Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v62i1.15456
Artículos originales

Glycemia-reducing effects of Bolivian nutraceutical plants

Efectos reductores de la glucemia de las plantas nutracéuticas bolivianas

Silvia Zambrana[1,2] 0000-0002-8661-2969
Orlando Mamani[1] 0000-0002-4163-8102
Mabel Canaviri[1] 0000-0000-0000-0000
Maria del Pilar Gutierrez[1] 0000-0000-0000-0000
Sergiu-Bogdan Catrina[2,3,4] 0000-0002-6914-3902
Claes-Goran Ostenson[2] 0000-0003-2888-9932
Eduardo Gonzales[1] 0000-0000-0000-0000

[1]Universidad Mayor de San Andrés, Facultad de Ciencias Farmacéuticas y Bioquímicas, Instituto de Investigaciones Fármaco Bioquímicas, Área de Farmacología, La Paz, Bolivia.

[2]Karolinska Institutet and Karolinska University Hospital, Department of Molecular Medicine and Surgery, 17176 Stockholm, Sweden.

[3]Karolinska University Hospital, Department of Endocrinology and Diabetes, 17176 Stockholm, Sweden.

[4]Center for Diabetes, Academic Specialist Centrum,11365 Stockholm, Sweden.

Correspondence

Eduardo Gonzales Dávalos
eduardo.gonzales@gmail.com

Received: 03.06.2020
Accepted: 15.10.2020
Published: 20.12.2020

Financial support

This research was funded by the Swedish International Development Cooperation Agency, SIDA. SZ is supported by grants from SIDA.

Conflict of interest

The authors declare no conflict of interest.

Abstract

Introduction: The prevalence of diabetes type 2 is increasing worldwide, thus the search of novel alternative therapies is needed. According to their traditional use, we selected five Bolivian plants *Chenopodium quinoa* (*CQ*) *Amaranthus caudatus* (*AC*), *Chenopodium pallidicaule* (*CP*), *Lupinus mutabilis* (*LM*) and *Smallanthus sonchifolius* (*SS*) that are traditionally used to control glycemia.

Methods: The effect of a single oral administration of Ethanolic (EtOH), hydro-ethanolic (EtOH70) and aqueous (Aq) extracts from all plant species were tested for their effect on blood glucose in non-fasted mice and during the oral glucose tolerance test (OGTT). The effect on insulin secretion was evaluated in mice pancreatic islets.

Results: EtOH70 extracts of all the plants showed glucose-reducing effect at the highest dose evaluated (2000 mg/kg b.w.). EtOH70 extracts improved the glucose tolerance evaluated by the OGTT in mice fasted for 12 hours. The extracts have different effects on glucose homeostasis since just extracts of *AC*, *LM* and *CQ* but not *CP* and *SS* increased insulin secretion as shown on mice pancreatic islets. The phytochemical qualitative characterization of EtOH70 extracts detected phenolic acids and flavonoids in *AC*, *CP* and *CQ*; alkaloids in *LM* and anthocyanidins in *SS*. None of EtOH70 extracts tested showed *in vitro* or *in vivo* acute toxicity at concentrations where they exhibit glucose lowering effects.

Conclusions: We report here that extracts from *AC*, *CQ*, *CP*, *LM* and *SS* exhibit glucose lowering effect while just *AC*, *CQ* and *LM* stimulate directly the insulin secretion.

Keywords: Nutraceutical; Natural product; Insulin secretion.

Resumen

Introducción: La prevalencia de diabetes tipo 2 está aumentando en todo el mundo, por lo que se necesita la búsqueda de nuevas terapias alternativas. Según su uso tradicional, seleccionamos cinco plantas bolivianas *Chenopodium quinoa* (*CQ*) *Amaranthus caudatus* (*AC*), *Chenopodium pallidicaule* (*CP*), *Lupinus mutabilis* (*LM*) y *Smallanthus sonchifolius* (*SS*) que se usan tradicionalmente para controlar la glucemia.

Métodos: Se evaluó el efecto de la administración oral única de extractos etanólicos (EtOH), hidroetanólicos (EtOH70) y acuosos (Aq) de las plantas mencionadas para determinar su efecto sobre la glucosa en sangre en ratones en o sin ayunas y durante la prueba de tolerancia a la glucosa oral (PTGO). El efecto sobre la secreción de insulina se evaluó en islotes pancreáticos de ratones.

Resultados: Los extractos de EtOH70 de todas las plantas disminuyeron la glucemia a la dosis más alta evaluada (2000 mg / kg b.w.). Los extractos de EtOH70 mejoraron la tolerancia a la glucosa evaluada mediante la PTGO en ratones con ayuno de 12 horas. Los extractos tienen diferentes efectos sobre la homeostasis de la glucosa, ya que solo los extractos de *AC*, *LM* y *CQ* pero no *CP* y *SS* aumentaron la secreción de insulina como se muestra en los islotes pancreáticos de los murinos. La caracterización cualitativa fitoquímica de extractos de EtOH70 detectó ácidos fenólicos y flavonoides en *AC*, *CP* y *CQ*, alcaloides en *LM* y antocianidinas en *SS*. Ninguno de los extractos de EtOH70 probados mostró toxicidad aguda *in vitro* o *in vivo* a concentraciones en las que exhiben efectos reductores de glucosa.

Conclusión: Los extractos de *AC*, *CQ*, *CP*, *LM* y *SS* exhiben un efecto reductor de la glucosa, mientras que solo *AC*, *CQ* y *LM* estimulan directamente la secreción de insulina.

Palabras clave: Nutracéutico; Producto natural; Secreción de insulina.

Introducción

The prevalence of non-communicable diseases such as metabolic syndrome, obesity, and type 2 diabetes mellitus (T2DM) has increased worldwide[1]. One main responsible factor is the dietary change habits to food rich in fat, and carbohydrates[2-4]. Anti-diabetic drugs are used to control blood glucose levels, but some of these drugs have adverse effects, including the risk of hypoglycemia[5].

Recently, the use of certain food to restore the metabolic balance has been reported[6]. Nutraceuticals, i.e. components from food sources provide not only nutritional but also health benefits by modulating pathophysiological processes to get favorable health outcomes[2,7-9]. This approach might be easy to be accepted by large number of patients and are potentially less coupled with severe secondary reactions compared to conventional drug therapies[2,10,11].

According to their traditional use, Bolivia has a huge diversity of food plants that could be considered as nutraceuticals. Thus, based on literature review, we selected five plants that are currently part of Bolivian diet to evaluate their property to reduce glycemia; andean pseudocereal seeds, *Chenopodium quinoa* (quinua), (*CQ*) *Amaranthus caudatus* (amaranto) (*AC*) and *Chenopodium pallidicaule* (kañawa) (*CP*), a legume, *Lupinus mutabilis* (tarwi) (*LM*) and a tuberous root *Smallanthus sonchifolius* (yacon) (*SS*). The pseudocereals are still the principal protein sources of the region; with similar content of essential amino acids[12]. *LM* and *SS* have compounds with therapeutic potential, such as phenolic compounds and antioxidants[13,14]. While *LM* has been reported to lower the blood glucose levels by an unclear mechanism[15-18], potential anti-diabetic properties were already reported for *CQ* that inhibits the α-glucosidase enzyme[19] and for *AC* that inhibits the α-amylase[17,20]. On the other hand, *SS* is an alternative food for patients with conditions that require dietary changes[13,21].

The present study evaluated the glycemia-reducing properties of extracts from *AC*, *CQ*, *CP* and *LM* seeds and the *SS* root in an experimental murine mouse model and investigated their effects on insulin secretion.

Methods

Animals

Male Swiss albino mice, weighing 20 ± 5 g were purchased from the Animal Facility of the Facultad de Ciencias Farmacéuticas y Bioquímicas, Universidad Mayor de San Andrés. Experiments were performed after one week of adaptation in the experimentation unit. Animals were kept at 22 °C with alternating 12 h light- dark cycle and had free access to food and fresh water. The study has ethical approval CEI-UMSA 0115 from the Ethics Committee of the Universidad Mayor de San Andrés[17,18].

Plant Species

Plant species were collected from local producers in Bolivia. *AC*, from Tomina municipality, Tomina Province, Chuquisaca (latitude 19°25´53.96´´S and longitude 64°15´5.44´´W). *CQ and CP* from Huancane (latitude 16°18´14.14´´S and longitude 68°32´35.88´´W) and Peñas (latitude 16°13´55.02´´S and longitude 68°29´41.70´´W) locality, respectively, Batallas municipality, Los Andes Province, La Paz. *LM* from Ancoraimes municipality, Omasuyos Province, La Paz (latitude 15°55´19.3´´S and longitude 68°53´50.1´´W). *SS* from Sorata municipality, Laracaja Province, La Paz (latitude 15°45´12.18´´S and longitude 68°39´19.46´´W). One voucher specimen of each plant, *AC* (No. EG-1, Amaranthaceae), *CQ* (No. EG-2, Amaranthaceae), *CP* (No. EG-3, Amaranthaceae), *LM* (No. EG-1, Fabaceae) and *SS* (No. EG-1, Asteraceae) was identified and certified by the Herbario Nacional de Bolivia from Universidad Mayor de San Andrés and has been deposited at the Department of Pharmacology at the Instituto de Investigaciones Farmaco Bioquímicas, UMSA, La Paz, Bolivia[17,18].

Plant Extracts Preparation

200 g of *AC, CP, CQ and LM*, seeds were powdered and macerated during 48 h in absolute ethanol (EtOH extract), 70% ethanol solution (EtOH70 extract) or water (Aq extract) (250 ml). To maximize the yield,

the maceration procedure was repeated 2 times for Aq extracts and 5 times for EtOH and EtOH70 extracts. *SS*, was prepared with 50 g of dry roots macerated during 48 h in 1000 ml of each solvent, previously mentioned; the maceration procedure was repeated 2 times for Aq extracts and 4 times for EtOH and EtOH70 extracts. Aq extracts were dried under pressure in a freeze dryer (Labconco, USA). EtOH and EtOH70 extracts were first concentrated by a rotary evaporator (Heidolph, Germany) and then dried under pressure in a freeze dryer (Labconco, USA). Crude extracts obtained had an appearance of a light powder with a yield of *AC* 6.5 % w/w, *CQ* 5.6% w/w, *CP* 5.5% w/w, *LM* 22.0% w/w and *SS* 6.0% w/w. For experiments, the extracts were dissolved in water and stock solutions were sterilized by a 0.22 µm Millipore filter, prior to use[17,18].

Phytochemical analysis

EtOH70 extracts from the studied species were screened for the presence of the phytoconstituents; alkaloids, tannins, phenolic compounds, flavonoids, anthocyanidins, saponins and coumarins where determined using standard qualitative procedures[22].

Oral Acute Toxicity

EtOH70 extracts were evaluated for their potential acute oral toxicity according to the guidelines set by the Organization for Economic Cooperation and Development (OECD) guideline No. 423[23]. Male Swiss albino mice (20 ± 5 g) received one single oral dose (2000 mg/kg) of each extract. Mortality and general behavior of treated groups were monitored for 14 days, then blood samples were collected to determine hematological and biochemical parameters.

Cytotoxicity

Cellular toxicity was evaluated in cell line BHK-21 (Hamster Kidney Fibroblast). Briefly, cells were seeded in 96 wells plates at $1x10^5$ cells/ml and were exposed to EtOH70 extracts (400 – 5 mg/ml) diluted in RPMI 1640 medium supplemented with antibiotics (100 IU/mL penicillin and 0.1 mg/mL streptomycin) (Invitrogen, USA) and heat-inactivated fetal calf serum (10%) (Sigma-Aldrich, USA). The cell viability was determined by MTT assay after 24 h[24,25].

Screening for glycemia-reducing effects

The effect of plant extracts on glycemia was evaluated in non-fasted mice by single oral administration by gavage. Several doses were tested: 4000 mg/kg and 2000 mg/kg b.w. for EtOH and Aq extracts and 2000 mg/kg, 1000 mg/kg and 500 mg/kg b.w for EtOH70 extracts of *AC, CQ, CP* and *LM*. For *SS* 1000 mg/kg b.w. for EtOH and Aq extracts and 1000 mg/kg, 500 mg/kg and 250 mg/kg b.w. for EtOH70. For experimentation Aq and EtOH70 extracts were dissolved in distillated water, and EtOH were dissolved in 1.5% water solution of Tween 20. The placebo groups received distillated water and 1.5 % water solution of Tween 20. Blood samples from the tip of the tail were collected 1, 2, 4 and 6 hours, after administration of the extracts[17,18,26]. Glycemia was measured using a glucometer (Clever Check, USA).

Oral Glucose Tolerance Test

Active extracts in screening evaluation were further evaluated during an oral glucose tolerance test (OGTT). The extracts were administrated orally, one hour before the test, to 10 h fasted mice. Blood glucose levels were determined at 0, 15, 30, 60, and 120 minutes after the oral administration of glucose (3 g/kg b.w.). Several doses were tested for *AC, CQ, CP* and *LM* (2000 mg/kg, 1000 mg/kg and 500 mg/kg b.w) and for *SS* (1000 mg/kg, 500 mg/kg and 250 mg/kg b.w). The placebo group received water, and the positive control group received glibenclamide (0.5 mg/kg b.w.)[17,18,26].

Pancreatic Islets Isolation

Mouse pancreatic islets were isolated by collagenase digestion followed by histopaque gradient separation. Briefly, 9 mg of collagenase dissolved in 10 mL of Hank's Balanced Solution (HBBS) were injected through the bile duct to insufflate the pancreas. Then the gland was collected in a test tube and digested in water bath without shaking for 24 min at 37°C. After the digestion, collagenase was washed

away with HBSS and digested tissue was then filtrated through a strainer. Finally, islets were separated by centrifugation at 2000 rpm for 20 min using a mixture of Histopaque 1119 and 1077 (Sigma-Aldrich, USA). For overnight culture, islets were hand-picked using a stereomicroscope and then cultured at 37°C, with an atmosphere of 5% CO2-95% air in RPMI 1640 supplemented with 30 mg L-glutamine (Sigma-Aldrich, USA), 11 mM glucose (Sigma-Aldrich, USA), antibiotics (100 IU/mL penicillin and 0.1 mg/mL streptomycin) (Invitrogen, USA) and heat-inactivated fetal calf serum (10%) (Sigma-Aldrich, USA)[27,28,29].

Islet Insulin Secretion

Overnight cultured islets were preincubated during 30 - 45 min in Krebs-Ringer bicarbonate (KRB) buffer (NaCl 118.4 mM, KCl 4.7 mM, MgSO4 1.2 mM, KH2PO4 1.2 mM, CaCl2 1.9 mM, NaHCO3 25 mM, HEPES 10 mM and 0.2% bovine serum albumin) (Sigma-Aldrich, USA) in 3.3 mM glucose and 37°C with an atmosphere of 5% CO2-95% air. Batches of 3 islets of similar size were incubated during 60 min in 300 µL of KRB in 3.3 mM or 16.7 mM glucose, with or without extracts at different concentrations (5 - 20 mg/mL) at 37°C in a shaking water bath[27,28,29]. After incubation, 200 µl of the solutions were collected to new tubes and kept in freezer −20°C for insulin quantification by ELISA (Thermo Fisher Scientific, USA).

Statistical Analysis

Results are presented as mean ± SEM. Statistical difference between groups was analyzed using two-way analysis of variance (ANOVA) for screening, OGTT and insulin secretion evaluations whereas paired Student's t-test was used for AUC analysis. Bonferoni's Post Hoc Test was used for correction in multiple testing. A *P* value of less than 0.05 was considered as significant. Data were analyzed using Prism Graph Pad Software (CA, USA)[17,18,27].

Results

Qualitative Phytochemical screening

Qualitative phytochemical characterization of crude EtOH70 extracts indicates the presence of phenolic compounds, tannins and antocianidins in all extracts (Table 1). Alkaloids were found only in *LM* and *CQ* extracts; flavonoids and coumarins were present in all extracts but *AC*. No saponins were detected in any of the extracts.

Table 1. Phytochemical screening. Constituents are represented according to their intensity. (-) Absent; (+) Present.

Name of constituents	*AC*	*CQ*	*CP*	*LM*	*SS*
Alkaloids	-	+	-	+++	-
Tannins & Phenolic compounds	+	+++	+	+	+
Flavonoids	-	+	++	++	+
Anthocyanidins	++	+	++	+++	+++
Saponin	-	-	-	-	-
Coumarins	-	++	+++	++	++++

Evaluated extracts have no toxic effect

The 50% cytotoxic concentration (CC_{50}) after 24 h of exposure evaluated by MTT assay were 208 ± 12.4 mg/ml for *AC*, 210 ± 22.2 mg/ml for *CQ,* 180± 11.1 mg/ml for *CP,* 52.3 ± 3.4 mg/ml for *LM* and 15 ± 1.1 mg/ml for *SS.*

No mice died during the observation period of the oral acute toxicity evaluation and normal behavior were noted in all treated animals. No significant differences in hematological parameters (red and white blood cells number, hematocrit and hemoglobin) or in biochemical indicators (aspartate transaminase, alanine transaminase, uric acid, cholesterol, triglycerides) were observed (data not shown).

Screening evaluation showed promising glycemia-reducing plant species

Single administration of extracts of pseudocereals *AC*, *CQ* and *CP* lowered the levels of glycemia in mice (Table 2).

Table 2. The effect of single oral administration of pseudocereals AC, CP and CQ extracts on glycemia in non-fasted mice (n=10)

Time (h)	0	1	2	4	6
AC EtOH70 extract (mg/kg b.w.)					
2000	9.5±0.4	8.6±0.5$^{\#}$	7.8±0.3$^{*\#\#\#}$	7.0±0.3$^{***\#\#\#\#}$	6.9±0.5$^{****\#\#\#\#}$
1000	9.2±0.2	9.0±0.3	9.0±0.5$^{\#}$	8.8±0.6	8.6±0.5
500	9.5±0.2	10.0±0.6	8.8±0.5	8.3±0.5$^{\#}$	8.2±0.2
AC Aq extract (mg/kg b.w.)					
4000	9.5±0.7	9.2±0.7	9.5±0.5	9.7±0.6	9.2±0.6
2000	9.6±0.8	9.4±0.8	9.6±0.6	9.8±0.5	9.3±0.6
AC EtOH extract (mg/kg b.w.)					
4000	10.5±0.6	9.1±0.8	9.0±0.7	6.6±0.5	8.4±0.2$^{****\#\#\#\#}$
2000	11.1±0.9	10.8±0.9	11.1±0.7	11.3±0.6	10.7±0.7
CQ EtOH70 extract (mg/kg b.w.)					
2000	10.0±0.2	9.3±0.2	8.9±0.1$^{\#}$	8.7±0.3$^{\#}$	9.3±0.3
1000	8.2±0.8	9.2±1	8.9±0.9$^{\#}$	8.4±0.9$^{\#}$	7.9±0.8
500	9.1±0.3	10.4±0.4	9.7±0.2	9.3±0.3	8.5±0.3
CQ Aq extract (mg/kg b.w.)					
4000	9.0±0.3	8.5±0.3$^{\#\#}$	8.8±0.3$^{\#\#}$	9.2±0.3	8.9±0.2
2000	8.8±0.3	8.9±0.3$^{\#}$	9.5±0.3	9.3±0.4	9.1±0.3
CQ EtOH extract (mg/kg b.w.)					
4000	11.1±0.5	10.0±0.3	9.2±0.2$^{**\#}$	9.5±0.3*	9.7±0.3*
2000	10.5±0.2	9.7±0.3	9.4±0.2	8.6±0.3$^{**\#}$	9.5±0.4
CP EtOH70 extract (mg/kg b.w.)					
2000	9.7±0.2	9.2±0.1$^{\#}$	9.0±0.2$^{\#}$	8.9±0.2$^{\#}$	9.1±0.3
1000	10.0±0.3	9.9±0.1	10.3±0.4	9.0±0.2	8.9±0.4
500	10.0±0.2	9.4±0.2	9.9±0.1	9.3±0.1	9.0±0.3
CP Aq extract (mg/kg b.w.)					
4000	9.3±0.3	9.1±0.1	9.4±0.2	9.9±0.3	9.1±0.1
2000	10.2±0.7	9.7±0.5	9.8±0.3	9.5±0.4	9.6±0.5
CP EtOH extract (mg/kg b.w.)					
4000	8.7±0.2	8.3±0.3$^{\#\#}$	8.1±0.2$^{\#\#\#}$	7.9±0.2$^{\#\#}$	7.8±0.1$^{\#\#}$
2000	10.4±0.7	9.9±0.5	10.0±0.3	9.7±0.5	9.7±0.5
Placebo					
1,5% Tween 20	10,7±0.3	10,4±0.2	10,2±0.3	10±0.4	9,6±0.4
H_2O	10.2±0.2	10.1±0.3	10.4±0.5	9.9±0.6	9.5±0.6

Data are presented as means ± SE (n = 10). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ compared to time 0; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ compared to placebo group at the same time point.

LM EtOH70 extract reduced glycemia 2 - 6 h after treatment in a dose-dependent manner (Table 3).

Table 3. The effect of single oral administration of *LM* extracts on glycemia in non-fasted mice (n=10)

Time (h)	0	1	2	4	6
LM EtOH70 extract (mg/kg b.w.)					
2000	9.0±0.6	8.0±0.4$^{\#}$	7.7±0.6$^{\#\#}$	7.2±0.8$^{\#\#\#}$	7.1±0.4$^{*\#\#}$
1000	9.8±0.6	9.2±0.6	8.2±0.6$^{\#\#}$	6.4±0.5$^{****\#\#\#\#}$	6.9±0.3$^{***\#\#}$
500	10.3±0.3	10.0±0.4	9.4±0.4	8.2±0.6*	7.2±0.5$^{***\#\#}$
LM Aq extract (mg/kg b.w.)					
4000	10.0±0.7	9.7±0.6	9.6±0.5	9.9±0.5	9.2±0.6
2000	9.7±0.2	9.5±0.2	9.5±0.5	9.3±0.5	9.3±0.3
LM EtOH extract (mg/kg b.w.)					
4000	9.8±0.3	10.1±0.5	8.8±0.3	8.7±0.4	8.1±0.5
2000	10.0±0.8	9.8±0.8	10.0±0.7	10.2±0.5	9.7±0.6
Placebo					
1,5% Tween 20	10,7±0.3	10,4±0.2	10,2±0.3	10±0.4	9,6±0.4
H_2O	10.2±0.2	10.1±0.3	10.4±0.5	9.9±0.6	9.5±0.6

Data are presented as means ± SE (n = 10). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ compared to time 0; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ compared to placebo group at the same time point.

No effect was observed in groups treated with *LM* EtOH and *LM* Aq extracts. *SS* EtOH70 extract (1000 mg/kg b.w.) reduced glycemia 2 - 4 h after treatment (Table 4). No effect was observed in groups treated with *SS* EtOH and Aq extracts.

Table 4. The effect of single oral administration of *SS* extracts on glycemia in non-fasted mice (n=10).

Time (h)	0	1	2	4	6
SS EtOH70 extract (mg/kg b.w.)					
1000	12.4±0.3	10.9±0.4	10.1±0,3**	8.7±0.4$^{****\#}$	10.7±0.6
500	12.4±0.6	10.7±0.5	9.3±0.5$^{***\#\#}$	9.7±0.8**	10.3±0.4*
250	12.6±0.5	11.6±0.7	10.1±0.4#	9.9±0.2	10.2±0.5
SS Aq extract (mg/kg b.w.)					
1000	11.6±0.4	10.2±0.7	10.1±0.3	8.9±0.7	9.7±0.5
SS EtOH extract (mg/kg b.w.)					
1000	11.6±0.8	10.4±0.5	9.2±0.4	8.6±0.6	9.6±0.5
Placebo					
1,5% Tween 20	11.5±0.2	11.0±0.2	10.7±0.4	10.8±0.5	10,6±0.7
H_2O	12.7±0.7	11.5±0.1	10.6±0.5	11.0±0.9	10.8±0.8

Data are presented as means ± SE (n = 10). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ compared to time 0; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ compared to placebo group at the same time point.

Extracts improved glucose tolerance in glucose-challenged mice

The extracts identified to have glucose lowering effect in screening evaluation were further tested in the OGTT. Among the pseudocereals, the *AC* EtOH70 extract (2000 mg/kg b.w.) improved the glucose tolerance (Figure 1A - 1B). The effect was dose-dependent and comparable to that of glibenclamide (0.5 mg/kg b.w.). The *CQ* EtOH70 extract (2000 mg/kg b.w.) improved also glucose tolerance compared to the placebo (Figure 1C - 1D).

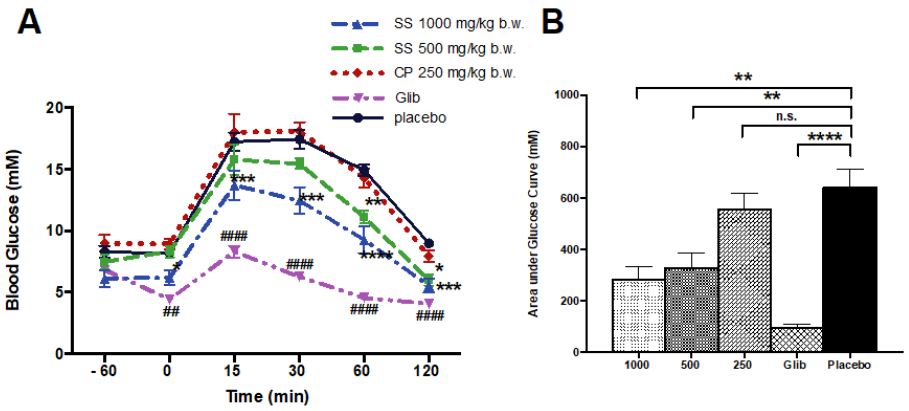

Figure 1. Effect of pseudocereals on glycemia levels during OGTT performed in fasted mice. Male Swiss mice (20 ± 5g), fasted for 12 h received *AC* (A), *CQ* (C) and *CP* (E) EtOH70 extracts, one hour before glucose-challenge, and glycemia was determined at 0, 15, 30, 60 and 120 min. The area under the curve was calculated from time 0 to 120 min for *AC* (B), *CQ* (D) and for *CP* (F). Data are presented as means ± SE (n = 10). **p< 0.05*, ** *p<0.01*, *** *p<0.001*, **** *p<0.0001* compared to placebo group.

Both *CP* (Figure 1E – 1F) and *LM* (Figure 2A – 2B) EtOH70 extracts improved glucose tolerance in specific time points after glucose challenge, but *CP* effect was not of enough magnitude to decrease the AUC of glucose.

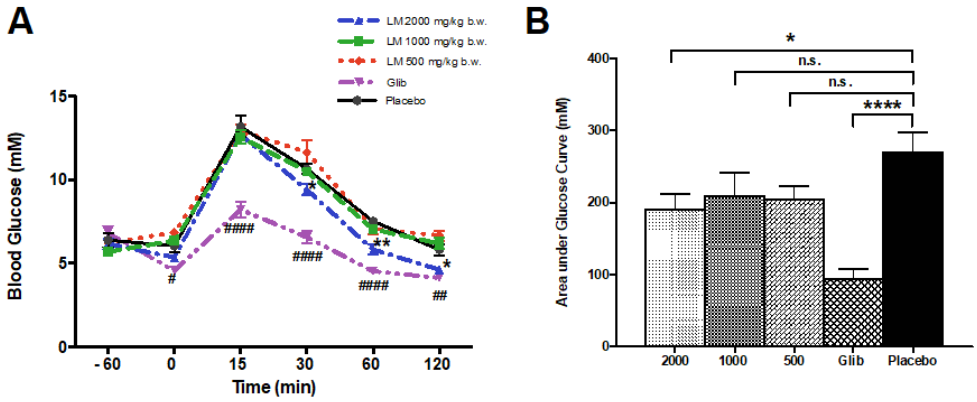

Figure 2. Effect of *LM* on glycemia levels during OGTT in fasted mice. Male Swiss mice (20 ± 5g) fasted for 12 h received *LM* EtOH70 extract (A) one hour before the OGTT and glycemia was determined at 0, 15, 30, 60 and 120 min. The area under the curve was calculated from time 0 to 120 min (B). Data are presented as means ± SE (n = 10). **p< 0.05*, ** *p<0.01*, *** *p<0.001*, **** *p<0.0001* compared to placebo group.

Finally, the *SS* EtOH70 extract (1000 mg/kg b.w.) improved the glucose tolerance in a dose-dependent manner (Figure 3A - 3B).

LM, *AC* and *CQ* EtOH70 extracts stimulate islets insulin secretion

Active extracts stimulated the *in vitro* insulin secretion in high glucose conditions (16.7 mM) by exposure to *AC* EtOH70 extract (Figure 4A), *CQ* EtOH70 extract (Figure 4 B) and *LM* EtOH70 extract (Figure 4 C) in concentration-dependent manner.

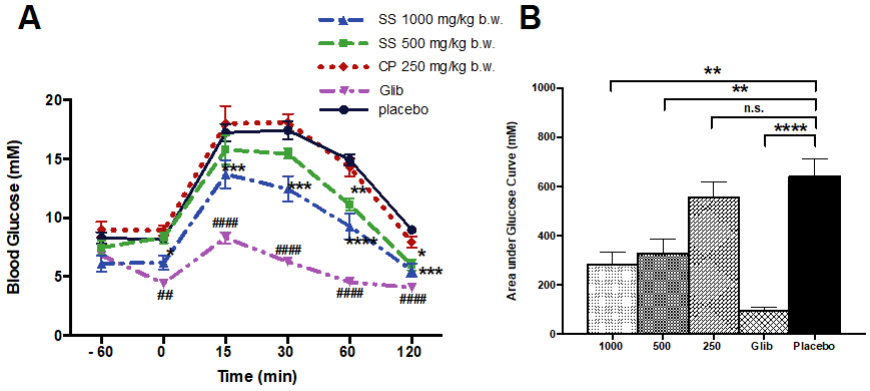

Figure 3. Effect of SS on glucose levels during OGTT in fasted mice. Male Swiss mice (20 ± 5g) fasted for 12 h received *SS* EtOH70 extract (A) one hour before OGTT and glycemia was determined at 0, 15, 30, 60 and 120 min. The area under the curve was calculated from time 0 to 120 min (B). Data are presented as means ± SE (n = 10). **p< 0.05*, *** p<0.01*, **** p<0.001*, ***** p<0.0001* compared to placebo group.

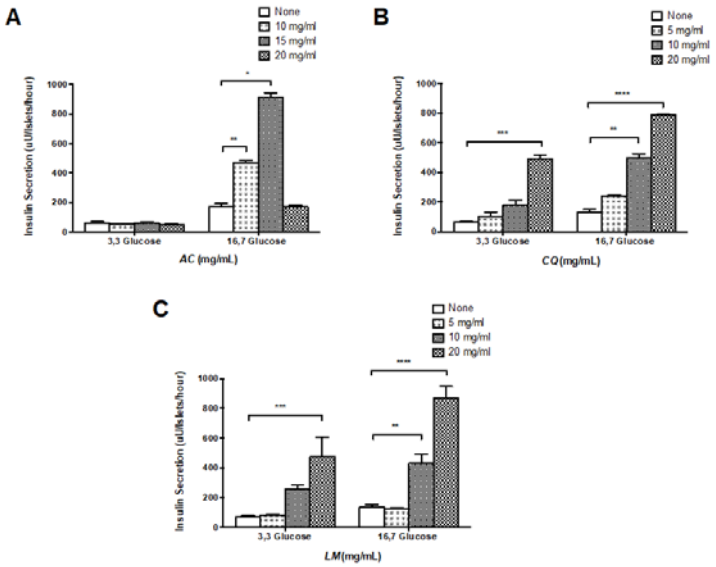

Figure 4. Effect of *LM*, *CQ* and *AC* on islets insulin secretion. The insulin secretion was evaluated in Swiss mice islets cultured at low (3.3 mM) and high (16.7 mM) glucose in presence of EtOH70 extracts of *LM* (5, 10 and 20 mg/ml) (A), *CQ* (5, 10 and 20 mg/ml) (B) and *AC* (10, 15 and 20 mg/ml) (C). Insulin concentration was measured by ELISA. The data are presented as means ± SE (n = 3), of duplicates from three independent experiments. **p< 0.05*, *** p<0.01*, **** p<0.001*, ***** p<0.0001* compared to negative control.

Discussion

Our results show that *AC*, *CQ*, *CP*, *LM* and *SS* have glycemia reducing effect in both non-fasted and in glucose-challenged mice. The effect was comparable to that of glibenclamide. Moreover, we show that the glucose-reducing effect of *LM*, *CQ* and *AC* is at least partially explained by the stimulation of insulin secretion.

Three kinds of extracts of each plant species were evaluated. During both, screening and OGTT experiments, in healthy animals; the EtOH70 extracts of *AC*, *CQ*, *LM* and *SS* showed major and significant glucose-reducing effect. Additionally, a slight but significant effect was found in for *CP* EtOH70 and *CQ* Aq extract. OGTT was performed with EtOH70 extracts, the most active ones. Extract preparation is an important factor that should be considered to assess the plant effects. If the purpose is to mimic the way that the plant is consumed, for the studied plants, the aqueous extract is the closest way to its use. However, we found that a mixture of water with ethanol enrich the extract with organic compounds, which are responsible for the effect.

The qualitative phytochemical analysis performed with EtOH70 extracts confirmed previous reports by identifying phenolic acids, and flavonoids for andean indigenous grains (*AC*, *CQ* and *CP*)[28,30] and alkaloids for *LM*[29,15]. For *SS* we found the presence of anthocyanidins, coumarins and phenolic compounds, constituents also previously reported[21].

The toxicity studies of EtOH70 extracts, were performed considering the OECD guidelines, that suggest to treat animals with doses equal or lower than 2000 mg/kg b.w. None of the EtOH70 extracts showed acute toxic effects; *in vitro* evaluation established parameters of safe concentrations. However, further evaluations need to be performed regarding possible sub-acute and chronic toxicity effect.

Amaranthus caudatus (*AC*) is a pseudocereal native of the andean Bolivian region. Its seeds are usually consumed in beverages, foods such soups or bread or as toasted flour[31]. Traditional medicine properties attributed to *AC* are "blood purifier", diuretic, and "digestive"[32]. Methanol extract of *AC* leaves has been reported to exert anti-diabetic activity in rats with streptozotocin (STZ)-induced diabetes[33]. Additionally, *AC* seeds inhibited α-amylase[34] and showed significant antioxidant activity[20]. In a previous study, using a non-obese diabetic model the Goto-kakisaki (GK) rats, the EtOH70 extract of *AC* improved the glucose tolerance[17,28]; in the present study our results confirm the glycemia-reducing effect for the seeds (EtOH70 extract) both in non-fasting mice as well during OGTT.

Chenopodium quinoa (*CQ*) seeds are also used in food preparations as a part of the diet of rural Bolivian families, including a variety of soups and refreshments[35]. Phenolic compounds responsible for the antioxidative activity represent the nutraceutical potential of *CQ*[36,37]. In addition, *CQ* inhibits α-glucosidase with no effect on pancreatic α–amylase[9]. In high fructose-treated Wistar rats, *CQ* reduced most of the adverse effects exerted by fructose on lipid profile and glycemia[38]. Our results confirmed the glycemia-reducing properties of *CQ,* for all kinds of extracts evaluated which improved the glucose levels both in non-fasting animals and during glucose-challenged mice.

Lupinus mutabilis (*LM*) seeds are usually consumed as washed and cooked seeds. The washing procedure abolishes the spicy taste that is attributed to the content in alkaloids[15]. To reduce glycemia in patients with diabetes the traditional use is to drink the water in which the seeds were washed[39]. It has been reported that *LM* decreases glycemia in patients with intolerance to glucose[16], and in patients with type 2 diabetes due to the alkaloid content[15]. For this purpose we used unwashed seeds with high alkaloid content that has been claimed to be active component. Furthermore, were did not cooked the seeds to preserve labile components. In a previous study we found that *LM* EtOH70 extract improves glucose tolerance in diabetic GK rats[29]. In this study we confirmed the effect of *LM* EtOH70 on the glucose tolerance, effect that could be attributed to its alkaloids content.

Smallanthus sonchifolius (*SS*) root is consumed in rural valley regions where is well known for their properties to reduce weight[40], due to their high content of components that cannot be digested (inulin and fructooligosacharides)[13,41,42]. Our findings showed also glycemia-reducing effect in non-fasting animals and glucose-challenged mice.

Chenopodium pallidicaule (*CP*) seeds are usually consumed in soups and refreshments[12].*CP* antioxidant properties and composition is closely related to *CQ* and *AC* as they belong from the same family[30,43,44]. In our evaluations, the extracts show a glycemia-reducing effect both during screening evaluations and during the glucose-challenged model that warrants further studies on the nutraceutical potential of *CP*.

To explore the mechanism behind the glycemia-reducing effect found in animals, experiments of insulin secretion by mice islets were performed in the presence of active EtOH70 extracts. *AC* promoted insulin secretion at high glucose (16.7 mM) but not at low (3.3 mM) glucose levels which is ideal for a potential therapeutic use, similar results to the previous findings[17,28]. *CQ* and *LM* stimulated insulin secretion however in both low and high glucose and these effects were concentration-dependent, effect similar with *sulphonylurea* drugs that have been used for decades for treatment of diabetes. *LM* effect seems to be non-glucose dependent, findings that could induce a hypoglycemic state. However, similar results were found in GK rats, where *LM* did not exert hypoglycemia[17,29]. No effect on insulin secretion was found for *CP* and *SS* (data not shown); it is therefore important to further explore their effect on the insulin sensitivity or glucose absorption since they exhibit clear effects on glycemia. The concentrations evaluated for all the extracts were below the CC_{50} found in cell cultures for 24 h.

Conclusion

We provide information about the glycemia-reducing properties of Bolivian food plants *Amaranthus caudatus*, *Chenopodium quinoa*, *Chenopodium pallidicaule*, *Lupinus mutabilis* and *Smallanthus sonchifolius* that exhibit glucose lowering effect, while only *Amaranthus caudatus*, *Chenopodium quinoa* and *Lupinus mutabilis* stimulate directly the insulin secretion. All studied plants are potential nutraceuticals for improving the glycemia when needed.

References

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Research and Clinical Practice.128:40-50. doi: 10.1016/j.diabres.2017.03.024

2. Alejandro R-H, María JC, Huguette R-O, Eduardo M, Guillermo C, Gabriela G-S. Obesity, Metabolic Syndrome, and Dietary Therapeutical Approaches with a Special Focus on Nutraceuticals (Polyphenols): A Mini-Review. International Journal for Vitamin and Nutrition Research. 2014;84(3-4):0113-23. doi: 10.1024/0300-9831/a000198

3. Wilding JP. The importance of weight management in type 2 diabetes mellitus. International journal of clinical practice. 2014;68(6):682-91. doi: 10.1111/ijcp.12384

4. Stanhope KL. Sugar consumption, metabolic disease and obesity: The state of the controversy. Critical Reviews in Clinical Laboratory Sciences. 2016;53(1):52-67. doi: 10.3109/10408363.2015.1084990

5. Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, et al. Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. Frontiers in Endocrinology. 2017;8:6. doi: 10.3389/fendo.2017.00006

6. Brown L, Poudyal H, Panchal SK. Functional foods as potential therapeutic options for metabolic syndrome. Obesity Reviews. 2015;16(11):914-41. doi: 10.1111/obr.12313

7. Magrone T, Perez de Heredia F, Jirillo E, Morabito G, Marcos A, Serafini M. Functional foods and nutraceuticals as therapeutic tools for the treatment of diet-related diseases. Canadian journal of physiology and pharmacology. 2013;91(6):387-96. doi: 10.1139/cjpp-2012-0307

8. Das L, Bhaumik E, Raychaudhuri U, Chakraborty R. Role of nutraceuticals in human health. Journal of food science and technology. 2012;49(2):173-83. doi: 10.1007/s13197-011-0269-4

9. Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. Journal of diabetes and metabolic disorders. 2013;12(1):43. doi: 10.1186/2251-6581-12-43

10. Gonzalez-Sarrias A, Larrosa M, Garcia-Conesa MT, Tomas-Barberan FA, Espin JC. Nutraceuticals for older people: facts, fictions and gaps in knowledge. Maturitas. 2013;75(4):313-34. doi: 10.1016/j.maturitas.2013.05.006

11. Brayden DJ, Baird AW. Opportunities for drug-delivery research in nutraceuticals and functional foods? Therapeutic delivery. 2013;4(3):301-5. doi: 10.4155/tde.12.152

12. Repo-Carrasco R, Espinoza C, Jacobsen SE. Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kaniwa (*Chenopodium pallidicaule*). Food Rev Int. 2003;19(1-2):179-89. doi: 10.1081/FRI-120018884

13. Caetano BFR, de Moura NA, Almeida APS, Dias MC, Sivieri K, Barbisan LF. Yacon (Smallanthus sonchifolius) as a Food Supplement: Health-Promoting Benefits of Fructooligosaccharides. Nutrients. 2016;8(7):436. doi: 10.3390/nu8070436

14. Dini I, Schettino O, Dini A. Studies on the constituents of *Lupinus mutabilis* (Fabaceae). Isolation and characterization of two new isoflavonoid derivatives. J Agr Food Chem. 1998;46(12):5089-92. doi:10.1021/jf980386l

15. Baldeon ME, Castro J, Villacres E, Narvaez L, Fornasini M. Hypoglycemic effect of cooked Lupinus mutabilis and its purified alkaloids in subjects with type-2 diabetes. Nutricion hospitalaria. 2012;27(4):1261-6. doi: 10.3305/nh.2012.27.4.5761

16. Fornasini M, Castro J, Villacres E, Narvaez L, Villamar MP, Baldeon ME. Hypoglycemic effect of Lupinus mutabilis in healthy volunteers and subjects with dysglycemia. Nutricion hospitalaria. 2012;27(2):425-33. doi: 10.1590/S0212-16112012000200012

17. Zambrana Santander ST. Evaluation of potential antidiabetic and antibacterial activities of Bolivian nutraceutical plants. Karolinska Institutet; 2019.

18. Mamani Calle O, Gonzales Davalos E, Zambrana Santander S. Evaluación de la modulación de la secreción de insulina en islotes pancreáticos y captación de glucosa en adipocitos mediado por Smallanthus sonchifolius y Lupinus mutabilis: Universidad Mayor de San Andres. Facultad de Ciencias Farmaceuticas y Bioquímicas; 2017.

19. Hemalatha P, Bomzan DP, Sathyendra Rao BV, Sreerama YN. Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities. Food chemistry. 2016;199:330-8. doi: 10.1016/j.foodchem.2015.12.025

20. Kumar A, Lakshman K, Jayaveera KN, Sheshadri Shekar D, Narayan Swamy VB, Khan S, et al. In Vitro alpha-Amylase Inhibition and Antioxidant Activities of Methanolic Extract of Amaranthus Caudatus Linn. Oman medical journal. 2011;26(3):166-70. doi: 10.5001/omj.2011.40

21. Choque Delgado GT, da Silva Cunha Tamashiro WM, Maróstica Junior MR, Pastore GM. Yacon (Smallanthus sonchifolius): A Functional Food. Plant Foods for Human Nutrition. 2013;68(3):222-8. doi: 10.1007/s11130-013-0362-0

22. Harborne J. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall; 1998. doi:10.1046/j.1365-3059.1999.00318.x

23. Oecd. Test No. 423: Acute Oral toxicity - Acute Toxic Class Method: OECD Publishing. doi:10.1787/9789264071001-en

24. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983;65(1-2):55-63. doi: 10.1016/0022-1759(83)90303-4

25. Catrina S-B, Catrina AI, Sirzén F, Griffiths W, Bergman T, Biberfeld P, et al. A cytotoxic, apoptotic, low-molecular weight factor from pineal gland. Life Sciences. 1999;65(10):1047-57.

26. Hoa NK, Phan DV, Thuan ND, Ostenson CG. Screening of the hypoglycemic effect of eight Vietnamese herbal drugs. Methods and findings in experimental and clinical pharmacology. 2009;31(3):165-9. doi: 10.1358/mf.2009.31.3.1362514

27. Hoa NK, Norberg A, Sillard R, Van Phan D, Thuan ND, Dzung DT, et al. The possible mechanisms by which phanoside stimulates insulin secretion from rat islets. Journal of Endocrinology. 2007;192(2):389-94. doi: 10.1677/joe.1.06948

28. Zambrana S, Lundqvist LCE, Veliz V, Catrina SB, Gonzales E, Östenson CG. Amaranthus caudatus Stimulates Insulin Secretion in Goto-Kakizaki Rats, a Model of Diabetes Mellitus Type 2. Nutrients. 2018;10(1). doi: 10.3390/nu10010094

29. Zambrana S, Lundqvist LCE, Mamani O. Lupinus mutabilis Extract Exerts an Anti-Diabetic Effect by Improving Insulin Release in Type 2 Diabetic Goto-Kakizaki Rats. 2018;10(7). doi:10.3390/nu10070933

30. Repo-Carrasco-Valencia R, Hellstrom JK, Pihlava JM, Mattila PH. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (Chenopodium quinoa), kaniwa (Chenopodium pallidicaule) and kiwicha (Amaranthus caudatus). Food chemistry. 2010;120(1):128-33. doi:10.1016/j.foodchem.2009.09.087

31. Escudero NL, Albarracin G, Fernandez S, De Arellano LM, Mucciarelli S. Nutrient and antinutrient composition of Amaranthus muricatus. Plant foods for human nutrition. 1999;54(4):327-36. doi: 10.1023/a:1008149721435

32. Girault L, Bustillos C, Alcocer R. Kallawaya: Curanderos Itinerantes de los andes, Investigación sobre prácticas medicinales y mágicas. Unicef; 1987.

33. Girija K, Lakshman K, Udaya C, Sabhya SG, Divya T. Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of Amaranthus. Asian Pacific journal of tropical biomedicine. 2011;1(2):133-8. doi: 10.1016/S2221-1691(11)60011-7

34. Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F. In Vitro antioxidant effect and inhibition of alpha-amylase of two varieties of Amaranthus caudatus seeds. Biological & pharmaceutical bulletin. 2005;28(6):1098-102. doi: 10.1248/bpb.28.1098

35. Cardozo A, Aliaga viuda de Vizcarra I. Bibliografía sobre la quinua (Chenopodium quinoa Willd.). La Paz, Bolivia1967. 10 l. p.

36. Gawlik-Dziki U, Swieca M, Sulkowski M, Dziki D, Baraniak B, Czyz J. Antioxidant and anticancer activities of Chenopodium quinoa leaves extracts - in vitro study. Food and chemical toxicology. 2013;57:154-60. doi: 10.1016/j.fct.2013.03.023

37. Filho AMM, Pirozi MR, Borges JTDS, Pinheiro Sant'Ana HM, Chaves JBP, Coimbra JSDR. Quinoa: Nutritional, functional, and antinutritional aspects. Critical Reviews in Food Science and Nutrition. 2017;57(8):1618-30. doi: 10.1080/10408398.2014.1001811

38. Pasko P, Zagrodzki P, Barton H, Chlopicka J, Gorinstein S. Effect of quinoa seeds (Chenopodium quinoa) in diet on some biochemical parameters and essential elements in blood of high fructose-fed rats. Plant foods for human nutrition. 2010;65(4):333-8. doi: 10.1007/s11130-010-0197-x

39. Jacobsen S-EM, Angel. El tarwi (Lupinus mutabilis Sweet.) y sus parientes silvestres. Botánica Económica de los Andes Centrales: Universidad Mayor de San Andrés; 2006. p. 458 - 82.

40. Honor, xe, Maris S, Grande MV, Gomez Rojas J, xe, et al. Smallanthus sonchifolius (Yacon) Flour Improves Visceral Adiposity and Metabolic Parameters in High-Fat-Diet-Fed Rats. Journal of Obesity. 2018;2018:15. doi.org/10.1155/2018/5341384

41. Lachman J, Havrland B, Fernandez EC, Dudjak J. Saccharides of yacon Smallanthus sonchifolius (Poepp. et Endl.) H. Robinson tubers and rhizomes and factors affecting their content. Plant Soil Environ. 2004;50(9):383-90. doi: 10.17221/4048-PSE

42. Genta SB, Cabrera WM, Mercado MI, Grau A, Catalan CA, Sanchez SS. Hypoglycemic activity of leaf organic extracts from Smallanthus sonchifolius: Constituents of the most active fractions. Chemico-biological interactions. 2010;185(2):143-52. doi: 10.1016/j.cbi.2010.03.004

43. Repo-Carrasco-Valencia R, de La Cruz AA, Alvarez JCI, Kallio H. Chemical and Functional Characterization of Kaiwa (Chenopodium pallidicaule) Grain, Extrudate and Bran. Plant foods for human nutrition. 2009;64(2):94-101. doi: 10.1007/s11130-009-0109-0

44. Penarrieta JM, Alvarado JA, Akesson B, Bergenstahl B. Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (Chenopodium pallidicaule): an Andean pseudocereal. Molecular nutrition & food research. 2008;52(6):708-17. doi: 10.1002/mnfr.200700189