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In Vitro evaluation of the antioxidant and hypoglycemic activities of leaves extracts from *Ambrosia arborescens*, *Buddleja incana*, *Aloysia citrodora*, and *Prunus serotina*

Evaluación *in vitro* de la actividad antioxidante e hipoglucemiante de extractos de hojas de *Ambrosia arborescens*, *Buddleja incana*, *Aloysia citrodora* y *Prunus serotina*

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ABSTRACT

Background: Diabetes mellitus is a chronic disease affecting many people in the world. The main symptom of diabetes is high blood glucose levels (hyperglycemia), which triggers an imbalance in the body, producing secondary pathologies associated with oxidative stress generated by this metabolic disorder. **Objective:** This research evaluated the antioxidant and hypoglycemic capacity of *Ambrosia arborescens, Buddleja incana, Aloysia citrodora,* and *Prunus serotina* ethanolic and aqueous leaf extracts. **Methods:** The phytochemical profile of each plant species was characterized through qualitative tests to determine the presence or absence of metabolites such as alkaloids, phenols, triterpenes, and flavonoids. Quantitative determinations of total phenols and flavonoid content were also conducted. The free radical scavenging assay with 2,2-diphenyl-1picrylhydrazil (DPPH) evaluated the antioxidant capacity. The hypoglycemic capacity was performed by quantifying the inhibition capacity of α-amylase and α-glucosidase enzymes. **Results:** All extracts showed a high concentration of phenols and flavonoids. Likewise, all extracts exhibited enzymatic inhibition at different concentrations, with 500 µg/mL showing the highest inhibitory effect. Additionally, the ethanolic extract of *A. arborescens* demonstrated the most excellent hypoglycemic capacity among all the extracts analyzed. **Conclusion:** The results of this study can serve as a basis for future research focused on utilizing medicinal plants to develop pharmaceutical formulations as an alternative treatment for hyperglycemia associated with diabetes.

Keywords: Diabetes mellitus, phytochemical screening, metabolites, antioxidants, hypoglycemic, α-amylase.

RESUMEN

Antecedentes: La diabetes mellitus es una enfermedad crónica que afecta a gran parte de la población mundial. El principal síntoma de la diabetes son los niveles elevados de glucosa en sangre (hiperglucemia), los cuales generan un desequilibrio en el organismo y producen patologías secundarias asociadas al estrés oxidativo derivado de este trastorno metabólico. **Objetivo:** Esta investigación evaluó la capacidad antioxidante e hipoglucemiante de los

extractos etanólicos y acuosos de hojas de *Ambrosia arborescens*, *Buddleja incana*, *Aloysia citrodora* y *Prunus serotina*. **Métodos:** El perfil fitoquímico de cada especie vegetal se caracterizó mediante pruebas cualitativas para determinar la presencia o ausencia de metabolitos como alcaloides, fenoles, triterpenos y flavonoides. Asimismo, se realizaron determinaciones cuantitativas del contenido de fenoles y flavonoides totales. La capacidad antioxidante se evaluó mediante el ensayo de captura del radical libre 2,2-difenil-1-picrilhidrazil (DPPH). La capacidad hipoglucemiante se determinó cuantificando la inhibición de las enzimas α-amilasa y α-glucosidasa. **Resultados:** Todos los extractos mostraron una alta concentración de fenoles y flavonoides. Del mismo modo, todos los extractos presentaron inhibición enzimática a diferentes concentraciones, siendo 500 µg/mL la que evidenció el mayor efecto inhibitorio. Adicionalmente, el extracto etanólico de *A. arborescens* demostró la mayor capacidad hipoglucemiante entre todos los analizados. **Conclusión:** Los resultados de este estudio pueden servir como base para futuras investigaciones orientadas al uso de plantas medicinales en la formulación de productos farmacéuticos como tratamiento alternativo para la hiperglucemia causada por la diabetes.

Palabras clave: Diabetes mellitus, tamizaje fitoquímico, metabolitos, antioxidantes, hipoglucemiante, α-amilasa.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose levels. In recent years, the number of people with DM has increased steadily. In 2019, it was reported that 9.3% (463 million) of the world's population had DM, and it is expected that by 2045, this figure will rise to 10.9% (700 million) (1).

It has been evidenced that abnormal glucose levels in the body caused by DM generate a metabolic imbalance responsible for the overproduction of ROS. This induces cellular damage in the structure of tissues (macromolecules, lipids, and proteins) and increases the complications encompassed by this disease (2). The organism's oxidative environment reduces patients' insulin sensitivity, producing resistance to this hormone and altering glucose tolerance at the cellular level (3).

Under normal conditions, the pancreatic islets of β cells synthesize insulin and store it in response to blood glucose levels (4). When blood glucose levels rise, there is a greater demand for insulin release. This increased demand can lead to the deterioration of pancreatic β cells and the development of insulin resistance. This process is exacerbated by oxidative stress in adipose tissues and skeletal muscles, which impairs glucose absorption (5).

Antioxidants are the primary defense mechanism used to neutralize ROS. However, in hyperglycemic conditions, the organism cannot counteract them in their entirety due to their high generation, resulting in a more significant interaction between ROS, macromolecules, and proteins, as well as glucose molecules. This leads to reactions of these compounds through oxidation and glycation, resulting in lipid peroxidation and the formation of advanced glycation products via the Maillard reaction, which can cause DNA damage and structural alterations in proteins (6).

On the other hand, plants have been used since ancient times in herbal medicine as a traditional therapeutic resource for the treatment of different diseases that affect health due to their high availability, reduced cost, and ancestral experience over time. They are an outstanding alternative to current pharmaceutical formulations (7). This practice is more frequently performed in rural areas due to the existing complications in accessing modern medicine (8).

Traditional medicine can be considered a viable option for the treatment and prevention of diabetes. It helps avoid the side effects associated with synthetic drugs produced by the pharmaceutical industry (9). Additionally, traditional medicine can be used alongside other medications to reduce the required doses and enhance their therapeutic effects. In some cases, it may even serve as a complete substitute for these medications due to its biological activity and the interactions it produces in metabolic processes (10). The main mechanisms used by plants for the treatment of diabetes are based on the improvement of intestinal flora, inhibition of α-amylase and α-glucosidase enzymatic activity, regulation of insulin levels and reduction of oxidative stress (11).

Ambrosia arborescens Mill (Asteraceae), known as "Marco"; *Buddleja incana* Ruiz & Pav (Scrophulariaceae), known as "Quishuar"; Aloysia citrodora Palaú (Verbenaceae), known as "Cedrón"; and *Prunus serotina* Ehrh (Rosaceae), known as "Capuli", although they are not endemic to Ecuador, have shown remarkable adaptability to the country's climatic conditions. This adaptability has facilitated their widespread use, especially among indigenous communities, and more recently in urban areas, thanks to their simple cultivation process. In Ecuadorian traditional medicine, leaf infusions are used to reduce fever, treat malaria, and

alleviate digestive and respiratory disorders. These infusions are also incorporated into therapeutic baths designed to relieve inflammation and pain. In addition, macerated leaves have long been applied directly to the skin to promote the healing of ulcers and superficial wounds. Although some reports mention their use in managing diabetes, this particular application remains anecdotal and lacks robust scientific validation (12). Thus, the current research aims to evaluate the traditional and empirical knowledge of the extracts from these four medicinal plants' leaves on the inhibition of α-amylase and α-glucosidase enzymes.

MATERIALS AND METHODS

Chemicals and reagents.

All the reagents used in this research were of the highest purity available from Sigma Aldrich (St. Louis, MO, USA).

Collection of plant material

Leaves of the different plants were collected in different locations in the city of Pelileo and Ambato (*A. arborescens:* 1°15'13.8"S 78°34'55.5"W; *B. incana:* 1°22'27.4"S 78°34'27.1"W; *A. citrodora:* 1°20'12.3"S 78°30'54.5"W; *P. serotina:* 1°15'13.8"S 78°34'55.5"W), after obtaining authorization for non-commercial collection from the Ministry of Environment, Water and Ecological Transition (No. MAATE-ARSFC-2022-2801).

The plant material was identified by Jorge Caraqui from the herbarium of the Escuela Superior Politécnica de Chimborazo (No-ESPOCH-HERB-035) and subsequently stored at the Research and Development Directorate Operating Unit (UODIDE) of the Faculty of Food Science and Engineering and Biotechnology.

Preparation of plant material

The leaves were disinfected with a 10% sodium hypochlorite solution and washed with abundant water to eliminate excess chlorine. It was then dried in a convection dehydrator (TROCKEN) at 40 °C for 24 to 48 hours. The dried material was crushed using a blender until a fine powder was obtained and stored in Ziplock bags (12).

Extract preparation

The infusion method was used to prepare the aqueous extract. For this, the leaves and distilled water were placed in a beaker in a 1:10 ratio (w/v). Then, it was taken for 10 min at 90 °C in a heating plate with magnetic stirring and waited to cool before being filtered. Finally, the solvent was removed by vacuum rotary evaporator (BUCHI) (60°C at 40 mbar with 100 RPM). The resulting product was stored in sterile plastic tubes under refrigeration until use (13).

The ethanolic extract was obtained using the maceration method. The leaves and 70% ethanol were placed in a glass container in a 1:10 ratio (w/v) and left to stand in the absence of light for 8 days, shaking occasionally to distribute the plant material in the solvent. After this time, the product was filtered. Finally, the solvent was removed by vacuum rotary evaporator (BUCHI) (40°C at 200 mbar with 100 RPM). The resulting product was stored in sterile plastic tubes under refrigeration until use (12).

Phytochemical Screening:

Identification of the chemical content (phenolics, flavonoids, saponins, triterpenoids/steroids) in the extracts was carried out following the procedure of Harborne (1987) (14). Identification of phenolics was carried out using 5% (w/v) $FeCl_3$ reagent, flavonoids using magnesium powder and concentrated hydrochloric acid, saponins using the foam test in water, and triterpenoids/steroids using Salkowski (chloroform and concentrated sulfuric acid) and Liebermann Burchard reagents (chloroform, concentrated sulfuric acid, and anhydrous acetic acid). Identification of alkaloids was carried out with Dragendorff.

Quantification of total phenols and flavonoid content

- **Total phenols**

The modified Folin-Ciocalteu method assay was followed to quantify the total phenolic compound content. For this, 10 µL of the extract in a 1:50 dilution was placed in a 96-well plate, to which 130 µL of distilled water was added, and 10 µL of the Folin-Ciocalteu reagent. It was left to react for 6 min to add 100 µL of a 7% (w/v) sodium carbonate solution. The reaction was incubated at room temperature for 90 min in the absence of light, and finally, the absorbance of each sample was measured at a wavelength of 750 nm (Multiskan Microplate Spectrophotometer - THERMO). A standard curve was prepared with serial solutions of standard gallic acid ranging from 10 to 100 mg/L for calibration. The content of total phenols was expressed as milligram

equivalents of gallic acid per gram of sample dry weight (mg GAE/g dry wt) (15).

- **Total flavonoids**

The aluminum chloride method was followed to determine the total flavonoid content. For this purpose, 60 µL of the extract in a 1:10 solution was mixed with 120 µL of a 2% (w/v) aluminum chloride solution, and the mixture was incubated at room temperature for 60 min. Subsequently, the absorbance was measured at a wavelength of 420 nm (Multiskan Microplate Spectrophotometer - THERMO). A quercetin (QE) standard curve was prepared with serial solutions ranging from 10 to 100 mg/L for calibration. The total flavonoid content was expressed as milligram quercetin equivalent per gram dry weight of the sample (mg QE/g dry wt) (16).

Analysis of Antioxidant Capacity

The DPPH technique described by Bobo et al. (2015) (17) with certain modifications, it was utilized to assess the antioxidant capacity of the extracts.

A stock solution of DPPH (150 µM) was prepared by dissolving 0.0059 g of the reagent with 100 mL of a diluent methanol-water solution in an 80:20 ratio. Subsequently, the solution was covered against light exposure and shaken for 30-40 min. Additionally, a stock solution of Trolox (500 µM) was made by dissolving 0.0125 g of reagent in 100 mL of methanol-water diluent solution in a 50:50 ratio and finally shaken to homogenize the components. Based on the Trolox stock solution, reagent concentrations of 50, 100, 200, 300, 400, and 500 µM were prepared to generate a calibration curve.

The percentage inhibition was calculated according to the following equation (1):

$$\%Inhibition\ DPPH = \left[1 - \left(\frac{A_m - A_b}{A_c - A_b}\right)\right] \times 100 \qquad (1)$$

Where A_m corresponds to the absorbance of the sample at 515 nm, A_b to the absorbance of the blank at 515 nm, and A_c to the absorbance of the control at 515 nm.

Evaluation of hypoglycemic capacity

To evaluate the hypoglycemic capacity of the plant extracts, the α-amylase and α-glucosidase inhibition assays were utilized, following the methodology of Coronado et al. (2021) (18), with specific modifications outlined below.

Enzymatic α-amylase assay

A volume of 50 µL of each extract made at different concentrations was mixed with 50 µL of α-amylase enzyme solution (5 U/mL), which was previously dissolved in 0.1 M phosphate-buffered saline (PBS) (pH 6.9). The mixture was incubated in a thermoblock at 37 °C for 60 min, and then 50 µL of a starch solution (0.5% (w/v)) dissolved in 0.1 M phosphate-buffered saline (pH 6.9) was added to incubate again at 37 °C for 5 min. After the time elapsed, the reaction was stopped by adding 50 µL of DNS reagent (3,5-dinitrosalicylic acid 96 mM), incubated in a thermoblock at 100 °C for 5 min, and allowed to cool for 5 min at room temperature. Subsequently, the absorbance was read at 540 nm using a UV-visible spectrophotometer. The final absorbance of the sample was obtained by subtracting the blank reading from that of the corresponding sample, and the percentage of enzyme inhibition generated was calculated according to equation (2):

$$\%Inhibición = \frac{A_c - A_m}{A_m} \times 100 \qquad (2)$$

Where A_c corresponds to the absorbance of the control at 540 nm and A_m to the absorbance of the sample at 540 nm.

Enzymatic α-glucosidase assay

A volume of 50 µL of each extract at different concentrations (100 - 500 µg/mL) was placed in a sterile microtube with 50 µL of potassium phosphate buffer (0.1 mol/L and pH 6.9), which contained the α-glucosidase solution (0.5 U/ml). The mixture was incubated for 30 min at 37 °C. Then 50 µL of 5 mmol/L pNPG (p-nitrophenyl-α-D-glucopyranoside) solution contained in potassium phosphate buffer (0.1 mol/L and pH 6.9) was added and incubated again at 37 °C for 5 min. Finally, 50 µL of 0.2 mol/L sodium carbonate was added and incubated at 37°C for 5 minutes. Subsequently, absorbance was measured at 405 nm in a UV-visible spectrophotometer. 50 µL of potassium phosphate buffer was used instead of the extract as a control, and the percentage inhibition produced was calculated according to the following Equation (3):

$$\%Inhibición = \frac{A_c - A_m}{A_m} \times 100 \qquad (3)$$

Where A_c will correspond to the absorbance of the control at 405 nm and y A_m to the absorbance of the sample at 405 nm.

Data analysis

For the statistical analysis of the activity of ethanolic and aqueous extracts, ANOVA (analysis of variance) and a Tukey comparison test ($p<0.05$) with a confidence level of 95% were performed using GraphPad Prism version 9.

RESULTS

Extract yield

In the comparative evaluation of extraction methods, variations in yield were evident among the studied species (Table 1), with maximum yield in the maceration extract of *P. serotina*.

Phytochemical analysis

The phytochemical analysis of eight plant extracts (four prepared by infusion and four by maceration) revealed the presence of various groups of secondary metabolites (Table 2). Alkaloids and saponins were found in high concentrations in nearly all the extracts. In contrast, the presence of phenols, triterpenes/steroids, and flavonoids showed no significant variation between the extracts, as each group demonstrated only a minimal presence.

Table 1. Extraction yield of infusion and ethanolic extracts.

EXTRACT YIELD		
Plant	**Extract type**	**Percentage**
A. arborescens	Maceration	10.3
	Infusion	12.5
B. incana	Maceration	11.1
	Infusion	10.6
A. citrodora	Maceration	15.5
	Infusion	18.3
P. serotina	Maceration	17.2
	Infusion	16.6

Table 2. Qualitative phytochemical analysis of secondary metabolites found in ethanolic and infusion extracts.

Plant	Extract type	Phytochemical				
		Alkaloids	**Phenols**	**Triterpenes/steroids**	**Flavonoids**	**Saponins**
A. arborescens	Maceration	++	+	+	+	+++
	Infusion	++	+	+	+	+++
B. incana	Maceration	++	+	+	+	+++
	Infusion	+	+	+	+	-
A. citrodora	Maceration	+++	+	+	+	++
	Infusion	+	+	+	+	-
P. serotina	Maceration	++	+	+	+	-
	Infusion	+	+	+	+	-

Note: -: absence; +: presence; ++: moderate presence; +++: abundant presence.

Determination of total phenolic and flavonoid content

The analysis highlighted the presence of phenols and flavonoids across all the plants, with *P. serotina* shining as the standout performer in both categories. Its infusion extract boasts a remarkable total phenol content of 156.543 ± 7.610 mg GAE/g dry wt, showcasing its potential. Meanwhile, the ethanolic extract showed a commendable flavonoid content of 41,217 ± 1.816 mg QE/g dry wt (Table 3).

Table 3. Total phenolic and flavonoid content of ethanolic and infusion extracts.

Plant	Extract type	Total phenolic content (mg GAE/g dry wt)	Total flavonoid content (mg EQ/g dry wt)
A. arborescens	Maceration	27.703 ± 6.735^{a}	18.991 ± 0.606^{a}
	Infusion	26.605 ± 7.501^{a*}	10.260 ± 0.315^{a*}
B. incana	Maceration	78.888 ± 8.523^{b}	21.077 ± 1.034^{b}
	Infusion	54.197 ± 3.867^{b*}	13.518 ± 0.206^{b*}
A. citrodora	Maceration	40.987 ± 4.128^{c}	26.271 ± 0.997^{c}
	Infusion	54.814 ± 2.000^{b*}	20.615 ± 1.785^{c*}
P. serotina	Maceration	82.160 ± 4.192^{b}	41.217 ± 1.816^{d}
	Infusion	156.543 ± 7.610^{c*}	25.625 ± 0.368^{d*}

Note: Data shows the mean ± SD of 6 replicates. Different letters (a, b, c, d) indicate significant differences. $p < 0.05$ ANOVA post hoc Tukey's test for percent inhibition. Statistical analyses were performed between ethanolic (Letter without asterisk) and aqueous extracts (*) separately.

Antioxidant activity

Similarly, as in the case of phenols and total flavonoids, it was possible to verify a high antioxidant activity in each of the extracts, particularly in *B. incana*. Thus, the ethanolic extract demonstrated an antioxidant activity of 84.619% ± 1.034, while the infusion extract showed an activity of 81.521% ± 1.017 (Table 4).

Table 4: Antioxidant activity (percentage) of ethanolic and infusion extracts.

Extraction method	***Plant***			
	A. arborescens	***B. incana***	***A. citrodora***	***P. serotina***
	% DPPH radical inhibition			
Maceration	79.915 ± 0446^{a}	84.619 ± 1.034^{b}	70.957 ± 0.712^{c}	80.028 ± 1.409^{a}
Infusion	77.408 ± 0.523^{a*}	81.521 ± 1.017^{b*}	65.408 ± 1.090^{c*}	77.464 ± 0.738^{a*}

Note: Data shows the mean ± SD of 6 replicates. Different letters (a, b, c, d) indicate significant differences. $p< 0,05$ ANOVA post hoc Tukey's test for percent inhibition. Statistical analyses were performed between ethanolic (Letter without asterisk) and aqueous extracts (*) separately.

Hypoglycemic Capacity

The α-amylase and α-glucosidase inhibition assays were conducted to evaluate the hypoglycemic potential of the plant extracts at concentrations ranging from 100 to 500 µg/mL. For α-amylase, the results indicated that the extracts exhibited a dose-dependent response, showing greater hypoglycemic activity at the highest concentration of 500 µg/mL (Table 5). Among the various extracts tested, *A. arborescens* demonstrated the highest inhibitory capacity ($p <0,05$), with an aqueous extract showing 41.196±1.457% inhibition and an ethanolic extract displaying 48.956±1.674% inhibition at the 500 µg/mL concentration (Figure 1).

Table 5: α-amylase hypoglycemic capacity (percentage) of ethanolic and aqueous extracts.

Plant	Extraction method	Concentration extracts				
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
A. arborescens	Maceration	12.942±2.984^{a}	19.107±3.645^{a}	23.787±3.513^{a}	34.017±5.204^{a}	48.956±1.674^{a}
	Infusion	22.034±2.899^{a*}	27.988±2.137^{a*}	29.637±2.232^{a*}	32.830±2.556^{a*}	41.196±1.457^{a*}
B. incana	Maceration	9.586±4.416^{a}	19.397±4.978^{a}	22.535±2.593^{a}	29.454±3.574^{a}	41.040±2.770^{b}
	Infusion	19.728±4.117^{a*}	22.076±3.038^{b*}	25.956±3.819^{a*}	27.826±0.390^{b*}	35.531±4.537^{b*}
A. citrodora	Maceration	10.129±1.253^{a}	13.630±3.797^{b}	16.040±2.116^{b}	26.719±4.698^{a}	33.695±2.690^{c}
	Infusion	10.763±4.952^{b*}	12.432±2.739^{c*}	16.227±2.487^{b*}	20.967±2.068^{c*}	26.946±2.005^{c*}
P. serotina	Maceration	4.378±1.667^{b}	9.434±2.414^{b}	12.195±4.219^{b}	16.769±2.927^{b}	23.620±3.622^{d}
	Infusion	5.794±2.036^{b*}	7.892±3.747 ^{c*}	8.859±1.876^{c*}	12.638±1.559^{d*}	19.359±1.283^{d*}

Note: Data show the mean ± SD of 6 replicates of α-amylase hypoglycemic capacity. Different letters (a, b, c, d) indicate significant differences $p < 0.05$, ANOVA post hoc Tukey's test for percent hypoglycemic capacity. Statistical analyses were performed between ethanolic (Letter without asterisk) and aqueous extracts (*) separately.

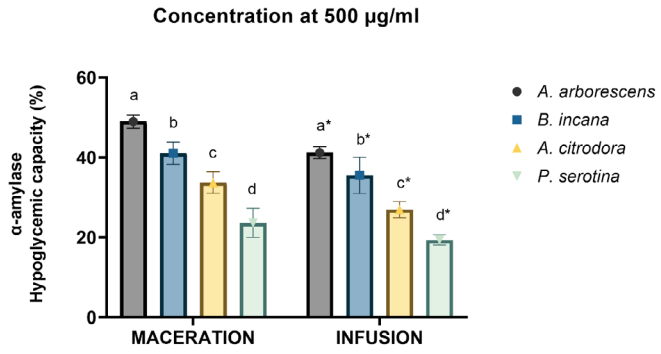

Figure 1: α-amylase hypoglycemic capacity in percentage of ethanolic and aqueous extracts at 500 µg/ml.

In the case of α-glucosidase, verifying a lower hypoglycemic capacity than that exhibited by the extracts in the quantification of α-amylase was possible. However, although the extracts showed a dose dependence, not all concentrations were significant, but a greater hypoglycemic capacity was observed at a concentration of 500 µg/ml (Table 6). Likewise, the ethanolic extracts showed a higher hypoglycemic activity than the infusion extracts (Figure 2).

Table 6: α-glucosidase hypoglycemic capacity of ethanolic and aqueous extracts.

Plant	Extraction method	Concentration				
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
A. arborescens	Maceration	12.942±2.984^{a}	15.762±3.214^{a}	19.874±3.215^{a}	29.674±3.512^{a}	39.874±4.215^{a}
	Infusion	12.348±2.674^{a*}	18.542±3.128^{a*}	22.763±3.412^{a*}	27.543±3.218^{a*}	32.764±3.412^{a*}
B. incana	Maceration	10.129±1.253a	13.489±2.876^{a}	17.562±2.843^{a}	25.389±2.974^{a}	35.562±3.843^{b}
	Infusion	9.215±3.102^{a*}	15.876±2.764^{a*}	18.529±2.874^{a*}	23.876±2.765^{b*}	28.539±2.874^{b*}
A. citrodora	Maceration	9.586±4.416^{a}	10.953±1.745a^{b}	14.739±1.987^{ab}	21.846±2.315^{ab}	28.739±2.987^{c}
	Infusion	7.589±2.431ab*	12.439±1.982^{ab*}	13.684±2.135^{b*}	18.492±2.134^{c*}	23.684±2.135^{c*}
P. serotina	Maceration	4.378±1.667^{b}	8.672±2.193^{b}	11.428±2.156^{b}	15.752±1.893^{b}	22.428±2.156^{d}
	Infusion	4.876±1.987^{b*}	9.215±2.347^{b*}	7.958±1.743^{c*}	11.759±1.987^{d*}	17.958±1.743^{d*}

Note: Data show the mean ± SD of 6 replicates of α α-glucosidase hypoglycemic capacity. Different letters (a, b, c, d) indicate significant differences, $p < 0,05$, ANOVA post hoc Tukey's test for percent hypoglycemic capacity. Statistical analyses were performed between ethanolic (Letter without asterisk) and aqueous extracts (*) separately.

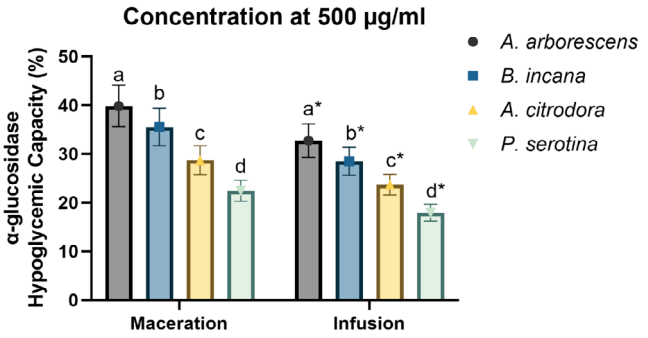

Figure 2: α-glucosidase hypoglycemic capacity in percentage of ethanolic and aqueous extracts at 500 µg/ml.

DISCUSSION

The present research was based on determining the hypoglycemic capacity of *Ambrosia arborescens* (Asteraceae), known as "Marco"; *Buddleja incana* (Scrophulariaceae), known as "Quishuar"; *Aloysia citrodora* (Verbenaceae), known as "Cedrón"; and *Prunus serotina* (Rosaceae), known as "Capuli". These four plants present in the Ecuadorian Andean zone are empirically used for treating diabetes (19).

Firstly, regarding the extracts yield, for *A. arborescens*, agitation during maceration favored a slightly higher yield than previously reported, reaching 12.5% with infusion (20). Similarly, *B. incana* presented yields close to 11%, consistent with earlier studies (21), while *A. citrodora* exhibited the highest values (15.5–18.3%), although differences with other authors suggest the influence of environmental and physiological factors (22). In *P. serotina*, the extraction yield was around 17%, aligning with findings from related species and confirming the importance of the extraction technique (23).

Overall, the results highlight that yield variability is strongly influenced by the solvent, extraction method, and operational conditions. As noted in prior studies, factors such as solvent polarity, temperature, exposure time, and concentration play a decisive role in metabolite solubilization (24,25). In this context, the higher yields obtained with aqueous infusions may be attributed to the strong polarity of water and the thermal conditions applied, which favor the recovery of bioactive compounds (26).

On the other hand, the determination of bioactive compounds allowed the identification of several secondary metabolites in each of the extracts made (ethanolic and aqueous). Thus, a large amount of alkaloids and saponins was observed in almost all the extracts, and only a minimal presence of phenols, flavonoids, and triterpenes (Table 2). These results are similar to those found by several authors, who agree that this group of metabolites possesses diverse biological activities, including antimicrobial, antitumor, antiviral, antioxidant, and hepatoprotective capacities (27,28).

A high concentration of phenols and flavonoids was verified in both ethanolic and aqueous extracts, with variations in concentration depending on the plant. In particular, the ethanolic extracts of *A. arborescens* and *B. incana* showed a higher concentration of these compounds. On the other hand, in *A. citrodora* and *P. serotina*, the highest concentrations varied between ethanolic and aqueous extracts (Table 3).

However, among the four species analyzed, *P. serotina* showed the highest content of phenols and flavonoids. For the most part, the results of this investigation coincide with those reported by other authors (28–30). However, in some cases, such as *A. arborescens* and *B. incana*, a lower phenol and flavonoid content was obtained compared to previous studies (31–33). This variation could be attributed to the extraction method used and the geographical conditions in which the plant material was collected (34).

The relationship between oxidative stress and diabetes is a complex interaction that significantly affects the pathophysiology and progression of diabetes mellitus. Oxidative stress is due to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, contributing to cellular damage and exacerbating diabetes-related complications (35). This imbalance is particularly pronounced in diabetes due to hyperglycemia and hyperlipidemia, which increase ROS production through various metabolic pathways (36). On the other hand, α-amylase and α-glucosidase are critical enzymes involved in carbohydrate metabolism, and their inhibition is an important strategy to control diabetes, particularly type II diabetes (37).

This relationship is reflected in the results obtained, where the extracts with the highest antioxidant capacity were ethanolic extracts, which also showed a remarkable hypoglycemic activity, especially in *A. arborescens* and *B. incana*. Although several authors have pointed out the ability of plant extracts to reduce enzyme activity, the hypoglycemic activity of the extracts analyzed has not been explicitly reported, but only at the level of their family or botanical genus in general (38–41)

Although the results provide a scientific basis for using the four medicinal plants studied as possible hypoglycemic agents, the present study has several limitations. Among them are the lack of specific identification of secondary metabolites and the absence of studies on their behavior in experimental animal models. This prevents, for the moment, the determination of pharmaceutical forms that optimize their absorption and metabolism. However, this represents the first research to generate a scientific basis for using these extracts as a possible enzymatic inhibitor and coadjuvant in the treatment of diabetes.

CONCLUSION

The present investigation has allowed us to determine that the extracts of *Ambrosia arborescens, Buddleja incana*, *Aloysia citrodora,* and *Prunus serotina* showed high content of phenols and flavonoids, highlighting the ethanolic ones for their higher antioxidant activity and inhibition of α-amylase and α-glucosidase, especially at 500 µg/mL. These results demonstrate their potential as sources of bioactive compounds with therapeutic applications, while also facilitating the generation of scientific knowledge on the traditional use of these medicinal plants in Ecuador.

Conflicts of interest: The authors of the manuscript declare that there are no conflicts of interest associated with this work.

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REFERENCES

[1] Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843. DOI: https://doi.org/10.1016/j.diabres.2019.107843

[2] Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders — A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis. 2017;1863(5):1066–1077. DOI: https://doi.org/10.1016/j.bbadis.2016.11.010.

[3] Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative stress: Pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. Bull Exp Biol Med. 2021;171(2):179-89. DOI: https://doi.org/10.1007/s10517-021-05191-7.

[4] Retnakaran R, Pu J, Emery A, Harris SB, Reichert SM, Gerstein HC, et al. Determinants of sustained stabilization of beta-cell function following short-term insulin therapy in type 2 diabetes. Nat Commun. 2023;14:4514. DOI: https://doi.org/10.1038/s41467-023-40287-w

[5] Borse SP, Chhipa AS, Sharma V, Singh DP, Nivsarkar M. Management of type 2 diabetes: Current strategies, unfocussed aspects, challenges, and alternatives. Med Princ Pract. 2021;30(2):109-21. DOI: https://doi.org/10.1159/000511002

[6] Masenga SK, Kabwe LS, Chakulya M, Kirabo A. Mechanisms of oxidative stress in metabolic syndrome. Int J Mol Sci. 2023;24(9):7898. DOI: https://doi.org/10.3390/ijms24097898

[7] Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5):559. DOI: https://doi.org/10.3390/molecules21050559

[8] Lim T, Davis EO, Crudge B, Roth V, Glikman JA. Traditional Khmer medicine and its role in wildlife use in modern-day Cambodia. J Ethnobiol Ethnomed. 2022;18(1):61. DOI: https://doi.org/10.1186/s13002-022-00553-5

[9] Li Y, Kong D, Fu Y, Sussman MR, Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem. 2020;148:80-9. DOI: https://doi.org/10.1016/j.plaphy.2020.01.006

[10] Zhaogao L, Yaxuan W, Mengwei X, Haiyu L, Lin L, Delin X. Molecular mechanism overview of metabolite biosynthesis in medicinal plants. Plant Physiol Biochem. 2023;204:108125. DOI: https://doi.org/10.1016/j.plaphy.2023.108125

[11] Yedjou CG, Grigsby J, Mbemi A, Nelson D, Mildort B, Latinwo L, et al. The management of diabetes mellitus using medicinal plants and vitamins. Int J Mol Sci. 2023;24(10):9085. DOI: https://doi.org/10.3390/ijms24109085

[12] De la Torre L, Navarrete H, Muriel M, Macía MJ, Balslev H, Editores. *Enciclopedia de las Plantas Útiles del Ecuador,* 1ª ed. Herbario QCA & Herbario AAU. Quito & Aarhus: Pontificia Universidad Católica del Ecuador & Universidad de Aarhus; 2008. 949 p.

[13] Salles BCC, da Silva MA, Taniguthi L, Ferreira JN, da Rocha CQ, Vilegas W, et al. *Passiflora edulis* Leaf Extract: Evidence of Antidiabetic and Antiplatelet Effects in Rats. *Biol Pharm Bull.* 2020;43(1):169–74. DOI: https://doi.org/10.1248/bpb.b18-00952.

[14] Urrego N, Sepúlveda P, Aragón M, Ramos FA, Costa GM, Ospina LF, et al. Flavonoids and saponins from *Passiflora edulis* f. *edulis* leaves (purple passion fruit) and its potential anti-inflammatory activity. J Pharm Pharmacol. 2021;73(11):1530–8. DOI: doi: https://doi.org/10.1093/jpp/rgab117.

[15] Harborne JB. General Procedures and Measurement of Total Phenolics. In: *Methods in Plant Biochemistry.* Vol 1. 1st ed. London: Academic Press; 1989. p. 1–28.

[16] Baek SH, Cao L, Jeong SJ, Kim HR, Nam TJ, Lee SG. The comparison of total phenolics, total antioxidant, and anti-tyrosinase activities of Korean Sargassum species. J Food Qual. 2021;2021:6640789. DOI: https://doi.org/10.1155/2021/6640789

[17] N'guessan B, Asiamah A, Arthur N, Frimpong-Manso S, Amoateng P, Amponsah S, et al. Ethanolic extract of Nymphaea lotus L. (Nymphaeaceae) leaves exhibits in vitro antioxidant, in vivo anti-inflammatory and cytotoxic activities on Jurkat and MCF-7 cancer cell lines. BMC Complement Med Ther. 2021;21(1):22. DOI: https://doi.org/10.1186/s12906-020-03195-w

[18] Bobo G, Davidov G, Arroqui C, Vírseda P, Marín M, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. J Sci Food Agric. 2015;95(1):204-9. DOI: https://doi.org/10.1002/jsfa.6706

[19] Coronado J, Carrasco R, Reategui O, Toscano E, Valdez E, Zimic M, et al. Inhibitory activity against α-amylase and α-glucosidase by phenolic compounds of quinoa (Chenopodium quinoa Willd.) and cañihua (Chenopodium pallidicaule Aellen) from the Andean region of Peru. Pharmacogn J. 2021;13(4):896-901. DOI: https://doi.org/10.5530/pj.2021.13.115.

[20] Huamanteca Manrique, Rodríguez Rodríguez. Efecto antiinflamatorio del gel a base del extracto hidroalcohólico de las hojas de *Ambrosia arborescens* Mill (Marco) en ratas albinas. [Trabajo de grado]. [Lima, Perú]: Universidad Interamericana para el Desarrollo. 2020. 81 p.

[21] Rodríguez C, Gabriela J. Actividad antioxidante y antimicrobiana de los extractos preparados con diferentes solventes de las hojas de *Ambrosia arborescens* (Marco). [Trabajo de grado]. [Quito, Ecuador]: Universidad Central del Ecuador. 2020. 65p.

[22] Tufinio Miranda K, Ames Canchaya H, Vergara Sotomayor A, Fukusaki Yoshizawa A, Paucar Cuba K, Tufinio Miranda K, et al. Determinación de la actividad antioxidante de extractos de hojas de Buddleja incana, Oreocallis grandiflora y Chuquiraga spinosa. Rev Soc Quím Perú. 2021;87(2):107-19. DOI: https://doi.org/10.37761/rsqp.v87i2.343.

[23] Bhat RA, Hakeem KR, Dervash MA. *Phytomedicine: A Treasure of Pharmacologically Active Products from Plants*. 1st ed. Amsterdam: Elsevier; 2021. 776p.

[24] Karabegović IT, Stojičević SS, Veličković DT, Todorović ZB, Nikolić NČ, Lazić ML. The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (Prunus laurocerasus) leaf and fruit extracts. Ind Crops Prod. 2014;54:142-8. DOI: https://doi.org/10.1016/j.indcrop.2013.12.047

[25] Oreopoulou A, Tsimogiannis D, Oreopoulou V. Extraction of polyphenols from aromatic and medicinal plants: An overview of the methods and the effect of extraction parameters. In: Watson RR, editor. Polyphenols in plants. 2nd ed. London: Academic Press; 2019. p. 243-59. DOI: https://doi.org/10.1016/B978-0-12-813768-0.00025-6

[26] Dirar AI, Alsaadi DHM, Wada M, Mohamed MA, Watanabe T, Devkota HP. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. S Afr J Bot. 2019;120:261-7. DOI: https://doi.org/10.1016/j.sajb.2018.07.003

[27] Plaskova A, Mlcek J. New insights into the application of water or ethanol-water plant extracts rich in active compounds in food. Front Nutr. 2023;10:1118761. DOI: https://doi.org/10.3389/fnut.2023.1118761

[28] Martino VS, Sülsen VP. Lactonas sesquiterpénicas: Promisorio grupo de compuestos naturales bioactivos. Academia Nacional de Farmacia y Bioquímica. 2019. Available from: https://ri.conicet.gov.ar/bitstream/handle/11336/120348/CONICET_Digital_Nro.8907dbe1-fdce-40d9-b45b-b1c67d12cdf0_A.pdf?sequence=2&isAllowed=y.

[29] Orlando J, Molina R, Maricela A, Castellano T, Ramiro E, Carvajal C, et al. Extracción hidroalcohólica de polifenoles a partir de las hojas de cedrón (Aloysia citrodora Paláu) como ingrediente alimentario natural. Rev Recur Nat Prod Sust. 2022;1(2):56-69. Available from: http://investigacion.utc.edu.ec/index.php/RENPYS/article/view/449/613

[30] Llanga Guamán BG. Determinación de la actividad antioxidante de los extractos de quishuar (Buddleja incana), aliso (Alnus acuminata) y romerillo (Hypericum laricifolium) localizados en tres zonas geográficas diferentes. [Trabajo de grado]. [Riobamba, Ecuador]: Escuela Superior Politécnica de Chimborazo; 2014. 106 p.

[31] Kazan A, Koyu H, Turu IC, Yesil-Celiktas O. Supercritical fluid extraction of Prunus persica leaves and utilization possibilities as a source of phenolic compounds. J Supercrit Fluids. 2014;92:55-9. DOI: https://doi.org/10.1016/j.supflu.2014.05.006.

[32] 30. Kazan A, Koyu H, Turu IC, Yesil-Celiktas O. Supercritical fluid extraction of Prunus persica leaves and utilization possibilities as a source of phenolic compounds. J Supercrit Fluids. 2014;92:55-9. DOI: https://doi.org/10.1016/j.supflu.2014.05.006

[33] Turvey T, Lall N. Anti-proliferative properties of various South African Buddleja species. In: Máthé Á, editor. Medicinal plants for cosmetics, health and diseases. Boca Raton: CRC Press; 2022. p. 175-98. DOI: https://doi.org/10.1201/9781003108375-10

[34] Silva-Correa CR, Villarreal-La Torre VE, González-Siccha AD, Cruzado-Razco JL, González-Blas MV, Sagástegui-Guarniz WA, et al. Acute toxicity of aqueous extract of Ambrosia arborescens Mill. on biochemical and histopathological parameters in rats. Toxicol Res. 2022;38(2):225-33. DOI: https://doi.org/10.1007/s43188-021-00106-0

[35] Tammar S, Salem N, Aidi Wannes W, Limam H, Bourgou S, Fares N, et al. Chemometric profiling and bioactivity of verbena (Aloysia citrodora) methanolic extract from four localities in Tunisia. Foods. 2021;10(12):2912. DOI: https://doi.org/10.3390/foods10122912

[36] Caturano A, D'Angelo M, Mormone A, Russo V, Mollica MP, Salvatore T, et al. Oxidative stress in type 2 diabetes: Impacts from pathogenesis to lifestyle modifications. Curr Issues Mol Biol. 2023;45(8):6651. DOI: https://doi.org/10.3390/cimb45080420.

[37] Chen X, Xie N, Feng L, Huang Y, Wu Y, Zhu H, et al. Oxidative stress in diabetes mellitus and its complications: From pathophysiology to therapeutic strategies. Chin Med J (Engl). 2024. DOI: 10.1097/CM9.0000000000003230.

[38] Ávila-Reyes JA, Almaraz-Abarca N, Alvarado EAD, Torres-Ricario R, Naranjo-Jiménez N, Gutierrez-Velazquez MV, et al. α-Glucosidase and α-amylase inhibition potentials of ten wild Mexican species of Verbenaceae. Trop J Pharm Res. 2019;18(1):31-6. DOI: https://doi.org/10.4314/tjpr.v18i1.5

[39] Bhosale HJ, Mamdapure SV, Panchal RB, Dhuldhaj UP. α-Amylase, α-glucosidase and aldose reductase inhibitory and molecular docking studies on Tinospora cordifolia (Guduchi) leaf extract. Future J Pharm Sci. 2024;10(1):1-13. DOI: https://doi.org/10.1186/s43094-024-00671-9

[40] Elhady SS, Youssef FS, Alahdal AM, Almasri DM, Ashour ML. Anti-hyperglycaemic evaluation of Buddleia indica leaves using in vitro, in vivo and in silico studies and its correlation with the major phytoconstituents. Plants. 2021;10(11):2351. DOI: https://doi.org/10.3390/plants10112351

[41] Kulkarni AA, Kamble RP. α-Amylase inhibitory secondary metabolites from Artemisia pallens Wall. ex DC: Biochemical and docking studies. Biol Life Sci Forum. 2022;11(1):73. DOI: https://doi.org/10.3390/IECPS2021-11978.

[42] Kidane Y, Bokrezion T, Mebrahtu J, Mehari M, Gebreab YB, Fessehaye N, et al. In vitro inhibition of α-amylase and α-glucosidase by extracts from Psiadia punctulata and Meriandra bengalensis. Evid Based Complement Alternat Med. 2018;2018:1-8. DOI: 10.1155/2018/2164345.