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Original Article

I. sonorae, *C. limetta*, and *B. media* phytoextracts and their antidiabetic potential

Fitoextractos de *I. sonorae*, *C. limetta* y *B. media* y su potencial antidiabético

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ABSTRACT

In Mexican traditional medicine, several herbs are used for their potential antidiabetic effects. This study aimed to investigate the mechanisms underlying the antihyperglycemic effects of *I. sonorae*, *C. limetta*, and *B. media*. Aqueous extracts of these herbs demonstrated a consistent reduction in post-prandial blood glucose levels, in healthy rats during a starch oral tolerance test. Notably, *B. media* and *I. sonorae* exhibited significant *in vitro* inhibitory effects against alpha-amylase activity (20.5 and 25.4 %, respectively), while *B. media* enhanced glucose uptake in adipocytes by 4.0-fold, which was related to the overexpression of key genes involved in insulin signaling cascade including *Glut4*, *Irs1*, and *Pi3k* (2.9-, 2.6-, and 3.2-fold, respectively). Furthermore, multivariate analysis highlighted that hydroxybenzoic acid hexoside and feruloylquinic acid were linked to their alpha-amylase inhibitory activity, while 17 distinct polyphenols were associated with the insulin mimetic activity. These findings propose a potential application of these herbs in the development of functional beverages with promising anti-diabetic attributes.

Keywords: Herbs; traditional medicine; adipocytes; glucose uptake; digestive enzymes; polyphenols.

RESUMEN

En la medicina tradicional mexicana, diferentes hierbas son utilizadas por su potencial efecto antidiabético. Este estudio tuvo como objetivo investigar los mecanismos subyacentes a los efectos antihiperglucémicos de *I. sonorae*, *C. limetta* y *B. media*. Los extractos acuosos de estas hierbas demostraron una consistente reducción en los niveles postprandiales de glucosa en sangre durante una prueba de tolerancia oral al almidón. Destacando que, los extractos de *B. media* e *I. sonorae* mostraron un efecto inhibitorio significativo *in vitro* contra la actividad de alfa-amilasa (20.5 y 25.4 %, respectivamente), mientras que *B. media* aumentó 4.0 veces la internalización de glucosa en adipocitos, lo cual fue relacionado con la sobreexpresión de genes claves involucrados en la cascada de señalización de la insulina incluyendo *Glut4, Irs1* y *Pi3k* (2.9, 2.6 y 3.2 veces, respectivamente). Además, el análisis multivariado resaltó que el ácido hidroxibenzoico hexódio y el ácido feruloilquínico fueron asociado con la actividad inhibitoria de alfa-amilasa, mientras que 17 polifenoles fueron asociados con la actividad mimética a la insulina. Estos hallazgos proponen la aplicación potencial de estas hierbas en el desarrollo de bebidas funcionales con prometedoras propiedades antidiabéticas.

Palabras clave: Hierbas; medicina tradicional; adipocitos; internalización de glucosa; enzimas digestivas; polifenoles.

INTRODUCTION

Type 2 diabetes (T2D) is a prevalent metabolic disorder, affecting over 537 million people globally in 2021 according to the International Diabetes Federation (IDF). Hyperglycemia arises due to impaired carbohydrate digestion, glucose uptake, and insulin signaling. Herbal extracts, rich in polyphenolic compounds, offer potential for managing hyperglycemia by inhibiting carbohydrate-digesting enzymes and improving insulin sensitivity (Tran *et al*., 2020).

According to Mexican traditional medicine, *Citrus limetta* roots, *Ibervillea sonorae* roots, and *Briza media* leaves are consumed for diabetes control. Several scientific reports have previously demonstrated their hypoglycemic potential. A methanol extract of *C. limetta* fruit peel reduced blood glucose levels in diabetic rats, which was associated with its polyphenolic composition and antioxidant capacity (Flores-Fernández *et al*., 2017). The juice and aqueous extract of *I. sonorae* roots decreased blood glucose in healthy mice, and its dichloromethane extract showed a similar effect in alloxan diabetic mice (Alarcón-Aguilar *et al*., 2005). Similarly, glucose levels were reduced when rats were fed with a high fat and fructose diet and were treated with aqueous extract of *I. sonorae* roots, which was partly associated with its flavonoid content (Rivera-Ramírez *et al*., 2011). Zapata-Bustos *et al*. (2014) reported that an aqueous extract of *I. sonorae* roots induces glucose uptake in insulin-sensitive and insulin-resistant adipocytes. However, the mechanisms associated with the anti-diabetic effect of *I. sonorae* and *C. limetta* has not been identified. Regarding *B. media*, its aqueous extract

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is used for diabetes treatment but there is no evidence of its effectiveness (Lane *et al.*, 2006).

There is an increasing interest in the identification of polyphenol-rich sources for the development of functional beverages, which are the main application of the global polyphenol market, valued in in about 1.68 billion USD in 2022 (Grand View Research on polyphenols market). Therefore, the aim of this study was to identify the mechanisms associated with the hypoglycemic effect of *C. limetta*, *B. media* and *I. sonorae* aqueous extracts, via the evaluation of *in vitro* inhibitory activity against carbohydrate digestion enzymes and the modulation of key transcripts involved in the insulin signaling pathway in 3T3 L1 adipocytes, and to associate these beneficial health effects with their polyphenol profile through a chemometric analysis. These plants are analyzed in parallel due to their shared traditional use for diabetes management and their hypothesized similar hypoglycemic effects. By comparing these extracts, we aim to understand their distinct and potentially complementary mechanisms of action in controlling blood glucose levels, providing a comprehensive evaluation of their therapeutic potential.

MATERIAL AND METHODS

Herbal material and preparation of herbal aqueous extracts

Roots of *Ibervillea sonorae* and *Citrus limetta* roots, leaves and stems of *Briza media* were obtained from local markets in Querétaro, México, based on their traditional use in Mexican medicine for diabetes management. Extracts were prepared using two g of dried material in 100 mL of water and boiled for 30 min. This preparation method was chosen according to the recommendations of local herbalists to ensure traditional relevance. After boiling, all extracts were filtered using a 0.5 mm pore size filter and were stored at 4 ºC protected from the light.

Animal protocol experiments

Thirty male Wistar rats of 220 - 240 g were acquired from the Universidad Nacional Autónoma de México (Juriquilla, Querétaro, Mexico). The housing room was maintained at 23 ºC and 50 % relative humidity, under a 12 h light-dark cycle, animals were housed individually with free access to standard diet (La Rodent Diet 5001) and water. Experiments were performed in accordance with the Animal Care and Use Protocol (NOM-062-ZOO-1999), and protocol was approved by the Bioethics Committee, Faculty of Natural Sciences of the Universidad Autónoma de Querétaro (Querétaro, México; approval number: 5FCN2015).

Oral starch tolerance test

After one week of acclimatization, rats were fasted for 12 h and then randomly allocated in five groups of six rats each. The negative control group was administered water (vehicle) via intragastric cannulation, the positive control group was administered acarbose (10 mg/kg b.w.), and the treatment groups were administered each herbal extract: *I. sonorae*, C. *limetta*, and *B. media* (6 mL/kg b.w.). This concentration of herbal extracts was based on previous studies conducted by our research group, which demonstrated effective results in similar experimental settings (Hernández-Saavedra *et al*., 2016). After 5 min, a starch solution (5 g/kg b.w.) was administered to all rats. Glucose was measured at 0, 15, 30, 60, 90, 120, 150, and 180 min., using blood obtained from the tail vein with an Accu-Chek System (Roche Diagnosis, Germany).

Evaluation of *in vitro* α-amylase and α-glucosidase enzymes inhibitory activity

Herbal extracts were evaluated against α-amylase and α-glucosidase according to the methods described by Apostolidis *et al.* (2007). Results were reported as percentage of inhibition.

Cell culture

3T3-L1 preadipocytes (1 x 104 cells) were cultured until confluence in Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum (FBS, Gibco by Life Technologies, New York, USA) and 1 % penicillin/streptomycin (Invitrogen). The differentiation of adipocytes was induced with 5 µg/mL insulin (Life Technologies), 0.25 µM dexamethasone (Sigma-Aldrich, Missouri, USA), 0.5 mM IBMX (Invitrogen), 2 % FBS, and 1 % penicillin/streptomycin in DMEM for 2 days. Then, the medium was replaced with DMEM with 5 µg/mL insulin, 2 % FBS, and 1 % penicillin/streptomycin for 1 day. Finally, the medium was replaced with DMEM with 2 % FBS and 1 % penicillin/streptomycin for 4 days. Mediums were changed every two days during adipocytes proliferation and differentiation.

In vitro glucose uptake assay

Differentiated cells were serum-starved for 4 h and were treated with 0.5 and 1.0 mg/mL of lyophilized herbal extracts dissolved in 0.1 % dimethyl sulfoxide (DMSO, Sigma-Aldrich). This concentration of herbal extracts was based on previous studies conducted by our research group, which demonstrated effective results in similar experimental settings (Pérez-Ramírez *et al*., 2017). Negative control cells were incubated with 0.1 % DMSO, whereas positive control cells were incubated with 100 nM of insulin. Glucose uptake assays were performed as described previously by Urso *et al*. (1999). Cells were incubated at 37 ºC for 30 min. Glucose concentration was determined using colorimetric-commercial kit (Spinreact, Girone, Spain). All experiments were assessed in triplicate.

Relative quantification of *Glut4*, *Irs1*, and *Pi3k* gene transcripts

As in glucose uptake assay, fully differentiated adipocytes were incubated at 37 ºC for 30 min with each treatment. Afterwards, cells were lysed with 1 mL of Trizol reagent (Thermo Fisher Scientific, Delaware, USA) for the isolation of total RNA according to the instructions of manufacturer. RNA was quantified with a NanoDrop 1000 spectrophotometer (Ther-

mo Fisher Scientific). RNA quality was assessed with 260/280 and 260-230 ratios, whereas RNA integrity was assessed by electrophoresis on a 1 % denaturing agarose gel.

The cDNA synthesis was carried out with 1 µg of total RNA, 4 µL of M-MLV 5X reaction buffer (Invitrogen, Ca, USA), 1 µL of 5 µM oligo dT (Invitrogen), 1 µL of 10 mM dNTP mix (Invitrogen), 1 µL (200 U) of M-MLV RT (Invitrogen), 3 µL of 0.1 M DTT (Invitrogen), 1 µL of RNase inhibitor (Invitrogen), and RNAse-free water (Sigma-Aldrich), reaching a final volume of 20 µL. The reaction was incubated at 42 °C for 50 min and was stopped by incubating at 70 °C for 15 min.

The RT-PCR reaction was carried out with Sybr Green qPCR master mix (Thermo Scientific) according to the instructions of the manufacturer using a StepOne Real-Time PCR System (Applied Biosystems. California, USA). Target transcripts were amplified using the following primers: *Glut4*: sense 5′-TCATCAGGATAAACAGCAG-3′ and antisense 5′-TACTATTGTGTTCCTTTGC-3′, *Pi3k*: sense 5′-CATGTTCTGGAAACTTCACCA-3′ and antisense 5′CCTGGGGAAACATAAACTTG-3′, and *Irs1*: sense 5′-CCTCACAGTCTTCAGTGGCT-3′ and antisense 5′-ATAGTCCCCATTTCCTTTGC-3′. Gene expression was assessed in duplicate. Relative quantification was carried out with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) using *Actin* (sense 5′- GCTACAGCTTCACCACCACA-3′ and antisense 5′- AGTTTCATGGATGCCACAGG-3′) and *18S* (sense 5′- GGAGCGATTTGTCTGGGTTA-3′ and antisense 5′- GTAGGGTAGGCACACGCTGA-3′) as reference genes.

Polyphenol profile

The polyphenol profile was assessed by Ultra-Performance Liquid Chromatography (UPLC) coupled to a Quadrupole-Time of Flight (QTOF) with an atmospheric pressure Electrospray Ionization (ESI) interphase (Vion, Waters Co, MA, USA). The column used was an Acquity BEH C18 (100 x 2.1 mm, 1.7 µm) using the conditions reported previously by Rodríguez-Gonzalez *et al.* (2018). Data acquisition was carried out with UNIFI Scientific Information System (Waters Co). Polyphenols were identified by comparison of elemental composition with the exact mass of the pseudo molecular ion (error mass < 5 ppm) using Phenol-Explorer database (http://phenol-explorer.eu), analysis of fragmentation pattern and isotopic distribution. The high-resolution mass spectra of the main polyphenols identified in the herbal extracts are shown in Figures 1S-9S.

Data analysis

Statistical significance was evaluated using ANOVA followed by Dunnet's test ($p < 0.05$). Identification of polyphenols linked to the extracts' antidiabetic potential was assessed by Partial Least Square-Discriminant Analysis (PLS-DA) and Variable Importance in the Projection (VIP) vs Coefficient score plots. JMP software (v11.0, SAS Institute, NC, USA) facilitated all statistical analyses.

RESULTS

Effect of herbal extracts on glucose absorption

Blood glucose levels peaked at 30 min after ingesting 5 g/kg of starch under fasting conditions (Figure 1). *C. limetta* extract slightly reduced the hyperglycemic peak (0.12-fold), while *B. media* and *I. sonorae* decreased ($p < 0.05$) postprandial glucose levels by 0.34 - 0.36-fold. This hints a mild postprandial antihyperglycemic activity. *In vitro* tests demonstrated that *I. sonorae* and *B. media* had the highest inhibitory α-amylase activity (25.4 and 20.5 %, respectively) as compared to *C. limetta* (5.3 %) (Table 1). Acarbose exerted even greater inhibitory activity (88.1 %). For α-glucosidase, *C. limetta* and *B. media* showed similar low inhibition (5.1 and 4.4 %, respectively), while *I. sonorae* lacked this effect.

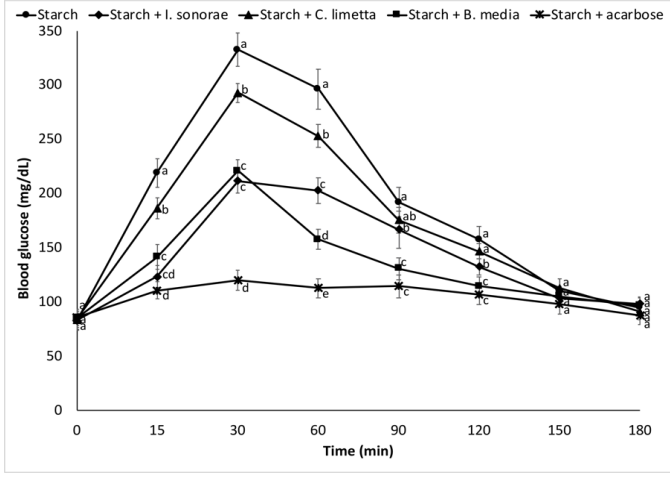

Figure 1. Effect of *I. sonorae*, *C. limetta*, and *B. media* aqueous extracts on postprandial blood glucose after a starch load (5 g/kg b.w.) in healthy Wistar rats. Data are shown as mean values (n = 6) and error bars represent standard error. Different letters in the same experimentation time indicate significant ($p < 0.05$) differences by Tukey's test.

Figura 1. Efecto de extractos acuosos de *I. sonorae*, *C. limetta* y *B. media* sobre la glucosa postprandial en sangre tras una carga de almidón (5 g/kg de peso corporal) en ratas Wistar sanas. Los datos son mostrados como media (n = 6) y las barras de error representan el error estándar. Letras diferentes en el mismo tiempo experimental indican diferencias significativas ($p < 0.05$) con la prueba de Tukey.

Effect of herbal extracts on glucose uptake in 3T3-L1 adipocytes

All herbal extracts increased ($p < 0.05$) glucose uptake as compared to control cells (Figure 2). *C. limetta* and *I. sonorae*

Table 1. *In vitro* inhibitory activity of *I. sonorae*, *C. limetta*, and *B. media* aqueous extracts against carbohydrates digestive enzymes.

Tabla 1. Actividad inhibitoria *in vitro* de extractos acuosos de *I. sonorae*, *C. limetta* y *B. media* contra enzimas de digestión de carbohidratos.

Herbal extracts	α-Amylase inhibition	α-Glucosidase inhibition
I. sonorae	25.4 ± 0.3b	ND
C. limetta	5.3 ± 0.5	5.1 ± 0.5b
B. media	20.5 ± 2.0c	4.4 ± 0.4b
Acarbose	88.1 ± 6.3a	81.9 ± 6.6a

Values are expressed as maximum percentage of inhibition (%). Data are expressed as mean values ± standard deviation (n = 3). Means within a column followed by the same letter are not significantly different ($p < 0.05$) by Tukey's test. ND: not detected.Los valores están expresados como porcentaje máximo de inhibición (%). Los datos son presentados como media ± desviación estándar (n = 3). Las medias dentro de una columna seguida de la misma letra no son significativamente diferentes ($p < 0.05$) por la prueba de Tukey. ND: no detectado.

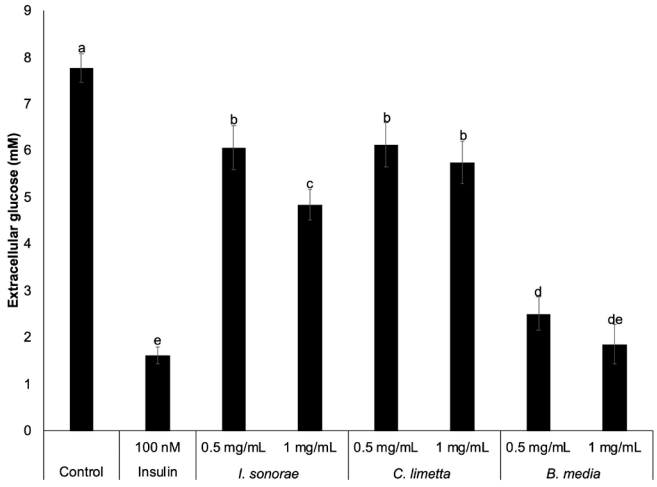

Figure 2. Effect of *I. sonorae*, *C. limetta*, and *B. media* aqueous extracts on glucose uptake in 3T3 L1 adipocytes. Data are showed as mean values (n = 3) and error bars represent standard error. Different letters indicate significant differences ($p < 0.05$) by Tukey's test.

Figura 2. Efecto de extractos acuosos de *I. sonorae*, *C. limetta* y *B. media* sobre la internalización de glucosa en adipocitos 3T3 L1. Los datos son mostrados como media (n = 3) y las barras de error representan el error estándar. Letras diferentes indican diferencias significativas ($p < 0.05$) por la prueba de Tukey.

led to lower extracellular glucose levels (0.22-0.38-fold) as compared to the control group. Remarkably, *B. media* significantly reduced extracellular glucose levels (0.68-0.76-fold) at both concentrations, comparable to effect of insulin at the highest *B. media* concentration (1.0 mg/mL). *B. media* extract induced significant overexpression of *Glut4*, *Irs1*, and *Pi3k* genes (2.27-2.85, 1.98 - 2.60, and 2.51 - 3.19-fold, respectively) as compared to control cells, showcasing a dose-response relationship (Figure 3).

Polyphenol profile of herbal extracts

A comprehensive analysis of the polyphenol profiles was conducted (Table 2). *I. sonorae* had 31 identified phenolic compounds, including eight hydroxybenzoic acids, twelve hydroxycinnamic acids, three flavanones, six flavonols, and two hydroxycoumarins. *C. limetta* roots extract had 39 phenolic compounds, including eleven hydroxybenzoic acids, twenty hydroxycinnamic acids, one flavanol, four flavanones, two flavonols, and one hydroxycoumarin. *B. media* contained 28 identified polyphenols, including twelve hydroxybenzoic acids, ten hydroxycinnamic acids, one flavanol, four flavanones, three flavonols, and two hydroxycoumarins. Multivariate analyses linked phenolic compounds to the previously described antidiabetic potential. *I. sonorae* and *B. media* extracts showed the highest *in vitro* α-amylase inhibitory activity (Table 1), which was linked mainly to hydroxybenzoic acid hexoside (PA_5), feruloylquinic acid (PA_24), and benzoic acid (PA_10) (Figure 4). Accordingly, these compounds were most abundant in these extracts (Table 2).

Eighteen polyphenols were associated with reduced glucose uptake (Figure 5): dihydroxybenzoic acid isomer I (PA_4), hydroxybenzoic acid isomer II (PA_7), rosmarinic acid (PA_36), protocatechuic acid (PA_8), dihydroxybenzoic acid isomer II (PA_9), caffeoylquinic acid isomer II (PA_21), hydroxybenzoic acid isomer I (PA_3), ellagic acid (PA_29), quercetin (F_17), naringin malonate (F_7), kaempferol hexoside-rhamnoside (F_13), narirutin (F_3), kaempferol sophoroside (F_12), naringenin (F_8), esculetin (C_3), hydroxycoumarin (C_4), and psoralen (C_1). These polyphenols were found in greater amount in *B. media* (Table 2), which exerted the greatest effect on glucose uptake in adipocytes (Figure 2). Additionally, eighteen polyphenols were linked to *Glut4*, *Irs1*, and *Pi3k* gene overexpression. Seventeen of these were also associated with an increase in glucose uptake of *B. media* (Fig. 5B), while sinapoylquinic acid isomer II (PA_32) was linked solely to gene overexpression in the insulin cascade pathway, not increased glucose uptake.

DISCUSSION

In summary, our study deepens into the anti-diabetic potential of three traditionally used herbal extracts (*I. sonorae*, *C. limetta*, and *B. media*) to elucidate their mechanisms of action and the associated polyphenols. Our findings align with the accumulated evidence of the role of polyphenols in metabolic disorders, particularly diabetes, beyond their antioxidant and anti-inflammatory properties. We adopted an *in vitro* multi-pronged approach to assess their mechanisms, focusing on their impact on glucose absorption and uptake.

Regarding glucose intestinal absorption, all extracts significantly mitigated postprandial hyperglycemia, with *B. media* and *I. sonorae* extracts exhibiting the most pronounced effects. This anti-hyperglycemic activity was particularly linked to their a-amylase inhibitory activity, an enzyme pivotal in starch digestion. In this regard, it has been previously reported that *C. limetta* peel extract inhibits α-amylase activity (Padilla-Camberos *et al*., 2014).

Notably, polyphenols such as hydroxybenzoic acid hexoside, benzoic acid, and feruloylquinic acid were key contributors to these effects, which can inhibit competitively or allosterically these digestive enzymes (Şöhretoğlu *et al*., 2023). Accordingly, it has been previously reported that an extract of phenolic compounds from green coffee beans, rich in feuroylquinic acids and caffeoylquinic acids, inhibited α-amylase activity (Cheng *et al*., 2019).

The second facet of our research, glucose uptake in 3T3 L1 adipocytes, produced interesting outcomes. *B. media* emerged as a potent inducer of glucose uptake, exerting a similar effect to insulin. These results suggest the insulin-mimetic potential of *B. media*, which was associated with the over-expression of *Glut4*, *Irs1*, and *Pi3k* genes, all critical elements in the insulin signaling cascade. This enhanced glucose uptake and expression of key genes in adipocytes is particularly valuable in the context of insulin resistance, a common characteristic of type 2 diabetes mellitus. Zapata-Bustos *et al.* (2014) reported that the extract of *I. sonorae* stimulates the glucose uptake in 3T3-F442A and 3T3-L1 adipocytes in a concentration-dependent manner, whereas this is the first study that reports this hypoglycemic mechanism for *C. limetta* roots and the *B. media* plant.

Table 2. Polyphenol profile of *I. sonorae*, *C. limetta*, and *B. media* aqueous extracts by UPLC-QTOF MS^E.
Tabla 2. Perfil de polifenoles de extractos acuosos de *I. sonorae*, *C. limetta* y *B. media* por UPLC-QTOF MS^E.

Code	Polyphenols	Rt (min)	Molecular Formula	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	*I. sonorae*	*C. limetta*	*B. media*
	Hydroxybenzoic acids								
PA_1	Hydroxybenzoic acid isomer I	1.22	C7H6O3	138.0317	138.0312	-3.3747	1293.3	1772.8	620.4
PA_2	Protocatechuic acid hexoside	1.63	C13H16O9	316.0794	316.0790	-1.4622	21512.3	347697.0	5984.6
PA_3	Vanillic acid†	1.70	C8H8O4	168.0423	168.0417	-3.2769	ND	26039.5	51512.0
PA_4	Dihydroxybenzoic acid isomer I	1.94	C7H6O4	154.0266	154.0266	-0.1933	668.5	446.8	20571.0
PA_5	Hydroxybenzoic acid hexoside	2.20	C13H16O8	300.0845	300.0834	-3.6896	3536.4	487.7	3027.3
PA_6	Gallic acid ethyl ester	2.36	C9H10O5	198.0528	198.0523	-2.5838	ND	96098.1	20600.6
PA_7	Hydroxybenzoic acid isomer II	3.13	C7H6O3	138.0317	138.0315	-1.4297	1614.8	966.4	87775.5
PA_8	Protocatechuic acid†	3.32	C7H6O4	154.0266	154.0259	-4.5348	853.3	444.1	1993.8
PA_9	Dihydroxybenzoic acid isomer II	4.21	C7H6O4	154.0266	154.0259	-4.7078	ND	ND	506.4
PA_10	Benzoic acid	4.27	C7H6O2	122.0358	122.0368	0.3424	2721.0	829.1	1300.0
PA_11	Syringic acid†	4.48	C9H10O5	198.0528	198.0523	-2.5853	ND	16507.5	11358.3
PA_12	Hydroxybenzoic acid isomer III	6.67	C7H6O3	138.0317	138.0316	-0.3344	489.8	3217.6	1763.7
	Hydroxycinnamic acids								
PA_13	Caffeoyl tartaric acid	1.08	C13H12O9	312.0481	312.0475	-1.9704	ND	938.1	ND
PA_14	Cinnamic acid†	1.45	C9H8O2	148.0524	148.0519	-3.6684	3911.7	ND	ND
PA_15	Caffeoylquinic acid isomer I	2.57	C16H18O9	354.0951	354.0954	0.8135	6010.1	450.2	ND
PA_16	Caffeic acid ethyl ester	3.52	C11H12O4	208.0736	208.0731	-2.1574	ND	2734.5	ND
PA_17	Coumaroylquinic acid isomer I	3.68	C16H18O8	338.1002	338.1005	1.0962	ND	747.8	ND
PA_18	Ferulic acid†	3.68	C10H10O4	194.0579	194.0576	-1.8077	ND	2984.7	ND
PA_19	Ferulic acid hexoside	3.69	C16H20O9	356.1107	356.1107	-0.1713	ND	8275.5	394.7
PA_20	Coumaroyl hexose	4.09	C15H18O8	326.1002	326.0996	-1.7866	1237.7	1070.8	ND
PA_21	Caffeoylquinic acid isomer II	4.17	C16H18O9	354.0951	354.0943	-2.0983	1451.5	999.5	10313.2
PA_22	Coumaroyl glycolic acid	4.45	C11H10O5	222.0528	222.0523	-2.4526	ND	879.9	5151.3
PA_23	Ellagic acid hexoside	4.73	C20H16O13	464.0591	464.0602	2.3819	ND	690.2	ND
PA_24	Feruloylquinic acid	5.12	C17H20O9	368.1107	368.1103	-1.1118	1715.0	ND	1050.0
PA_25	Coumaric acid†	5.18	C9H8O3	164.0473	164.0472	-0.8242	613.7	1563.4	780.1
PA_26	Sinapoylquinic acid isomer I	5.23	C18H22O10	398.1213	398.1200	-3.3438	ND	446.0	ND
PA_27	Ellagic acid pentoside	5.32	C19H14O12	434.0485	434.0492	1.4832	ND	2870.2	ND
PA_28	Coumaroylquinic acid isomer II	5.34	C16H18O8	338.1002	338.0999	-0.7703	ND	502.8	369.2
PA_29	Ellagic acid†	5.63	C14H6O8	302.0063	302.0067	1.2807	ND	1194.1	1477.4
PA_30	Isoferulic acid	5.73	C10H10O4	194.0579	194.0576	-1.8164	539.7	4217.5	2327.3
PA_31	Dicaffeoylquinic acid isomer I	6.29	C25H24O12	516.1268	516.1257	-2.0080	72712.9	2850.5	ND
PA_32	Sinapoylquinic acid isomer II	6.36	C18H22O10	398.1213	398.1231	4.6479	ND	1554.8	1459.7
PA_33	Dicaffeoylquinic acid isomer II	6.39	C25H24O12	516.1268	516.1260	-1.5250	28039.8	1634.2	2519.1
PA_34	Caffeoylquinic acid isomer III	6.40	C16H18O9	354.0951	354.0947	-0.9726	4404.7	ND	ND
PA_35	Dicaffeoylquinic acid isomer III	6.73	C25H24O12	516.1268	516.1272	0.8322	75354.8	2355.7	ND
PA_36	Rosmarinic acid†	6.82	C18H16O8	360.0845	360.0841	-1.1864	586.0	ND	1831112.9
	Flavanols								
F_1	(Epi)-catechin hexose	5.20	C21H24O11	452.1319	452.1328	2.0200	ND	1731.3	678.3
	Flavanones								
F_2	Neoeriocitrin	4.50	C27H32O15	596.1741	596.1736	-0.8151	679.8	802.3	ND
F_3	Narirutin	6.37	C27H32O14	580.1792	580.1788	-0.7378	ND	ND	5223.6
F_4	Naringin†	6.57	C27H32O14	580.1792	580.1796	0.6403	ND	190414.1	ND
F_5	Hesperidin†	6.76	C28H34O15	610.1898	610.1892	-0.9442	ND	662410.3	1141.0
F_6	Eriodictyol	7.73	C15H12O6	288.0634	288.0636	0.5717	191.1	ND	ND
F_7	Naringin malonate	8.03	C36H44O22	828.2324	828.2318	-0.7540	ND	ND	788.0
F_8	Naringenin†	8.68	C15H12O5	272.0685	272.0686	0.3929	816.7	724.7	917.0
	Flavonols								
F_9	Myricetin rutinoside	5.17	C27H30O17	626.1483	626.1494	1.6816	358.1	ND	ND
F_10	Rhamnetin hexoside	5.25	C22H22O12	478.0747	478.0757	2.1079	391.5	ND	ND
F_11	Myricetin hexoside	5.27	C21H20O13	480.0904	480.0905	0.1751	2583.4	3125.6	ND
F_12	Kaempferol sophoroside	5.72	C27H30O16	610.1534	610.1535	0.1290	ND	ND	5046.0
F_13	Kaempferol hexoside-rhamnoside	5.86	C27H30O15	594.1585	594.1605	3.4310	ND	ND	1449.4
F_14	Quercetin hexoside	5.90	C21H20O12	464.0955	464.0953	-0.4036	16498.3	735.0	ND
F_15	Quercetin malonyl-hexoside	6.18	C24H22O15	550.0959	550.0948	-2.0132	246.8	ND	ND
F_16	Kaempferol malonyl-hexoside	6.75	C24H22O14	534.1010	534.0992	-3.2217	402.2	ND	ND
F_17	Quercetin†	7.90	C15H10O7	302.0427	302.0422	-1.5475	ND	ND	431.4
	Furanocoumarins								
C_1	Psoralen	6.92	C11H6O3	186.0317	186.0320	1.3986	ND	ND	29060.3
	Hydroxycoumarins								
C_2	Esculin	3.20	C15H16O9	340.0794	340.0786	-2.5525	1690.6	1427.8	ND
C_3	Esculetin	4.06	C9H6O4	178.0266	178.0260	-3.3713	403.6	ND	26338.5
C_4	Hydroxycoumarin	6.82	C9H6O3	162.0317	162.0318	0.7271	ND	ND	112663.3

Results are expressed as arbitrary units. Data are shown as mean values (n = 3). Rt: retention time; ND: not detected; PA: phenolic acids; F: flavonoids; C: coumarins. †Identification confirmed with commercial standards.
Los resultados están expresados como unidades arbitrarias. Los datos son mostrados como media (n = 3). Rt: tiempo de retención; ND: no detectado; PA: ácidos fenólicos; F: flavonoides; C: cumarinas. †Identificación confirmada con estándares comerciales.

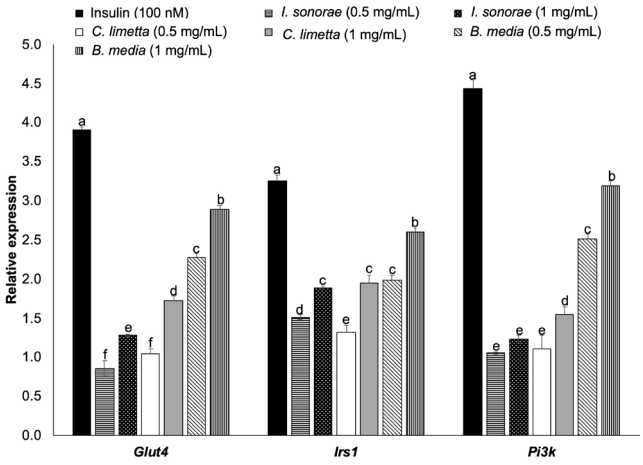

Figure 3. Effect of *I. sonorae*, *C. limetta*, and *B. media aqueous* extracts on *Glut4*, *Irs1*, and *Pi3k* relative expression in 3T3 L1 adipocytes. Relative expression was estimated in comparison with negative control cells. Data are showed as mean values (n = 3) and error bars represent standard error. Different letters for each gene indicate significant differences ($p < 0.05$) by Tukey's test. *Glut4*: glucose transporter type 4, *Irs1*: insulin receptor substrate-1, *Pi3k*: phosphatidylinositol 3-kinase.

Figura 3. Efecto de extractos acuosos de *I. sonorae*, *C. limetta* y *B. media* sobre la expresión relativa de *Glut4*, *Irs1* y *Pi3k* en adipocitos 3T3 L1. La expresión relativa fue estimada en comparación con las células de control negativo. Los datos son mostrados como media (n = 3) y las barras de error representan el error estándar. Letras diferentes para cada gen indican diferencias significativas ($p < 0.05$) por la prueba de Tukey. *Glut4*: transportador de glucosa tipo 4, *Irs1*: sustrato del receptor de insulina 1; *Pi3k*: fosfatidilinositol 3-quinasa.

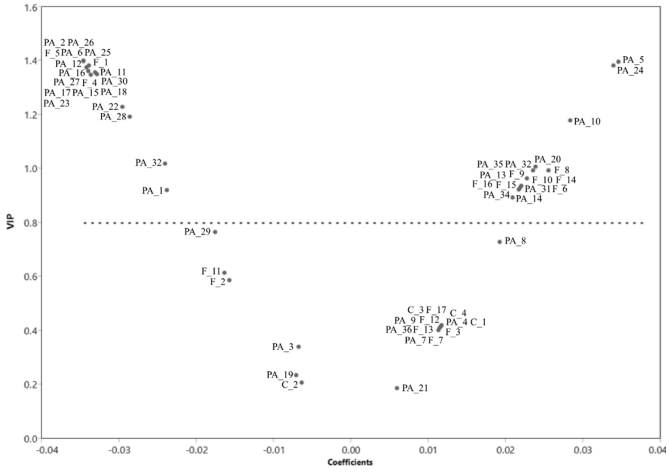

Figure 4. Association between the polyphenol profile of *I. sonorae*, *C. limetta*, and *B. media* aqueous extracts and their antidiabetic potential assessed by α-amylase inhibitory activity. VIP: variable importance in the projection.

Figura 4. Asociación entre el perfil de polifenoles de extractos acuosos de *I. sonorae*, *C. limetta* y *B. media* y su potencial antidiabético determinado por la actividad inhibitoria de α-amilasa. VIP: importancia de la variable para la proyección.

Our detailed multivariate analysis suggested several polyphenols associated with the observed effects, some of which have been previously reported to impact glucose uptake and insulin signaling. For instance, it has been reported that rosmarinic acid (Vlavcheski *et al*., 2017), naringin (Dayarathne *et al*., 2021), ellagic acid (Kábelová *et al*., 2021) and kaempferol (Moore *et al*., 2023) increase glucose uptake

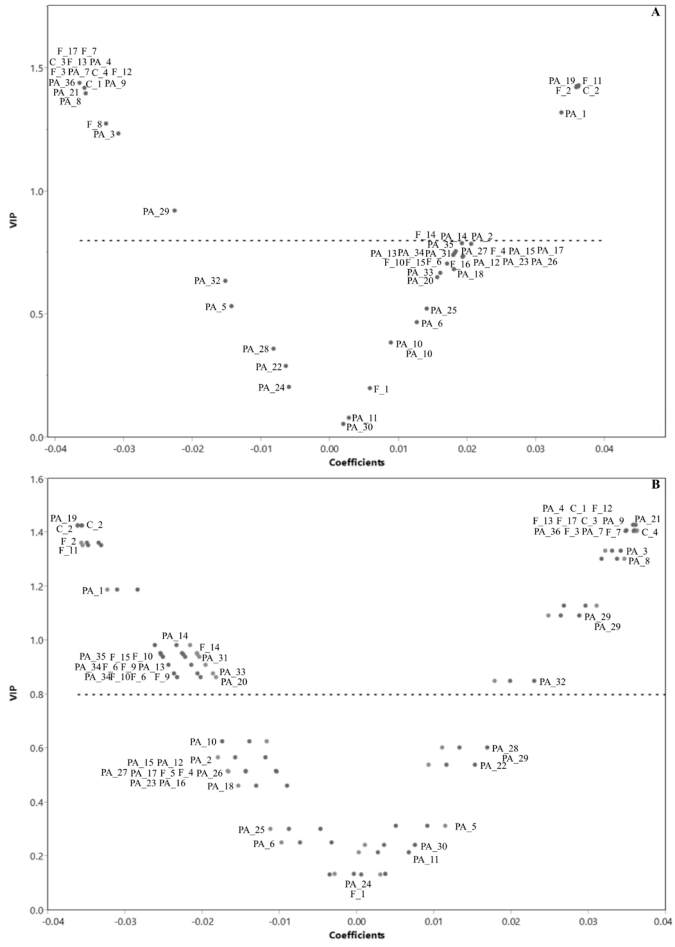

Figure 5. Association between the polyphenol profile of *I. sonorae*, *C. limetta*, and *B. media* aqueous extracts and their antidiabetic potential assessed in 3T3 L1 adipocytes by glucose uptake (A) and the relative expression of *Glut4*, *Irs1*, and *Pi3k* (B). *Glut4*: glucose transporter type 4, *Irs1*: insulin receptor substrate-1, *Pi3k*: phosphatidylinositol 3-kinase, VIP: variable importance in the projection.

Figura 5. Asociación entre el perfil de polifenoles de extractos acuosos de *I. sonorae*, *C. limetta* y *B. media* y su potencial antidiabético determinado por la internalización de glucosa en adipocitos 3T3 L1 (A) y la expresión relativa de *Glut4*, *Irs1* y *Pi3k* (B). *Glut4*: transportador de glucosa tipo 4, *Irs1*: sustrato del receptor de insulina 1; *Pi3k*: fosfatidilinositol 3-quinasa; VIP: importancia de la variable para la proyección.

in skeletal muscle cells and adipocytes. Protocatechuic acid is an hydroxybenzoic acid which stimulates insulin signaling pathways in myocytes, hepatocytes and adipocytes. Specifically, this phenolic acid significantly increased the expression and protein level of IRS-1 and GLUT4 in insulin resistant adipocytes (Shakoor *et al*., 2023).

Numerous studies have reported the ability of polyphenols to increase insulin-dependent glucose uptake via GLUT4 activation by up-regulating *Pi3k* expression (Williamson and Sheedy, 2020). Chlorogenic acid and quercetin promote glucose uptake by increasing *Glut4* mRNA levels and its translocation to cell membrane (Gannon *et al*., 2014). Raciti *et al.* (2018) evaluated a citrus extract rich in hesperidin and narirutin in adipocytes 3T3-L1 and reported increased *Glut4* mRNA levels; however, the administration of the isolated compounds did not show the same effect, suggesting a

synergistic mechanism. Altogether, these results further credence to the notion that polyphenols play a pivotal role in the mechanisms behind the anti-diabetic effects of herbal extracts.

CONCLUSIONS

This study identifies the mechanism underlying the potential glucose-regulating properties of *B. media* and *I. sonorae*, suggesting their viability as anti-diabetic agents in traditional medicine or as ingredients for functional beverages. Furthermore, the identification of polyphenols associated with these effects adds to the growing evidence highlighting the potential of these natural compounds in mitigating metabolic disorders, offering a promising opportunity for the development of novel therapeutic strategies. Further investigations are warranted to validate the impact of these herbal extracts on insulin resistance and glucose intolerance through intervention studies.

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CONFLICTS OF INTEREST

The authors declare no actual or potential conflict of interests, including financial, personal or relationship with other organizations.

REFERENCES

Alarcón-Aguilar, F.J., Calzada-Bermejo, F., Hernandez-Galicia, E., Ruiz-Angeles, C. and Roman-Ramos, R. 2005. Acute and chronic hypoglycemic effect of *Ibervillea sonorae* root extracts-II. Journal of Ethnopharmacology. 97: 447-452.

Apostolidis, E., Kwon, Y.I. and Shetty, K. 2007 Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science & Emerging Technologies. 8: 46-54.

Cheng, K., Wenjiang, D., Long, Y., Zhao, J., Hu, R., Zhang, Y. and Zhu, K. 2019. Evaluation of the impact of different drying methods on the phenolic compounds, antioxidant activity, and *in vitro* digestion of green coffee beans. Food Science & Nutrition. 7: 1084-1095.

Dayarathne, L.A., Ranaweera, S.S., Natraj, P., Rajan, P., Lee, Y.J. and Han, C.H. 2021. The effects of naringenin and naringin on the glucose uptake and AMPK phosphorylation in high glucose treated HepG2 cells. Journal of Veterinary Science, 22: e92.

Flores-Fernández, J., Barragán-Álvarez, C., Díaz-Martínez, N., Villanueva-Rodríguez, S. and Padilla-Camberos, E. 2017. *In vitro* and *in vivo* postprandial glycemic activity of *Citrus limetta* peel flour. Pharmacognosy Magazine. 13: 613-616.

Gannon, N., Conn, C. and Vaughan, R. 2014. Dietary stimulators of GLUT4 expression and translocation in skeletal muscle: a mini-review. Molecular Nutrition & Food Research. 59: 48-64.

Hernández-Saavedra, D., Pérez-Ramírez, I.F., Ramos-Gómez, M., Mendoza-Díaz, S., Loarca-Pina, G. and Reynoso-Camacho, R. 2016. Phytochemical characterization and effect of *Calendula officinalis*, *Hypericum perforatum*, and *Salvia officinalis* infusions on obesity-associated cardiovascular risk. Medicinal Chemistry Research. 25: 163-172.

Kábelová, A., Malínská, H., Marková, I., Oliyarnyk, O., Chylíková, B. and Šeda, O. 2021. Ellagic acid affects metabolic and transcriptomic profiles and attenuates features of metabolic syndrome in adult male rats. Nutrients. 13: 804.

Lane, J., Strich, H., Taylor, A., Timmermann, B., Malone, D., Teufel-Shone, N., Drummond, R., Woosley, R., Pereira, E. and Martinez, A. 2006. Use of herbal remedies by diabetic Hispanic women in the southwestern United States. Phytotherapy Research. 20: 250-255.

Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods. 25: 402-408.

Moore, W.T., Luo, J. and Liu, D. 2023. Kaempferol improves glucose uptake in skeletal muscle via an AMPK-dependent mechanism. Food Science and Human Wellness. 12: 2087-2094.

Padilla-Camberos, E., Lazcano-Díaz, E., Flores-Fernandez, J.M., Owolabi, M.S., Allen, K. and Villanueva-Rodríguez, S. 2014. Evaluation of the inhibition of carbohydrate hydrolyzing enzymes, the antioxidant activity, and the polyphenolic content of *Citrus limetta* peel extract. The Scientific World Journal. 2014: 121760.

Pérez-Ramírez, I.F., González-Dávalos, M.L., Mora, O., Gallegos-Corona, M.A. and Reynoso-Camacho, R. 2017. Effect of *Ocimum sanctum* and *Crataegus pubescens* aqueous extracts on obesity, inflammation, and glucose metabolism. Journal of Functional Foods. 35: 24-31.

Raciti, G.A., Fiory, F., Campitelli, M., Desiderio, A., Spinelli, R., Longo, M., Nigro, C. and Miele, C. 2018. *Citrus aurantium* L. dry extracts promote *C/ebpβ* expression and improve adipocyte differentiation in 3T3-L1 cells. PloS One. 13: e0193704.

Rivera-Ramírez, F., Escalona-Cardoso, G.N., Garduño-Siciliano, L., Galaviz-Hernández, C. and Paniagua-Castro, N. 2011. Antiobesity and hypoglycaemic effects of aqueous extract of *Ibervillea sonorae* in mice fed a high-fat diet with fructose. Journal of Biotechnology and Biomedicine. 2011: 968984.

Rodríguez-González, S., Pérez-Ramírez, I.F., Castaño-Tostado, E., Amaya-Llano, S., Rodríguez-García, M.E., Reynoso-Camacho, R. 2018. Improvement of physico-chemical properties and phenolic compounds bioavailability by concentrating dietary fiber of peach (*Prunus persica*) juice by-product. Journal of the Science of Food and Agriculture. 98: 3109.

Shakoor, H., Hussein, H., Al-Hassan, N., Alketbi, M., Kizhakkayil, J. and Platat, C. 2023. The muscle-conditioned medium containing protocatechuic acid improves insulin resistance by modulating muscle communication with liver and adipose tissue. International Journal of Molecular Sciences. 24: 9490.

Şöhretoğlu, D., Renda, G., Arroo, R., Xiao, J. and Sari, S. 2023. Advances in the natural α-glucosidase inhibitors. eFood. 4: e112.

Urso, B., Cope, D., Kallo, H., Hayward, A., Whitehead, J., O'Rahilly, S. and Siddle, K. 1999. Differences in signaling properties of the cytoplasmic domains of the insulin receptor and insulin-

like growth factor receptor in 3T3-L1 adipocytes. Journal of Biological Chemistry. 274: 30864-30873.

Tran, N., Pham, B. and Le, L. 2020. Bioactive compounds in anti-diabetic plants: from herbal medicine to modern drug discovery. Biology. 9: 252.

Vlavcheski, F., Naimi, M., Murphy, B., Hudlicky, T. and Tsiani, E. 2017. Rosmarinic acid, a rosemary extract polyphenol, increases skeletal muscle cell glucose uptake and activates AMPK. Molecules, 22: 1669.

Williamson, G. and Sheedy, K. 2020. Effects of polyphenols on insulin resistance. Nutrients. 12: 3135.

Zapata-Bustos, R., Alonso-Castro, A., Gómez-Sánchez, M. and Salazar-Olivo, L. 2014. *Ibervillea sonorae* (Cucurbitaceae) induces the glucose uptake in human adipocytes by activating a PI3K-independent pathway. Journal of Ethnopharmacology. 28: 546-52.