the viability of B16F10 cells showed that 22 of the 24 extracts did not present significant toxicity at 200 µg/mL, being the ethyl acetate extract of *C. imbricata* seed the one that presented the lowest toxicity (13.01%), while the methanolic extract of *I. sonorae* had a high toxicity (94.47%).

To know the toxicity pattern of the methanolic extract of *I. Sonorae*, which presented toxicity at concentrations of 100 µg/mL and 200 µg/mL, this extract was evaluated at concentrations of 20, 40, 60, 80 and 100 µg/mL (Figure 1), observing a positive relationship where increasing the dose increased the toxic response, and the mean lethal dose (IC₅₀) was determined at 76.90 µg/mL.

**Antioxidant activity**

The antioxidant activity of the 24 extracts was evaluated using the DPPH method at a concentration of 200 µg/mL, and six extracts showed an activity greater than 50% at the concentration evaluated with four extracts being the most active, two from *O. engelmannii* (A, M) seed with 93.67% and 91.95%, respectively, one from *C. imbricata* seed (A) with 56.80%, and one from *T. cacao* seed (M) with 91.52% inhibition of DPPH (Figure 2).

![Figure 1. Effect of the methanolic extracts of *I. sonorae* at concentrations of 20, 40, 60, 80 and 100 µg/mL on the viability (percentage) of B16F10 cells. Means and standard deviation of treatments in triplicate experiments. Different literals indicate statistically significant differences between treatments (p≤0.05). ST, without treatment; DMSO, dimethylsulfoxide (C₂H₆OS); M, methanolic extract; *I. sonorae*; R, root.](image-url)
The extracts with the greatest activity were evaluated at a lower concentration of 50 µg/mL; these had an activity similar to vitamin E as the positive control (p≥0.05) with an activity of 93.87%. Of the six extracts evaluated, three were statistically similar (p≤0.05) to vitamin E; two were from *O. engelmannii* (A, M) seed and one from *T. cacao* (M). Table I shows the IC₅₀ values of the evaluated extracts; only *T. cacao* showed a theoretical IC₅₀ (5.96 µg/mL) lower than vitamin E (7.53 µg/mL). The extracts with the greatest activity were evaluated at a lower concentration of 50 µg/mL. These presented an activity similar to that of vitamin E, the positive control (p≥0.05), with an activity of 93.87% (Figure 3).

Figure 2. Antioxidant activity of 24 extracts using the DPPH method at a concentration of 200 µg/mL. Means and standard deviation of treatments in triplicated experiments. Different literals indicate statistically significant differences between treatments (p≤0.05). Positive control: Vitamin E. DMSO, dimethylsulfoxide (C₂H₆OS); H, hexanic extract, D, dichloromethane extract; A, ethyl acetate extract; M, methanolic extract; Oe, *Opuntia engelmannii*; Ci, *Cylindropuntia imbricata*; TC, *Theobroma cacao*; Is, *Ibervillea sonorae*; C, cladode; S seed; R, root.

Figure 3. Antioxidant activity of six extracts using the DPPH method at a concentration of 50 µg/mL. Means and standard deviation of treatments in triplicated experiments. Different literals indicate statistically significant differences between treatments (p≤0.05). Positive control: Vitamin E. DMSO, dimethylsulfoxide (C₂H₆OS); A, ethyl acetate extract; M, methanolic extract; Oe, *Opuntia engelmannii*; Ci, *Cylindropuntia imbricata*; TC, *Theobroma cacao*; Is, *Ibervillea sonorae*; C, cladode; S seed; R, root.
**α-glucosidase inhibition**

α-glucosidase inhibition was evaluated in the 24 extracts at a concentration of 200 µg/mL and enzyme inhibition was determined in the extracts with the greatest activity. Acarbose was used as a positive control with an inhibition of 18.77%. The extracts that showed inhibitory activity greater than the control was *O. engelmannii* (A, M) seed with 18.34 and 93.59%, respectively; *C. imbricata* cladode (H) with 94.57%; *C. imbricata* (A) seed with 38.45% and *T. cacao* (A, M) seed with 95.79 and 95.92%, respectively. The six extracts had a theoretical IC$_{50}$ lower than acarbose; one of the drugs used for the treatment of type 2 diabetes mellitus. Table II shows the calculated IC$_{50}$ in which the extracts had a result lower than acarbose.

**DISCUSSION**

Flavonoids, which are a subgroup of bioactive compounds, were found during the phytochemical screening of the methanolic extract of the cladode of *C. imbricata*. Lee, Kim, Kim & Jang (2002), found that the ethanolic extracts of the cladode of *Opuntia ficus-indica* var. Saboten had a large quantity of phenols and attributed its antioxidant activity to flavonoids, which could have a therapeutic action, as in this case, inhibiting α-glucosidase and DPPH. Flavonoids have been studied as the possible structures responsible for these effects. In this regard, Yamashita, Okabe, Natsume & Ashida (2012), found that the liquor of *T. cacao* showed this activity, it could be that the same type of molecules is present; however, the studied extract was obtained by continuous extraction from *T. cacao* seed with no prior alteration, which makes it different from the previously studied liquor. In phytochemical tests, this extract showed the presence of molecules from the flavonoid family, while extracts with lower polarity did not show activity that inhibits DPPH.

Hatano *et al.* (2002), showed that in the purified polar fractions of *T. cacao* liquor extracts there were proanthocyanadin glycosides and related phenolic compounds where 11 from a total of 17 compounds showed antioxidant activity when inhibiting DPPH. Because the methanolic extract of *T. cacao* showed this activity, it could be that the same type of molecules is present; however, the studied extract was obtained by continuous extraction from *T. cacao* seed with no prior alteration, which makes it different from the previously studied liquor. In phytochemical tests, this extract showed the presence of molecules from the flavonoid family, while extracts with lower polarity did not show activity that inhibits DPPH.

With regard to the genus *Opuntia*, Bayar, Kriaa & Kammoun (2016), showed this activity in three polysaccharides extracted from the cladode of *Opuntia ficus-indica*, which were DPPH inhibitors and therefore, had antioxidant properties; this genus normally has molecules with this activity. In our study, the ethyl acetate and methanolic extracts of *O. engelmannii* seed were active.

The ethyl acetate and methanolic extracts of *T. cacao* seed showed inhibitory activity on α-glucosidase, demonstrating that these extracts have molecules with an antihyperglycemic effect. In this regard, Yamashita, Okabe, Natsume & Ashida (2012), found that the liquor of *T. cacao* contained molecules responsible for the inhibitory effect of α-glucosidase; these molecules were procyanidins. Therefore, it is possible that molecules similar to those found in cocoa liquor are present in unfermented *T. cacao* seed, and these were extracted with more polar solvents (A, M). Phytochemical tests showed that compounds such as flavonoids form the procyanidins that are present in these extracts.

Similarly, the ethyl acetate extracts of *O. engelmannii* and *C. imbricata* seeds, as well as the methanolic extract of *O. engelmannii* seed, showed an inhibitory effect of α-glucosidase, with more polar extracts being those that showed flavonoids, which could have a therapeutic action, as in this case, inhibiting α-glucosidase and DPPH. Flavonoids have been studied as the possible structures responsible for these activities.

**Table I. Theoretical 50% inhibitory concentration (IC$_{50}$) in µg/mL of the antioxidant activity of the most active extracts and vitamin E.**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Part used/extract</th>
<th>IC$_{50}$ µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. imbricata</em></td>
<td>Cladodium/Methanolic</td>
<td>176.49</td>
</tr>
<tr>
<td><em>C. imbricata</em></td>
<td>Seed/Ethyl acetate</td>
<td>43.05</td>
</tr>
<tr>
<td><em>O. engelmannii</em></td>
<td>Seed/Ethyl acetate</td>
<td>18.71</td>
</tr>
<tr>
<td><em>O. engelmannii</em></td>
<td>Seed/Methanolic</td>
<td>15.77</td>
</tr>
<tr>
<td><em>I. sonorae</em></td>
<td>Root/Ethyl acetate</td>
<td>50.65</td>
</tr>
<tr>
<td><em>T. cacao</em></td>
<td>Seed/Methanolic</td>
<td>5.96</td>
</tr>
<tr>
<td>E Vitamin</td>
<td>Control</td>
<td>7.53</td>
</tr>
</tbody>
</table>

**Table II. 50% Inhibitory concentration in µg/mL (IC$_{50}$) of the most active plants extracts under study and acarbose in the inhibition of α-glucosidase.**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Part used/extract</th>
<th>IC$_{50}$ µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. imbricata</em></td>
<td>Cladodium/Hexanic</td>
<td>55.75</td>
</tr>
<tr>
<td><em>C. imbricata</em></td>
<td>Seed/Ethyl acetate</td>
<td>222.09</td>
</tr>
<tr>
<td><em>O. engelmannii</em></td>
<td>Seed/Ethyl acetate</td>
<td>372.77</td>
</tr>
<tr>
<td><em>O. engelmannii</em></td>
<td>Seed/Methanolic</td>
<td>76.81</td>
</tr>
<tr>
<td><em>T. cacao</em></td>
<td>Seed/Ethyl acetate</td>
<td>85.17</td>
</tr>
<tr>
<td><em>T. cacao</em></td>
<td>Seed/Methanolic</td>
<td>18.40</td>
</tr>
<tr>
<td>Acarbose</td>
<td>Control</td>
<td>843.20</td>
</tr>
</tbody>
</table>
therapeutic effects (Di Carlo, Mascolo, Izzo & Capasso, 1999). Therefore, it is recommended to isolate the active compounds of these extracts to verify if flavonoids are responsible for the antidiabetic effect. On the other hand, the hexanic extract of the cladode of *C. imbricata* presented inhibitory activity against α-glucosidase but did not show the presence of flavonoids, and it is a nonpolar extract; therefore, its antidiabetic effect could be related to other types of molecules.

In the *in vitro* antihyperglycemic activity (α-glucosidase inhibition), the ethyl acetate and methanolic extracts of the cladodes of *O. engelmannii* and *C. imbricata* did not inhibit this enzyme. This effect is similar to the results obtained by Becerra-Jiménez & Andrade-Cetto (2012), who report that the aqueous extracts of *O. streptacantha* did not present relevant inhibitory activity on this enzyme. However, it is important to point out that the authors mentioned that they did not obtain the nonpolar extracts of the cladode. In this regard, in this study, it was found that the hexanic extract of the cladode of *C. imbricata* showed an inhibitory effect on α-glucosidase. Furthermore, when the inhibitory effect of acarbose (positive control) was compared, the extract showed a greater effect when both were tested at 200 µg/mL, with an IC₅₀ of 56 µg/mL; this was less than acarbose with an IC₅₀ of 843.20 µg/mL and it was not toxic to B16F10 cells at 200 µg/mL.

On the other hand, Ennouri, Fetoui, Bourret, Zeghal, Guermazi & Attia (2006), performed an *in vivo* study with *Opuntia ficus-indica* seed in Wistar rats, in which a reduction in serum glucose occurred. The seed was administered in a continuous diet for 63 days, demonstrating that it is not toxic. Similar to this result, in the *in vitro* experiment we performed, we found that the ethyl acetate extract of *C. imbricata* seed and the methanolic extract of *O. engelmannii* seed showed an antidiabetic effect by inhibiting α-glucosidase with an IC₅₀ of 222 µg/mL and 77 µg/mL, respectively, with no toxicity in the cell line at 200 µg/mL. To prove the antidiabetic effect of *C. imbricata* and *O. engelmannii* seeds in the *in vitro* tests, we plan to continue research and perform *in vivo* tests.

Additionally, in the *in vitro* tests performed, it was found that the ethyl acetate and methanolic extracts of *T. cacao* seed inhibit α-glucosidase more effectively than acarbose, with an IC₅₀ of 85 µg/mL and 20 µg/mL, respectively. Particularly, the methanolic extract of *T. cacao* seed was the most effective in inhibiting the enzyme. In this extract, a significant antioxidant activity similar to that of vitamin E; thus, it had a dual activity in *in vitro* tests and did not present toxicity on B16F10 cells. This makes it a promising extract for future *in vivo* studies of its antihyperglycemic effect. The above is consistent with Ruzaidi *et al.* (2005) and Bellesia & Tagliazucchi (2014) who report hypoglycemic effect in fermented *T. cacao* and cocoa drink, respectively.

In the B16F10 cell viability results obtained from the studied plants, 22 of the 24 extracts did not show significant toxicity at 200 µg/mL; the extract that was most toxic was the methanolic extract of *I. sonorae* root with 6.01% viability.

Regarding the inhibition of α-glucosidase, the ethyl acetate extracts of *C. imbricata* and *O. engelmannii* seeds showed an IC₅₀ of 222 µg/mL and 373 µg/mL, respectively, and both extracts presented antioxidant activity with an IC₅₀ of 45 µg/mL and 18 µg/mL, respectively. Furthermore, the ethyl acetate extract of *C. imbricata* seeds showed low toxicity at a concentration of 200 µg/mL in B16F10 cells. The above seems to indicate that at least the ethyl acetate extract of *C. imbricata* seeds is safe and effective at this concentration as an inhibitor of α-glucosidase and DPPH, and without significant toxic effects.

On the other hand, the methanolic extract of *O. engelmannii* showed an IC₅₀ of 77 µg/mL as a α-glucosidase inhibitor and an IC₅₀ of 16 µg/mL as a DPPH inhibitor making the methanolic extract more effective and therefore, making it advisable to investigate its effect *in vivo* in the future.

**Conclusions**

In this study, four of the 24 extracts tested had antioxidant and antihyperglycemic activity, the methanolic extracts of *O. engelmannii* and *T. cacao* seeds and the ethyl acetate extracts of *T. cacao* and *C. imbricata* seeds. These extracts did not present toxicity on B16F10 cells. This is the first time that the activities of the ethyl acetate and methanolic extracts of unprocessed *T. cacao* seed and the hexanic extract of the cladode and the ethyl acetate extract of the seed of *C. imbricata* are reported. The extracts obtained with more polar solvents showed significant biological activity with an *in vitro* antidiabetic potential; therefore, they should be considered for future *in vivo* studies of diabetes control.

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**References**


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