IN VITRO HYPOGLYCEMIC AND ANTIOXIDANT ACTIVITIES OF SOME MEDICINAL PLANTS USED IN TREATMENT OF DIABETES IN SOUTHERN ECUADOR

Ximena Jaramillo-Fierro[1]*, Santiago Ojeda-Riascos[1]

[1]Universidad Técnica Particular de Loja, Departamento de Química y Ciencias Exactas, , P.O, Loja-Ecuador.

*Autor para correspondencia: e-mail: xvjaramillo@utpl.edu.ec

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RESUMEN

En el proceso de identificar plantas promisorias con actividades hipoglucémicas y antioxidantes, evaluamos las actividades inhibidoras de la α-glucosidasa y la α-amilasa, el contenido fenólico soluble total (TSPC), la actividad de eliminación de radicales libres (DPPH, ABTS) y la capacidad inhibidora de la autooxidación linoleica, de doce plantas utilizadas en la medicina tradicional del Ecuador: *Siparuna eggersi* (Monte de oso), *Croton wagneri* (Moshquera), *Ilex guayusa* (Guayusa), *Baccharis genistelloides* (Tres filos), *Neonelsonia acuminada* (Zanahoria blanca), *Oreocallis grandiflora* (Cucharillo), *Verbena litoralis* (Verbena), *Justicia colorata* (Insulina), *Artocarpus altilis* (Fruto del pan), *Adiantun poiretii* (Culantrillo), *Costus comosus* (Caña agria) y *Piper crassinervium* (Guabiduca). *O. grandiflora* se encontró superior a las otras plantas, especialmente en términos de su actividad inhibidora sobre α-glucosidasa (IC_{50} = 2.8 ± 0.4 µg / mL) y α-amilasa (IC_{50} = 161.5 ± 1.3 µg / mL), así como para los radicales libres (DPPH, ABTS) actividad de barrido ($IC_{50\text{-}DPPH}$ = 9.9 ± 0.06 µg / mL; $IC_{50\text{-}TEAC}$ = 6.6 ± 0.55 µg / mL). Por otro lado, *A. altilis* tuvo la mayor capacidad inhibidora de la autooxidación linoleica ($IC_{50\text{-}β\text{-}CLAMS}$ = 3.1 ± 0.20 µg / mL), y *O. grandiflora,* nuevamente, obtuvo el valor más alto de contenido fenólico soluble total (TSPC = 185.9 ± 0.09 GAE / g extracto). Estos hallazgos sugieren que *O. grandiflora* podría considerarse como un agente inhibidor y antioxidante enzimático alternativo para el tratamiento de la diabetes mellitus.

Palabras clave: α-amilasa, α-glucosidasa, DPPH, TEAC, β-CLAMS, FOLIN-CIOCALTEU

ABSTRACT

In the course of identifying promissory plants with hypoglycemic and antioxidant activities we evaluated the α-glucosidase and α-amylase inhibitory activities, the total soluble phenolic content (TSPC), the free radicals (DPPH, ABTS) scavenging activity and the linoleic autoxidation inhibitory capacity, of twelve plants used in traditional medicine of Ecuador: *Siparuna eggersi* (Monte de oso), *Croton wagneri* (Moshquera), *Ilex guayusa* (Guayusa), *Baccharis genistelloides* (Tres filos), *Neonelsonia acuminate* (Zanahoria blanca), *Oreocallis grandiflora* (Cucharillo), *Verbena litoralis* (Verbena), *Justicia colorata* (Insulina), *Artocarpus altilis* (Fruto del pan), *Adiantun poiretii* (Culantrillo), *Costus comosus* (Caña agria) and *Piper crassinervium* (Guabiduca).The study has shown that *O. grandiflora* was superior in comparison to the others plants, especially, in terms of its inhibitory activity on α-glucosidase (IC_{50} =

2.8 ± 0.4 µg/mL) and α-amylase (IC_{50} = 161.5 ± 1.3 µg/mL), as well as for free radicals (DPPH, ABTS) scavenging activity ($IC_{50\text{-}DPPH}$ = 9.9 ± 0.06 µg/mL; $IC_{50\text{-}TEAC}$ = 6.6 ± 0.55 µg/mL). On the other hand, *A. altilis* had the major linoleic autoxidation inhibitory capacity ($IC_{50\text{-}\beta\text{-}CLAMS}$ = 3.1 ± 0.20 µg/mL), and *O. grandiflora* again had the highest value of total soluble phenolic content (TSPC = 185.9 ± 0.09 GAEs/g extract). These findings suggest that *O. grandiflora* might be considered as an alternative enzyme inhibitory and antioxidative agent for the treatment of diabetes mellitus.

Keywords: α-amylase, α-glucosidase, DPPH, TEAC, β-CLAMS, FOLIN-CIOCALTEU

INTRODUCTION

Diabetes is recognized as a group of heterogeneous disorders with the common elements of hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action, or both (Hernandez-Galicia et al., 2002) (Shai et al., 2010).

Anti-diabetic or hypoglycemic compound or composition, generally refers to an agent that lowers blood glucose levels. In traditional medicine, diabetes mellitus is treated with diet, physical exercise and medicinal plants (Alarcón-Aguilar, Roman-Ramos, Flores-Sánchez, & Aguirre-García, 2002). More than 1200 plant species from 725 genera and 183 families have been used in ethnopharmacology or experimentally around the world in the control of diabetes mellitus (Hasenah, Houghton, & Soumyanath, 2006); and, approximately, 30% of the traditionally used antidiabetic plants has been pharmacologically and chemically investigated (Alarcón-Aguilar et al., 2002; Andrade-Cetto & Heinrich, 2005; Rao, Sudarshan, Rajasekhar, Nagaraju, & Rao, 2002; Soumyanath, 2006) (Deutschländer, Lall, Venter, & Dewanjee, 2012).

There are more than 200 pure compounds from plant sources that have been reported to show blood glucose lowering activity (Hasenah et al., 2006). An interesting finding is that plants typically have more than one active component, often associated with more than one mode of action. Certain groups, such as alkaloids, saponins, xanthones and flavonoids, and non starch polysaccharides, appear to have effects of particular significance in diabetes treatment, therefore the identification of activities and modes of action are important for drug development, and for the validation, standardization, and rational use of traditional herbal remedies (Soumyanath, 2006).

The mechanisms involved in hypoglycemic activity from antidiabetic plants are numerous, including direct competitive antagonism with insulin, stimulation of insulin secretion, stimulation of glycogenesis and hepatic glycolysis, pancreatic beta cell potassium channel blockers, cAMP stimulation, among others. (Liu et al., 2017). Another therapeutic approach of medicinal plants for treating diabetes is to decrease the post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. It is now believed that inhibition of these enzymes involved in the digestion and absorption of carbohydrates can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet, and therefore can be an important and potentially natural and safe approach in the management of type 2 diabetes as well as chronic vascular complications (McCue, Kwon, & Shetty, 2004; Shim et al., 2003). Examples of such inhibitors, which are in clinical use, are acarbose, miglitol, emigitate, voglibose, (Bailey, 2003; Onal, Timur, Okutucu, & Zihnioglu, 2005). These inhibitors are widely used, as monotherapy as well as combination therapy with other antidiabetic agents (Fujisawa, Ikegami, Inoue, Kawabata, & Ogihara, 2005). A main drawback of currently used α-glucosidase and α-amylase inhibitors are side effects such as abdominal distention, flatulence, meteorism and possibly diarrhea. It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic **α**-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Kwon, Apostolidis, & Shetty, 2007). Therefore, it becomes necessary to identify glucosidase inhibitors, from natural sources, having lesser side-effects (Conforti et al., 2005); (Bhat, Zinjarde, Bhargava, Kumar, & Joshi, 2008).

Diabetes is a major risk factor for premature atherosclerosis, and oxidative stress plays an important role (Conforti et al., 2005). In fact, numerous studies demonstrated that oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and related contributions, thus it became clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications (Cunningham, 1998; Johansen, Harris, Rychly, & Ergul, 2006); (Kaleem et al., 2006).

Several valuable reviews on the ethnobotanical use of plants of Southern Ecuador are available (Bejár, Russman, Roa, & Sharon, 2002; Bussmann & Sharon, 2006; PFN-133, 2006; Tene et al., 2007), nevertheless, studies dedicated to either antioxidants or antidiabetics have been published so far. The objective of this investigation was to ascertain the scientific basis for the use of plants in the treatment of diabetes mellitus. Therefore, this study was designed to investigate the α-glucosidase and α-amylase inhibitory capacity, free radicals (DPPH, ABTS) scavenging activity and linoleic autoxidation inhibitory capacity of twelve plants advocated in traditional Southern Ecuador medicine.

MATERIALS AND METHODS

Chemicals

α-amylase (E.C. 3.2.1.1) type VI-B from porcine pancreas, α-glucosidase (E.C. 3.2.1.20) type I from Saccharomyces cerevisiae, p-Nitrophenyl-α-D-glucopyranoside (pNPG) as a synthetic substrate of α-glucosidase, 2,2 Diphenyl-1-picryhydrazyl (DPPH), 6 hydroxi-2,5,7,8-tetrametil-cromo-2-carboxilico (TROLOX), 3,5-Dinitrosalisylic acid (DNS), linoleic acid, (±)-α-tocopherol, potassium chloride, sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, sodium phosphate monobasic, potassium peroxodisulfate, 2,2'-Azio-bis (3-etilbenzotiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium sodium tartrate tetrahydrate, β-carotene Type I, sodium carbonate, were purchased from Sigma Chemical Co., and other chemicals including Tween 40 emulsifier, Folin ciocalteu and maltose were obtained from Merck Co., Sodium hydroxide and starch soluble purum were purchased from Riedel-deHaën. Finally, UltraPure Tris was obtained from Invitrogen.

Selection and collection of traditional antidiabetic plants

The traditional antidiabetic plants in this study were collected from various locations of Loja and Zamora Chinchipe, two provinces of southern Ecuador. Table 1 shows the scientific name, family, herbarium voucher, vernacular name(s) and therapeutical applications of each plant (Tene et al., 2007).

Table 1. Medicinal plants used in treatment of diabetes in southern Ecuador

No.	Scientific name	Family	Herbarium voucher	Vernacular name(s)
1	*Oreocallis grandiflora* (Lam.) R.Br.	Proteaceae	PPN-pe-001	Cucharillo
2	*Siparuna eggersii* Hieron	Monimiaceae	PPN-mn-001	Monte del oso
3	*Artocarpus altilis* (Parkinson) Fosberg	Moraceae	PPN-mo-003	Fruto del pan
4	*Adiantum poiretii* Wikstr.	Pteridaceae	PPN-pt-001	Culantrillo
5	*Costus comosus* (Jacq.) Roscoe	Costaceae	PPN-cs-001	Caña
6	*Piper crasinervium* Kunth.	Piperaceae	PPN-pi-002	Guabiduca
7	*Baccharis genistelloides* (Lam.) Pers.	Asteraceae	PPN-as-013	Tres filos
8	*Croton wagneri* Müll. Arg.	Euphorbiaceae	PPN-eu-001	Moshquera
9	*Ilex guayusa* Loes.	Aquifoliaceae	PPN-aq-001	Guayusa
10	*Neonelsonia acuminata* (Benth.) blancaJ.M.Coult & Rose ex Drude	Apiaceae	PPN-ap-007	Zanahoria
11	*Verbena litoralis* Kinth.	Verbenaceae	PPN-ve-001	Verbena
12	*Justicia colorata* (Nees) Wassh	Acanthaceae	PPN-ac-004	Insulina

Preparation of methanol extracts

Plants were dried, pulverized and extracted with methanol 10:1 (methanol:plant) for 5 h at room temperature and constant stirring. The extracts were then concentrated using a rotary evaporator after filtration and kept at -20 °C until the assay experiments.

Hypoglycemic activity

Inhibition assay for α-amylase activity

The α-amylase activity was determined by the method of Tsujida et al. (2006) (Takahiro & Takeshi, 2006). A volume of 125 µL of α-amylase was premixed with 125 µL of extract at various concentrations (10-1000 µg/mL) and 125 µL of starch as a substrate was added as a 0.5 % starch solution in 20 mM phosphate buffer (pH 6.9) to start the reaction. The reaction was carried out at 25 °C for 10 min and terminated by addition of 125 µL of the DNS reagent (96 mM 3,5-dinitrosalicylic acid, 12% sodium potassium tartrate in 2 M NaOH). The reaction mixture was heated for 15 min at 100 °C and then diluted with 1 mL of distilled water in an ice bath. α-amylase activity was determined by measuring absorbance at λ=540 nm.

Inhibition assay for α-glucosidase activity

The inhibitory activity of total extracts against α-glucosidase was measured according to Matsui et al. (2001), (Matsui, 1996). Briefly, 35 µL of α-glucosidase (0.075 unit) was premixed with 35 µL of extract at various concentrations (10-1000 µg/mL). A volume of 930 µL of 10 mM p-nitrophenyl-D-glucopyranoside (pNPG) as a substrate in 67 mM phosphate buffer (pH = 6.9) was added to the mixture to start the reaction. The reaction was incubated at 37 °C for 15 min and stopped by adding 1 mL of 0.5 M TRIS buffer? (pH = 7.4). The α-glucosidase activity was determined by measuring the p-nitrophenol release from pNPG at λ=400 nm. The enzymatic inhibitory activity (%) in each reaction was calculated from the absorbance A, B, C and D by the following equation:

$$\% \text{ inhibition} = \{1-[(B-D)/(A-C)]\} \times 100$$

Where:
B = Sample absorbance
D = Blank 1 absorbance (without enzyme)
A = Blank 2 absorbance (without inhibitor)
C = Blank 3 absorbance (without inhibitor and enzyme)

Total Soluble Phenolic Content of the extracts and antioxidant activity

Total Soluble Phenolic Content assay (TSPC)

The total phenolic content was determined by an assay modified from Shetty et al. (1995) (Kwon et al., 2007). The total extract (250 µL) was transferred into a test tube and mixed with 1.5 mL of distilled water. To each sample, 125 µL of 50% (v/v) Folin-Ciocalteu reagent was added and mixed. After 5 min, 250 µL of 20% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 760 nm. The absorbance values were converted to total phenolics and were expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract (Zengin et al., 2015). Standard curves were established using various concentrations of gallic acid (0.625-20 µg/mL).

Determination of free radical scavenging activity using DPPH assay

The antioxidant activity of each total extract was determined as the ability of the extract to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Buenger et al., 2005). A 0.1 mM DPPH radical solution in methanol was prepared. Immediately prior to measurement, this stock solution is diluted with methanol to an absorbance of 0.70 ± 0.02, determined by UV-Vis spectrophotometry at λ=517 nm. A volume of 1960 µL of this DPPH solution was mixed with 40 µL of sample or methanol (as control), and incubated for 15 min at RT.

The investigated antioxidant solutions were prepared in concentrations ranging from 5-500 µg/mL. A stock solution of α-tocopherol was prepared and serially diluted to concentrations ranging from 0.1-10 µg/mL. The absorbance of each sample at λ=517 nm was measured. This antioxidant activity is given as percentage (%) of DPPH scavenging, calculated as:

$$\text{DPPH scavenging (\%)} = \{(Ac - As)/(Ac)\} \times 100.$$

Where
Ac = Control absorbance
As = Sample absorbance

Determination of free radical scavenging activity using TEAC assay

The determination of the antioxidative activity of substances using the TEAC assay is based on their capability to reduce the stable radical 2,2'-Azio-bis (3-etilbenzotiazoline-6-sulfonic acid) diammonium salt (ABTS), in comparison with the standard TROLOX (Buenger et al., 2005). An ABTS solution (7 mm in water) is mixed in the with potassium peroxydisulfate solution (2.45 mM in water) and incubated for 12–16 h at room temperature in darkness. Immediately prior to measurement, this stock solution is diluted with water to an absorbance of 0.70 ± 0.02. A stock solution of TROLOX was prepared and serially diluted to concentrations ranging from 0.1-10 µg/mL.The investigated antioxidant solutions were prepared to concentrations ranging from (5-500 µg/mL). Water is used as solvents for both TROLOX and the samples. For the measurement, 40 µL of the samples or standards are mixed with 1960 µL of the reaction solution and the absorbance at 734 nm was measured after exactly 6 min against the solvent. This antioxidant activity was given as percentage (%) of ABTS scavenging, calculated as:

$$\text{ABTS scavenging (\%)} = \{(Ac - As)/(Ac)\} \times 100.$$

Where

Ac = Control absorbance
As = Sample absorbance

Antioxidant activities by β-Carotene-linoleic acid assay

The antioxidant activity of the methanol extracts was evaluated following the method of Miller (Duarte-Almeida, Santos, Genovese, & Lajolo, 2006). Briefly, 600 µL of β-carotene (0.2 mg/mL) dissolved in chloroform was pipetted into a small round bottom flask with 500 mg of Tween 40 and 50 µL of linoleic acid. After removing the chloroform using a rotary evaporator under reduced pressure and temperature, less than 45 °C, 60 ml of H_2O_2 were added to the flask with vigorous shaking. Aliquots (1960 µL) of the prepared emulsion were transferred to a series of tubes each containing 40 µL of extract or positive control (α-tocopherol). The investigated antioxidant solutions were prepared to concentrations ranging from (5-500 µg/mL). A stock solution of α-tocopherol was prepared and serially diluted to concentrations ranging from 0.05-5 µg/mL. A control sample was prepared exactly as before but without adding antioxidants. Each type of sample was prepared in triplicate. The test systems were placed in a water bath at 50 °C for 2 h. The absorbance of each sample was read spectrophotometerically at 470 nm, immediately after sample preparation (0 min) and at 120 min of the experiment. The antioxidant activity expressed as antioxidant protection factor (APF) of the extracts was evaluated in terms of bleaching of β-carotene using the following formula (Duarte-Almeida et al., 2006):

$$\textbf{APF} = \{[(Ac_0 - Ac_{120}) - (As_0 - As_{120})]/(Ac_0 - Ac_{120})\} \times 100$$

Where:
Ac_0 = absorbance of control at t = 0 min
Ac_{120} = absorbance of the control at t = 120 min
As_0 = absorbance of the sample at t = 0 min
As_{120} = absorbance of the sample at t = 120 min

IC_{50} Calculation

The IC_{50} value from the hypoglycemic assays was defined as the concentration of α-amylase or α-glucosidase inhibitors to inhibit 50% of activity under the assay conditions (Matsui, 1996). The IC_{50} value from the antioxidants assays, was defined as the concentration of antioxidant that causes 50% loss of the DPPH or ABTS activity (color) or inhibit 50% of linoleic autoxidation under the assay conditions (Molyneux, 2004).

Statistical analysis

All experiments were performed in triplicate. Results are expressed as mean ± S.D. (Table 2) and were compared using a one-way analysis of variance (ANOVA). Comparisons between groups were made according to the Duncan and Fisher's test. p-values less than 0.05 ($p<0.05$) were considered as statistically significant. The 50% inhibitory concentration (IC_{50}) was calculated from the XLSTAT dose–response curve (statistical program) obtained by plotting the percentage of inhibition against the concentrations.

RESULTS AND DISCUSSION

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances, which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect (Malviya, Jain & Malviya, 2010).

Pancreatic and intestinal glucosidases are the key enzymes of dietary carbohydrate digestion and inhibitors of theses enzymes may be effective in retarding glucose absorption to suppress postprandial hyperglycemia (Bhat et al., 2008). On the other hand, it is well known that a compound having antioxidant properties can prevent oxidative stress, which plays an important role in the prevention of diabetes complications (Akhter et al., 2013).

The in vitro hypoglycemic and antioxidant activities of *Siparuna eggersi*, *Croton wagneri*, *Ilex guayusa*, *Baccharis genistelloides*, *Neonelsonia acuminate*, *Oreocallis grandiflora*, *Verbena litoralis*, *Justicia colorata*, *Artocarpus altilis*, *Adiantun poiretii*, *Costus comosus* and *Piper crassinervium* were investigated, and the results expressed as inhibition percentage for each concentration (µg/mL) of extract are shown in Table 2. As can be seen in the α-amylase assay, only *S. eggersi*, *C. wagneri* and *O. grandiflora* had activity, being the latter the most active. In comparison, all the plant extracts had activity in the α-glucosidase assay; however, *O. grandiflora* had very high activity and for this reason, lower concentrations (1-100 µg/ml) were used for this plant. Table 2 also shows a dose-dependent response in all antioxidant assays.

Table 2. Results of hypoglycemic and antioxidant activities of medicinal plants expressed as inhibition percentage ± S.D.

Results from inhibition assay for α-amylase activity

µg/mL	Acarbose	µg/mL	A.p.	C.c.	A.a	P.c.	I.g.	J.c.	S.e.	C.w.	O.g.	B.g.	V.l.	N.a.
10	10±0.8	10	NA	NA	NA	NA	NA	NA	21±0.5	27±0.3	1±1.0	NA	NA	NA
50	50±2.4	50	NA	NA	NA	NA	NA	NA	26±0.3	30±0.5	18±0.3	NA	NA	NA
100	69±2.2	100	NA	NA	NA	NA	NA	NA	29±0.9	31±0.9	53±0.3	NA	NA	NA
500	84±2.1	500	NA	NA	NA	NA	NA	NA	30±0.7	32±0.4	75±0.4	NA	NA	NA
1000	93±2.1	1000	NA	NA	NA	NA	NA	NA	49±1.0	36±0.4	83±0.5	NA	NA	NA

Results from inhibition assay for α-glucosidase activity

µg/mL	Acarbose	µg/mL	A.p.	C.c.	A.a	P.c.	I.g.	J.c.	S.e.	C.w.	O.g.*	B.g.	V.l.	N.a.
10	3±0.9	10	1±0.9	6±1.3	7±1.0	11±0.3	6±0.6	NA	17±0.9	8±0.4	8±0.8	3±0.0	NA	13±0.9
50	7±2.0	50	55±0.7	49±0.7	60±0.7	21±0.3	8±1.2	NA	66±0.7	13±0.3	77±0.3	19±0.2	NA	15±1.3
100	13±2.0	100	91±0.9	71±1.5	84±0.5	39±0.1	15±0.7	NA	95±0.4	27±0.4	97±0.4	36±1.0	0±0.2	21±0.7
500	33±2.3	500	95±0.9	91±0.5	96±0.5	93±0.9	90±0.7	37±0.9	96±0.0	87±0.9	98±1.0	77±1.2	81±0.9	73±0.2
1000	52±2.7	1000	99±6.6	96±1.1	99±1.2	98±0.8	100±0.0	78±0.6	100±0.0	93±1.1	99±0.5	98±0.0	99±0.8	91±1.0

***Concentration: 1-5-10-50-100 µg/mL**

Results of free radical scavenging activity using DPPH assay

µg/mL	α-Tocopherol	µg/mL	A.p.	C.c.	A.a	P.c.	I.g.	J.c.	S.e.	C.w.	O.g.	B.g.	V.l.	N.a
3.125	27±1.7	10	15±1.3	11±0.4	16±0.8	6±0.8	31±0.4	20±0.7	29±0.5	8±0.7	47±1.1	10±0.7	62±0.4	18±0.7
6.25	46±3.1	50	61±0.8	42±0.5	56±0.3	19±0.7	88±1.0	71±1.1	82±1.1	26±0.4	90±0.9	45±0.5	89±0.8	64±1.0
12.5	77±2.1	100	92±1.0	69±1.1	87±0.3	33±0.3	92±0.9	90±0.5	90±0.5	48±0.7	91±0.7	78±1.4	95±1.3	86±0.5
25	92±1.2	500	93±1.3	91±1.0	97±0.6	75±0.8	94±0.6	99±0.2	93±0.6	83±0.5	96±0.8	97±0.9	95±0.6	94±0.5
50	95±1.1	1000	95±0.8	94±0.4	97±0.6	88±1.2	97±1.2	99±0.5	94±0.8	93±1.2	96±0.6	98±1.4	96±0.8	96±0.5

Results of free radical scavenging activity using TEAC assay

µg/mL	TROLOX	µg/mL	A.p.	C.c.	A.a	P.c.	I.g.	J.c.	S.e.	C.w.	O.g.	B.g.	V.l.	N.a
0.1	5±1.0	5	17±1.3	15±1.0	17±0.6	5±1.2	20±0.7	13±0.4	25±1.1	13±1.1	40±1.2	14±0.6	20±1.0	11±0.7
0.5	15±1.0	10	27±0.3	25±0.8	29±0.4	8±1.4	36±1.1	24±0.8	43±1.1	22±0.8	66±0.4	21±1.1	38±0.8	20±0.9
1	26±0.9	50	75±0.9	64±0.4	72±0.6	28±1.6	97±0.5	77±1.0	94±1.0	55±0.8	100±0.6	60±0.7	99±0.8	70±0.7
5	98±0.9	100	90±1.2	94±0.9	95±0.2	49±1.3	99±0.8	95±0.9	99±0.6	69±0.4	100±0.6	94±1.1	100±0.2	96±1.2
10	100±0.1	500	99±0.8	100±0.7	96±0.4	96±1.1	99±0.4	98±1.0	100±0.2	97±0.4	100±0.6	100±1.0	100±0.4	100±0.2

Results of antioxidant activities by β-Carotene-linoleic acid assay

µg/mL	α-Tocopherol	µg/mL	A.p.	C.c.	A.a	P.c.	I.g.	J.c.	S.e.	C.w.	O.g.	B.g.	V.l.	N.a
0.05	28±2.1	1	10±1.0	21±0.6	36±0.4	6±0.3	26±0.6	19±0.4	14±1.1	18±3.4	NA	2±0.1	22±1.1	13±0.7
0.25	67±2.1	5	15±1.3	36±0.3	53±0.9	8±0.7	37±1.2	34±1.1	27±0.6	23±3.6	NA	3±1.1	34±1.0	14±1.2
0.5	82±1.4	10	21±0.6	51±0.9	68±0.5	12±0.2	46±1.1	43±0.7	40±0.7	36±5.2	NA	11±0.8	45±0.9	18±1.1
2.5	90±1.0	50	45±1.2	78±1.2	84±0.7	42±0.2	64±0.5	59±1.1	78±0.5	74±6.2	1±1.9	20±0.7	77±0.4	55±0.6
5	92±0.5	100	57±0.6	84±1.1	92±0.6	59±0.6	74±0.8	65±0.2	88±1.0	91±2.3	39±1.6	43±0.5	82±0.6	68±1.3

Siparuna eggersi (S.e.), Croton wagneri (C.w.), Ilex guayusa (I.g.), Baccharis genistelloides (B.g.), Neonelsonia acuminate (N.a.), Oreocallis grandiflora (O.g.), Verbena litoralis (V.l.), Justicia colorata (J.c.), Artocarpus altilis (A.a.), Adiantun poiretii (A.p.), Costus comosus (C.c.) and Piper crassinervium (P.c).

α-amylase/α-glucosidase inhibitory activity

The α-glucosidase and α-amylase inhibitor effectiveness of extracts of the different plant species were compared on the basis of their resulting IC_{50} values (Table 3). *O. grandiflora* inhibited the activity of both α-glucosidase and α-amylase with an IC_{50} of 2.8 ± 0.40 µg/mL and 161.5 ± 1.30 µg/mL, respectively. *S. eggersii* and *C. wagneri*, also inhibited the activity of α-amylase, but their inhibitor effectiveness was lower (IC_{50} > 1000 ug/mL) compared to *O. grandiflora*. The other extracts only inhibited the activity of α-glucosidase. *S.eggersii* (IC_{50} = 28.3 ± 0.60 µg/mL) was the next best after *O. grandiflora* to inhibit the activity of α-glucosidase (Table 3).

Table 3. IC_{50} values (µg/mL) of α-glucosidase (AGH) and α-amylase (AAH) inhibition assays

PLANT	AGH (IC_{50})	AAH (IC_{50})
Oreocallis grandiflora	2.8 ± 0.40	161.5 ± 1.30
Siparuna eggersii	28.3 ± 0.60	>1000
Artocarpus altilis	40.9 ± 0.38	NA
Adiantum poiretii	46.3 ± 0.92	NA
Costus comosus	57.9 ± 0.71	NA
Piper crasinervium	108.5 ± 1.00	NA
Baccharis genistelloides	154.6 ± 1.28	NA
Croton wagneri	162.4 ± 1.34	>1000
Ilex guayusa	176.5 ± 1.50	NA
Neonelsonia acuminata	198.7 ± 1.59	NA
Verbena litoralis	337.9 ± 1.75	NA
Justicia colorata	622.1 ± 2.52	NA
POSITIVE CONTROL*	964.6 ± 2.80	56.8 ± 2.50

NA: Non active.
***Acarbose for AGH and AAH**

The extracts of *I. guayusa* (IC_{50} = 176.5 ± 1.50 µg/mL) and *V. litoralis* (IC_{50} = 337.9 ± 1.75 µg/mL), had less inhibitory activity on α-glucosidase than *O. grandiflora*, however these plants could also be of interest in the treatment of diabetes mellitus due to their high antioxidant activity (Table 3).

Total Soluble Phenolic Content (TSPC) of the extracts and antioxidant activity

To better understand the mechanism(s) of action of the extracts against α-amylase and α-glucosidase, the total soluble phenolic content and antioxidant activity were measured for all of the extracts. Table 4 shows the total soluble phenolic contents (TSPC) of the extracts determined as gallic acid equivalents per gram of extract (GAEs/g extract), and the IC_{50} values obtained in the antioxidant assays, DPPH, TEAC and β-CLAMS, expressed in µg/mL.

Table 4. IC_{50} values (µg/mL) of antioxidant activity by DPPH, TEAC and β-CLAMS assays and (GAEs/g extract) of TSPC assay

PLANT	$IC_{50\text{-}DPPH}$	$IC_{50\text{-}TEAC}$	$IC_{50\text{-}\beta CLAMS}$	TSPC
Oreocallis grandiflora	9.9 ± 0.06	6.6 ± 0.55	108.5 ± 1.00	185.9 ± 0.09
Siparuna eggersii	17.1 ± 1.19	10.5 ± 0.89	13.0 ± 1.02	143.3 ± 0.06
Artocarpus altilis	11.7 ± 0.96	19.1 ± 1.56	3.1 ± 0.20	73.5 ± 0.08
Adiantum poiretii	32.2 ± 1.51	19.9 ± 1.63	69.8 ± 0.46	73.3 ± 0.09
Costus comosus	60.3 ± 0.47	22.5 ± 1.86	8.9 ± 0.06	52.3 ± 0.08
Piper crasinervium	167.8 ± 1.36	87.3 ± 0.72	73.9 ± 0.54	37.4 ± 0.04
Bacharis genistelloides	14.8 ± 1.22	25.4 ± 0.21	176.6 ± 1.15	44.3 ± 0.04
Croton wagneri	113.1 ± 0.91	37.8 ± 0.30	13.7 ± 1.07	82.6 ± 0.04
Ilex guayusa	14.2 ± 0.99	11.8 ± 1.01	13.0 ± 0.85	116.8 ± 0.05
Neonelsonia acuminata	31.6 ± 0.24	23.0 ± 1.93	43.1 ± 0.31	81.6 ± 0.05
Verbena litoralis	3.6 ± 0.12	10.9 ± 0.95	10.6 ± 0.07	159.8 ± 0.08
Justicia colorata	26.0 ± 0.21	20.4 ± 1.69	21.8 ± 0.14	64.9 ± 0.07
POSITIVE CONTROL*	6.3 ± 0.53	1.3 ± 0.10	0.1 ± 0.01	135.8 ± 0.00

***α-Tocopherol for DPPH, TEAC and β-CLAMS and TROLOX for TSPC**

Taking into account the complex nature of phytochemicals, the antioxidant activities of plant extract cannot be evaluated using a single method. Thus, commonly accepted assays were employed to evaluate the antioxidant effects of the methanol extracts and free radical scavenging was determined by DPPH and TEAC assays (Table 2). The effect of antioxidants on DPPH and ABTS radicals is due to their hydrogen donating ability. Though the DPPH and ABTS radical scavenging abilities of the extracts were less than that of positive controls (α-tocopherol and TROLOX, respectively). The study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Proton radical scavenging is an important attribute of antioxidants (Adedapo, Jimoh, Koduru, Afolayan, & Masika, 2008).

Although Wang et al. (1998) found that some compounds which have ABTS scavenging activity did not show DPPH scavenging activity (Adedapo et al., 2008), in this study, the extracts showed comparable scavenging activities against DPPH and ABTS radicals. This demonstrates the capability of the extracts to scavenge different free radicals in different systems, suggesting that they may be useful therapeutic agents for treating radical-related pathological damage (Adedapo et al., 2008).

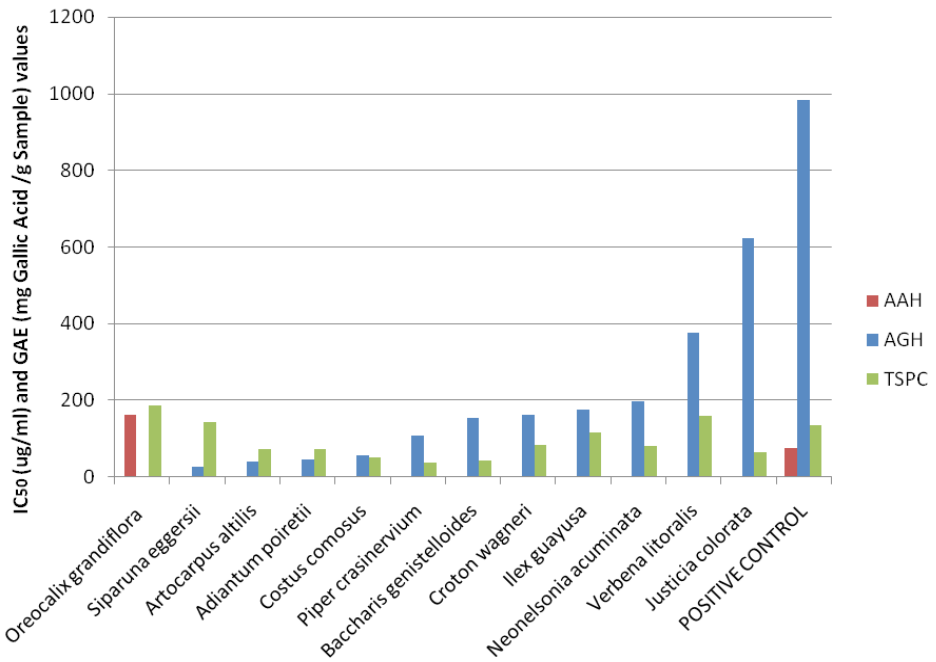
The antioxidant potential of the extracts also was evaluated using model systems based on β-carotene coupled with autoxidized linoleic acid. Regarding antioxidant activity, all studied extracts, except *O. grandiflora* ($IC_{50\text{-}DPPH}$ = 9.9 ± 0.06 µg/mL ; $IC_{50\text{-}TEAC}$ = 6.6 ± 0.55 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 108.5.6 ± 1.00 µg/mL), *B. genistelloides* ($IC_{50\text{-}DPPH}$ = 14.8 ± 1.22 µg/mL ; $IC_{50\text{-}TEAC}$ = 25.4 ± 0.21 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 176.6 ± 1.15 µg/mL) and *A. poiretii* ($IC_{50\text{-}DPPH}$ = 32.2 ± 1.51 µg/mL ; $IC_{50\text{-}TEAC}$ = 19.9 ± 1.63 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 69.8 ± 0.40 µg/mL), showed to have high correlation between scavenging activities and β-CLAMS assays. However, *O. grandiflora*, *B. genistelloides* and *A. poiretii* had best scavenging activity than autoxidized linoleic acid inhibition capacity, therefore these plant acts better as primary antioxidants than as secondary. The other plants have both primary and secondary (preventive) antioxidant activity being *A. altilis* ($IC_{50\text{-}DPPH}$ = 11.7 ± 0.96 µg/mL ; $IC_{50\text{-}TEAC}$ = 19.1 ± 1.56 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 3.1 ± 0.20 µg/mL), *S. eggersii* ($IC_{50\text{-}DPPH}$ = 17.1 ± 1.19 µg/mL ; $IC_{50\text{-}TEAC}$ = 10.5 ± 0.89 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 13.0 ± 1.02 µg/mL), *I. guayusa* ($IC_{50\text{-}DPPH}$ = 14.2 ± 0.99 µg/mL ; $IC_{50\text{-}TEAC}$ = 11.8 ± 1.01 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 13.0 ± 0.85 µg/mL), *V. litorialis* ($IC_{50\text{-}DPPH}$ = 3.6 ± 0.12 µg/mL ; $IC_{50\text{-}TEAC}$ = 10.9 ± 0.95 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 10.6 ± 0.07 µg/mL) and *J. colorata* ($IC_{50\text{-}DPPH}$ = 26.0 ± 0.21 µg/mL ; $IC_{50\text{-}TEAC}$ = 20.4 ± 1.69 µg/mL ; $IC_{50\text{-}\beta\text{-}CLAMS}$ = 21.8 ± 0.14 µg/mL) the most active ($p<0.05$). The free radical scavenging method is quantitatively more reliable than β-CLAMS; however, the latter assay provides an alternative mechanism by measuring the capability of a compound to resist peroxidation and free radical chain reaction (Joaquim Mauricio Duarte-Almeida, Negri, Salatino, Carvalho, & Lajolo, 2007).

Regarding to the Total Soluble Phenolic Content (TSPC) of the extracts, the highest value of TSPC (GAEs/g extract) corresponds to *O. grandiflora* (185.9 ± 0.09), followed by *V. litoralis* (159.8 ± 0.08), *S. eggersii* (143.3 ± 0.06) and *I. guayusa* (116.8 ± 0.05), therefore these plant species are interesting since phenolic phytochemicals have positive effect on health because they counteract the effects of reactive oxygen species (ROS) generated during cellular metabolism (Huan-xia, Hai-sheng, & Shu-fang, 2014; Miguel et al., 2014). The results of the hypoglycemic and antioxidant activities obtained here, support several traditional therapeutic uses reported for the species studied (Bejár, et al., 2002; Bussmann & Sharon, 2006; PFN-133, 2006; Tene et al., 2007).

Comparison of hypoglycemic and antioxidant activities and total soluble phenolic content of extracts

The data suggests that high phenolic content does not always confer a high anti-amylase or anti-glucosidase activity of an extract (Figure 1).

Figure 1. Comparison between α-amylase/α-glucosidase inhibitory capacity and Total Soluble Phenolic Content.



It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds (Huan-xia, et al., 2014; Miguel et al., 2014). Therefore, it is important to consider the effect of the total phenolic content on the antioxidant activity of the extracts assayed. The antioxidant activity of the extracts was monitored using the DPPH and ABTS radical inhibition assays and β-carotene-linoleate model system. In recent studies with traditional plants, polymeric polyphenols were reported as contributing to strong glucosidases inhibition (Onal et al., 2005), but in this study the antioxidant activity of the extracts assayed was not proportional to both α-amylase or α-glucosidase inhibitory activity. There is, however, a significant correlation between Total Soluble Phenolic Content and free radicals (DPPH, ABTS) scavenging activity (Figure 2), except in *Croton wagneri* case; and linoleic autoxidation inhibitory capacity (Figure 3), except in few cases: *Verbena litoralis, Siparuna eggersii, Ilex guayusa, Croton wagneri, Artocarpus altilis, Justicia colorata, Costus comosus*.

Figure 2. Comparison between free radicals (DPPH, ABTS) scavenging activity and Total Soluble Phenolic Content.

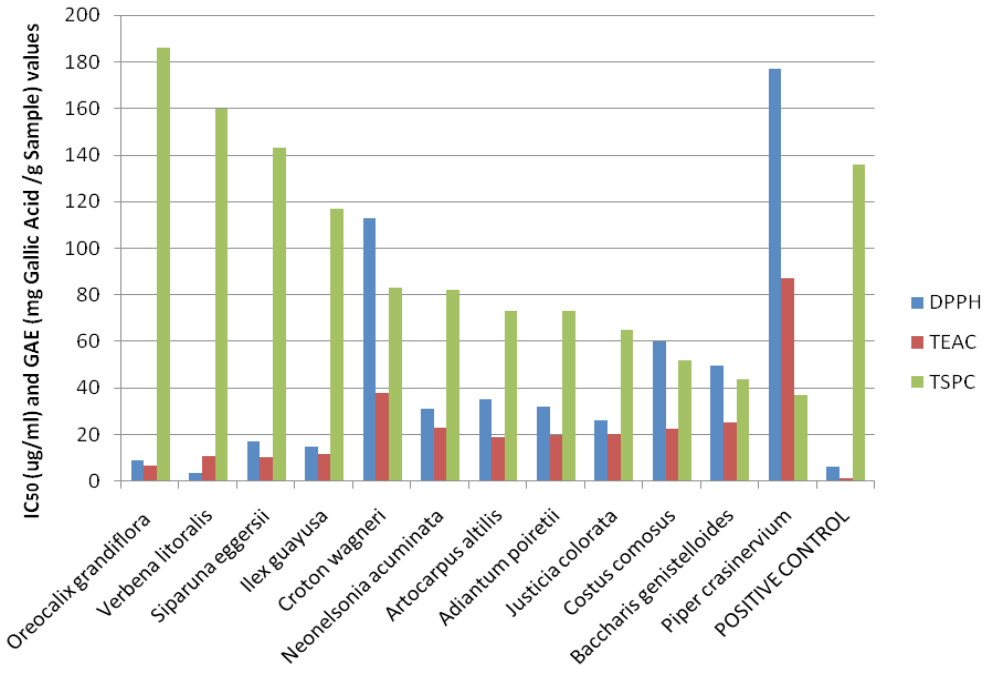
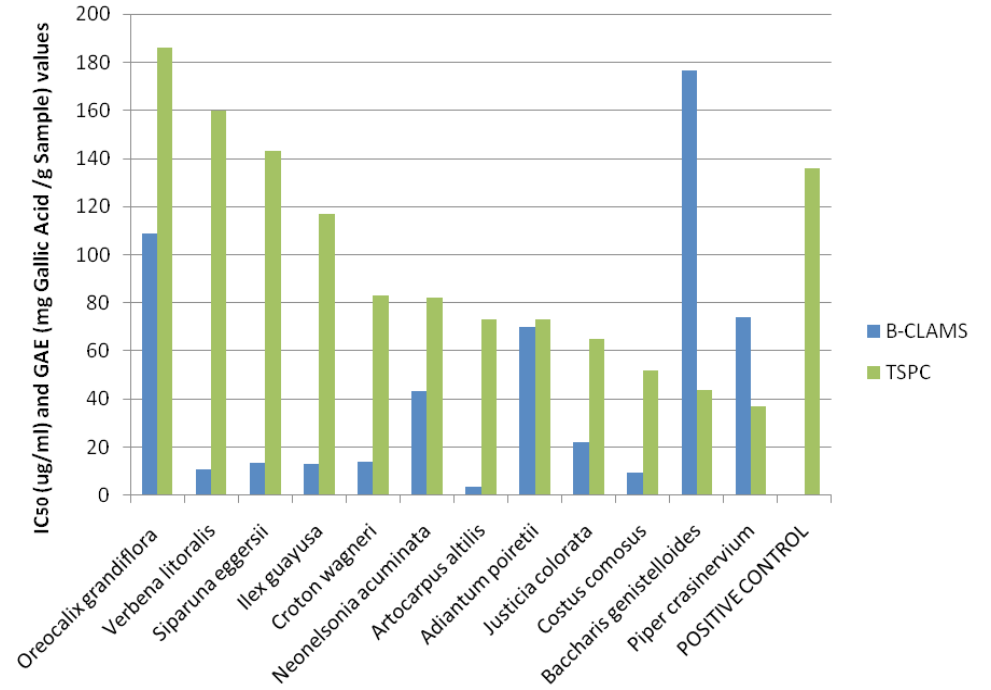


Figure 3. Comparison between linoleic autoxidation inhibitory capacity and Total Soluble Phenolic Content.



CONCLUSION

In vitro hypoglycemic and antioxidant activities of extracts from twelve medicinal plants used in treatment of diabetes in southern Ecuador were investigated based on biochemical techniques. In the α-amylase assay, only *S. eggersi*, *C. wagneri* and *O. grandiflora* had activity, being the last the most active. All the plant extracts had activity in the α-glucosidase assay; however, *O. grandiflora* had very high activity. *S. eggersii* was the next best after *O. grandiflora* to inhibit the activity of α-glucosidase.

Regarding antioxidant activity, almost all the extracts showed to have high correlation between scavenging activities and β-CLAMS assays; however, *O. grandiflora*, *B. genistelloides* and *A. poiretii* had best scavenging activity than autoxidized linoleic acid inhibition capacity, therefore these plants act better as primary antioxidants than as secondary ones. The other plants have both primary and secondary (preventive) antioxidant activity being *A. altilis*, *S. eggersii*, *I. guayusa, V. litorialis* and *J. colorata* the most active ($p<0.05$).

The results of the hypoglycemic and antioxidant activities obtained here based on biochemical techniques, support several traditional therapeutic uses reported for the species studied. This results, suggest that all species studied, especially *O. grandiflora*, are excellent candidates for future research on determining the mechanisms of their hypoglycemic or antioxidant activity, as well as for the isolation and identification of active hypoglycemic and antioxidant substances. In addition, further comprehensive pharmacological investigations, including experimental chronic studies, should be carried out to assess the possible toxicological effects of these plants.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adedapo, A. A., Jimoh, F. O., Koduru, S., Afolayan, A. J., & Masika, P. J. (2008). Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. *BMC Complement Alternative Medicine,* 8(53).

Akhter, F., Hashim, A., Khan, M. S., Ahmad, S., Iqbal, D., Srivastava, A. K., & Siddiqui, M. H. (2013). Antioxidant, α-amylase inhibitory and oxidative DNA damage protective property of Boerhaavia diffusa (Linn.) root. *South African Journal of Botany,* 88, 265-272.

Alarcón-Aguilar, F. J., Roman-Ramos, R., Flores-Sánchez, J. L., & Aguirre-García, F. (2002). Investigation on the Hypoglycaemic Effects of Extracts of Four Mexican Medicinal Plants in Normal and Alloxan-diabetic Mice. *Phytotheraphy Research,* 16, 383-386.

Andrade-Cetto, A., & Heinrich, M. (2005). Mexican plants with hypoglycaemic effect used in the treatement of diabetes. *Journal of Ethnopharmacology,* 99, 325-348.

Bailey, C. J. (2003). New approaches to the pharmacotherapy of diabetes. In J. C. Pickup & G. William (Eds.), *Textbook of Diabetes* (Third ed., Vol. 2, pp. 73.71-73.21). UK: Blackwell Science Ltd.

Bejár, E., Russman, R., Roa, C., & Sharon, D. (2002). Medicinal Herbs of Southern Ecuador: A field guide to the medicinal plants of Vilcabamba. California. Spring Vallety

Bhat, M., Zinjarde, S. S., Bhargava, S. Y., Kumar, A. R., & Joshi, B. N. (2008). Antidiabetic Indian Plants: a Good Source of Potent Amylase Inhibitors. *eCAM Advance Access published,* 1-6.

Buenger, J., Ackermann, H., Jentzsch, A., Mehling, A., Pfitzner, I., Reiffen, K. A., Wollenweber, U. (2005). An interlaboratory comparison of methods used to assess antioxidant potentials. *International Journal of Xosmetic Science,* 28, 135-146.

Bussmann, R. W., & Sharon, D. (2006). Traditional medicinal plant use in Loja province, Southern Ecuador. J*ournal of Ethnobiology and Ethnomedicine*, 2(44).

Conforti, F., Statti, G., Loizzo, M. R., Sacchetti, G., Poli, F., & Menichini, F. (2005). In Vitro Antioxidant effect and Inhibition of a-amylase of two varieties of Amaranths caudatus Seeds. *Biological and Pharmaceutical Bulletin*, 28(6), 1098-1102.

Cunningham, J. (1998). Micronutrients as nutriceutical interventions in diabetes mellitus. Journal of the *American College of Nutrition*, 17, 7-12.

Deutschländer, M. S., Lall, N., Venter, M. V. d., & Dewanjee, S. (2012). The hypoglycemic activity of Euclea undulata Thumb. var. myrtina (Ebenaceae) root bark evaluated in a streptozotocin-nicotinamide induced type 2 diabetes rat model. *South African Journal of Botany*, 80, 9-12.

Duarte-Almeida, J. M., Negri, G., Salatino, A., Carvalho, J. E. d., & Lajolo, F. M. (2007). Antiproliferative and antioxidant activities of a tricin acylated glycoside from sugarcane (Saccharum officinarum) juice. *Phytochemistry,* (68), 1165-1171.

Duarte-Almeida, J. M., Santos, R. J. d., Genovese, M. I., & Lajolo, F. M. (2006). Avaliacão da atividade antioxidante utilizando sistema β-caroteno/ácido linoléico e método de seqüestro de radicais DPPH•. *Ciencia e Tecnologia de Alimentos,* 26(2), 446-452.

Fujisawa, T., Ikegami, H., Inoue, K., Kawabata, Y., & Ogihara, T. (2005). Effect of two a-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdomina symptoms. *Metabolism Clinical and Experimental,* 54, 387-390.

Hasenah, A., Houghton, P. J., & Soumyanath, A. (2006). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with paricular reference to Phyllanthus amarus. *Journal of Ethnopharmacology,* 107, 449-455.

Hernandez-Galicia, E., Aguilar-Contreras, A., Aguilar-Santamaria, L., Roman-Ramos, R., Chavez-Miranda, A. A., García-Vega, L. M., Flores-Saenz, J.L., Alarcón-Aguilar, F. J. (2002). Studies on hypoglycemic activity of mexican medicinal plants. *Proceedings of the Wester Pharmacology Society*, (45), 118-124.

Huan-xia, Z., Hai-sheng, Z., & Shu-fang, Y. (2014). Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. *Food Science and Human Wellness*, 3(3-4), 183-190

Johansen, J. S., Harris, A. K., Rychly, D. J., & Ergul, A. (2006). Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovascular Diabetology,* 4(5).

Kaleem, M., Asif, M., Ahmed, Q. U., & Bano, B. (2006). Antidiabetic and antioxidant activity of Annona squamosa extracto in streptozotocin-induced diabetic rats. *Singapore Medical Journal,* 47(8), 670.

Kwon, Y., Apostolidis, E., & Shetty, K. (2007). Evaluación of pepper (Capsicum Annuum) for managemente of diabetes and hypertension. *Journal of Food Biochemistry,* 30, 699-717.

Marles, R. J., & Farnsworth, N. (1996). Diabetes Mellitus: Report of a WHO Study Group, 1985. Protocol *Journal of Botanical Medicine,* 1(3), 85-135.

Liu, Y., Zhong, X., Ding, Y., Ren, L., Bai, T., Liu, M., Guo, Y., Guo, Q., Zhang, Y, Yang, J., & Zhang, Y., (2017). Inhibition of voltage-dependent potassium channels mediates cAMP-potentiated insulin secreteion in rat pancreatic β cells. Islets, 9(12), 11-18.

Malviya, N., Jain, S. & Malviya, S. (2010) Antidiabetic potential of medicinal plants. Acta Poloniae Pharmaceutica, 67(2):113–118.

McCue, P., Kwon, Y., & Shetty, K. (2004). Anti-amylase, anti-glucosidase and anti-angiotensin I-converting enzyme potential of selected foods. *Journal of Food Biochemistry,* 29, 278-294.

Miguel, M., Nunes, S., Dandlen, S., Cavaco, A., & Antunes, M. (2014). Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (Apis mellifera L.) from Algarve, South Portugal. *Food Science and Technology,* 34(1), 16-23.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology,* 26(2), 211-219.

Onal, S., Timur, S., Okutucu, B., & Zihnioglu, F. (2005). Inhibition of a-Glucosidase by Aqueous Extracts of Some Potent Antidiabetic Medicinal Herbs. *Preparative Biochemistry & Biotechnology,* 35, 29-36.

PFN-133, I. d. P. (2006). *Bioactividad de aceites esenciales y extractos de plantas medicinales y aromáticas de la Región Sur del Ecuador.* Loja-Ecuador: Universidad Técnica Particular de Loja.

Rao, B. K., Sudarshan, P. R., Rajasekhar, M. D., Nagaraju, N., & Rao, C. A. (2002). Antidiabetic activity of Terminalia pallida fruit in alloxan induced diabetic rats. *Journal of Ethnopharmacology,* 85, 169-172.

Shai, L. J., Masoko, P., Mokgotho, M. P., Magano, S. R., Mogale, A. M., Boaduo, N., & Eloff, J. N. (2010). Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African Journal of Botany*, 76, 465-470.

Shim, Y.-J., Doo, H.-K., Ahn, S.-Y., Kim, Y.-S., Seong, J.-K., Park, I.-S., & Min, B.-H. (2003). Inhibitory effect of aqueous extracto from the gall of Rhus Chinensis on alpha-glucosidase activity and postprandial blood glucose. *Journal of Ethnopharmacology,* 85(2-3), 283-287.

Soumyanath, A. (2006). Traditional Medicines for Modern Times. Antidiabetic Plants. USA: CRC Press.

Takahiro, T., & Takeshi, T. (2006). Bureau Patent No. W.I.P.O.I.

Tene, V., Malagón, O., Finzi, P. V., Vidari, G., Armijos, C., & Zaragoza, T. (2007). An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. *Journal of Ethnopharmacology,* 111(63-81).

Zengin, G., Sarikurkcu, C., Gunes, E., Uysal, A.,Ceylan, R., Uysal, S., Güngör, H., & Aktümsek, A. (2015). Two Ganoderma species: Profiling of phenolic compounds by HPLC- DAD, antioxidant, antimicrobial and inhibitory activities on key enzymes linked to Diabetes mellitus, Alzheimer's disease and skin disorders. *Food & Function,* 6(8), 2794-2802.