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Original Article

Characterization, antioxidant capacity and erythroprotective effect of Zaya (*Amoreuxia palmatifida*) extracts

Caracterización, capacidad antioxidante y efecto eritroprotector de extractos de Zaya *(Amoreuxia palmatifida)*

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ABSTRACT

Zaya (*Amoreuxia palmatifida*) is a herbaceous plant found in northwest Mexico, particularly in the state of Sonora, where it has been historically consumed by its first settlers due to its nutritional and medicinal properties. Despite its importance, there is little information about the phytochemical compounds present in Zaya, its antioxidant activity and erythroprotective effect. Therefore, this study aimed to identify and quantify some of the compounds of interest related to the antioxidant capacity and erythroprotective effect of the plant. The results show that ethanolic extracts of zaya leaf (LE), root (RE) and stem (SE) contain the highest amount of phenols, tannins, flavonoids, chlorophylls and carotenoids, compared to extracts with leaf ethyl acetate (LEA), root (REA), and stem (SEA). Antioxidant activity was highest in LE by DPPH (61.27 ± 1.03 % of inhibition), ABTS (58.21 ± 0.48 % of inhibition) and FRAP (132.44 ± 5.23). All Zaya extracts had erythroprotective effect on O+ (80 to 87 %), followed by B+ (22 - 85 %) and then with A+ (38 - 60 %). In the cytotoxic assay, the highest percentage of hemolysis (41 - 50 %) occurred in blood type B+ with REA, RE, LEA and LE, while the lowest was in blood type O+. Therefore, LE contains the best secondary metabolites that confer greater antioxidant and erythroprocteric capacity without causing toxicity.

Keywords: antioxidants; secondary metabolites; erythrocytes.

RESUMEN

La Zaya (*Amoreuxia palmatifida*) es una planta herbácea que se encuentra en el noroeste de México, particularmente en el estado de Sonora, donde ha sido consumida históricamente por sus primeros pobladores debido a sus propiedades nutricionales y medicinales. A pesar de su importancia, existe escasa información sobre los compuestos fitoquímicos presentes en la Zaya, su actividad antioxidante y su efecto eritroprotector. Por lo tanto, este estudio tuvo como objetivo identificar y cuantificar algunos de los compuestos de interés relacionados con la capacidad antioxidante de la planta y efecto eritroprotector. Los resultados muestran que extractos etanólicos de zaya de hoja (LE), raíz (RE) y tallo (SE) contienen la mayor cantidad de fenoles, taninos, flavonoides, clorofilas y carotenoides en comparación con los extractos con etil acetato de hoja (LEA), raíz (REA) y tallo (SEA). La actividad antioxidante fue mejor en LE en DPPH (61,27 ± 1,03 % de inhibición), ABTS (58,21 ± 0,48 % de inhibición) y FRAP (132,44 ± 5,23). Todos los extractos de Zaya tuvieron efecto eritroprotector sobre el grupo O+ (80 - 87 %), seguido del B+ (22 - 85 %) y luego del A+ (38 - 60 %). En el ensayo citotóxico, el mayor porcentaje de hemólisis (41 - 50 %) ocurrió en el grupo sanguíneo B+ con REA, RE, LEA y LE, mientras que el más bajo fue en el grupo sanguíneo O+. Por tanto, LE contiene los mejores metabolitos secundarios que le confieren mayor capacidad antioxidante y eritroprotectora sin provocar toxicidad.

Palabras clave: antioxidante; metabolitos secundarios; eritrocitos.

INTRODUCTION

Zaya (*Amoreuxia palmatifida*), also known as Saya, is an herbaceous plant that reaches a maximum height of 50 centimeters. It has yellow-orange petals measuring 5-7 cm in diameter, a capsule fruit, tuberous and elongated roots, and green palm-shaped leaves (Poppendieck, 1981; Michel *et al*., 2023). All parts of the Zaya plant are edible, including the root, stem, leaf, petals, and fruits (Hodgson, 1993), with the root being the most consumed part and having various preparation techniques, such as cooking methods and soaking to avoid a bitter taste. This species is particularly mentioned

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for its use in treating spider bites and as a potential treatment for diabetes (Castro-Montoya *et al*., 2012; Michel *et al*., 2023). Currently, *Amoreuxia palmatifida* is classified as "Subject to Special Protection" according to the NOM 059 (SEMARNAT, 2010), highlighting the importance of studying it in the field of agriculture for reproduction purposes and exploring bioactive compounds of interest in the areas of food and pharmaceuticals for consumption.

Phytochemical compounds can be understood as chemical substances naturally produced by plants. Not all of them are considered beneficial for human metabolism, but some, such as flavonoids, carotenoids, chlorophylls, and phenols, are associated to antioxidant or anti-inflammatory properties (González-Gallego *et al*., 2010; Pandey and Rizvi, 2009; Tiwari *et al*., 2015). The use of bioactive compounds from plants has increased for various purposes, including pharmacological and nutraceutical applications (Cameron *et al*., 2005), being the antioxidant activity one of the most studied properties in this field.

Antioxidant activity is an area of interest in plants for the prevention of cellular damage caused by free radicals. This activity is important for the integrity of vital biomolecules such as DNA, proteins, and lipids (Lobo *et al*., 2010). The detrimental effects of free radicals in the body have driven the search for compounds with antioxidant properties as potential therapeutic treatments. Given the increasing concern about the potential toxic effects of synthetic antioxidants used in food preservation, there is a need to develop safer and more versatile antioxidants for application in the food and pharmaceutical industries (Barlow, 1990). However, one of the current challenges in the field of nutrition and medicine is understanding the mechanisms followed by antioxidant compounds in different blood types.

In recent years, research has focused on ABO antigens to describe their role in various diseases and the mechanisms involved (Cooling, 2015; González-Vega *et al*., 2023). Since antigens are proteins and carbohydrates bound to other proteins or lipids found in different organs such as the kidney, intestine, or heart, it is important to analyze whether the Zaya plant can affect these mechanisms to predict whether it may have beneficial or detrimental effects on specific blood types.

Therefore, the objective of this study was to analyze phytochemical compounds of Zaya, including quantification of phenols, flavonoids, carotenoids, and chlorophylls, as well as qualitatively analyze the presence of saponins, tannins, and coumarins. Additionally, the study aimed to gain an initial understanding of the plant's antioxidant capacity through three assays: FRAP, ABTS, and DPPH. The erythroprotective effect of Zaya was done on three blood types, A+, B+, and O+. Furthermore, the cytotoxicity of the plant was also investigated.

MATERIAL AND METHODS

Vegetal material and extract obtention

Zaya *(Amoreuxia palmatifida*) was obtained from the Department of Agriculture and Livestock at the University of Sonora (29.01451° N, 111.13378° W). The climate was characterized as very dry and warm, with a mean annual temperature of 25.4°C. Maximum temperatures are recorded in June, while the lowest temperatures occur in February. Annual precipitation averages 399.4 mm (data from 1986 to 2022), predominantly during the summer monsoon season between July and August, with occasional winter rainfall (INEGI, 2022). The soil at the site is classified as sandy loam, with a bulk density of approximately 1.50 g/cm^3 (WRB, 2022). The plant was cultivated outdoors in agricultural soil under drip irrigation. A weekly irrigation rate of 1.0 cm was applied, though irrigation was suspended during weeks with rainfall exceeding 20 millimeters. Growth occurred during summer under warm, full-sun conditions without shading. Stems, leaves and roots were extracted from one-year-old plants. Samples were washed with water, and left to dry for two weeks in darkness at room temperature.

Roots, leaves and stems were grinded with a mortar and pestle, and also a grinder machine Hamilton Beach® was used for thirty seconds. A 50-mesh sieve was used to homogenize the remaining part. One gram of sample was weighed and added to a 50 mL Falcon® tube. Two different solvents were used for extraction, ethyl acetate and ethanol. Ten milliliters of ethyl acetate were poured into three different tubes with the pulverized samples of roots, leaves and stems. The same process was done with 10 mL of ethanol. A vortex was used to homogenize and samples were sonicated for thirty minutes at 27 °C. Extracts were filtered and the volume was adjusted at 35 mL with both ethyl acetate and ethanol. Samples of zaya were named as follows: ethyl acetate extracts from roots (REA), leaves (LEA) and stem (SEA) and ethanolic extracts from roots (RE), leaves (LE) and stems (SE). A phytochemical profile was carried out as follows: saponins, coumarins and tannins were determined qualitatively, whilst phenols, flavonoids, chlorophyll and carotenoids, quantitatively.

Phytochemical profile

Saponins

Nine mL of distilled water were added to 1 mL of extract in a test tube (13 x100), shaken vigorously for 30 seconds by hand, and samples were left to rest for 10 minutes. The amount of foam per millimeter suggests a higher amount of saponins: less than 5 mm indicates their absence, between 5 and 10 mm a moderate content, and more than 15 mm high amount (Galindo *et al*., 1989; Guajardo-Flores *et al*., 2012, García *et al*., 2009).

Coumarins

A volume of 2 mL of extract was added to each test tube (13x100). Five milliliters of NaOH dissolution (1.5 M) were prepared and added with 5mL distilled water. Filter paper was cut off at a length of 5 cm and a width of 1 cm, and placed between the glass tube and the thread without touching the extract. The tubes were heated with a Fischer burner until gases finally evaporated. Subsequently, the filters were exposed to UV (UVP® 95-0279-01 Model UVLS-26 EL Series 2UV)

at 254 and 365 nm to observe the possible fluorescent dots indicating the presence of coumarins (García *et al*., 2009).

Tannins

In a Falcon tube, 0.02 mL of extract and 5 mL of $K_4[Fe(CN)_6]$ (4mM) were mixed in the absence of light and centrifuged at 100 rpm for 15 minutes. A volume of 1 mL of $FeCl_3$ (8 mM) was added to the test tube. Dark green pigment indicates presence of condensed tannins, blue color suggests hydrolyzable tannins (Alcaráz-López, 2009; García *et al*., 2009; Sánchez *et al*., 2010).

Phenols and flavonoids quantification

For phenol quantification, a 10 µL aliquot of distilled water was mixed with 25 µL of Na_2CO_3 (1.89 M) in a microplate. A mix of 25 µL Folin-Ciocalteu (1 N) reactive and 10 µL of each sample extract were put in the same recipient. After 30 minutes, the samples were read on a spectrophotometer at 760 nm (García *et al*., 2009). A gallic acid curve (0- 1 mg/mL) was performed. Data was reported as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

Quantification of flavonoids was carried out by adding 80 µL of each sample to 80 µL of $AlCl_3$ ethanolic solution (20 g/L). After an hour without light, the samples were read on a spectrophotometer at 415 nm (García *et al*., 2009). A quercetin curve (1 mg/mL) was performed, and the results were reported as milligrams of quercetin equivalents per gram of sample (mg QE/g).

Chlorophyll and carotenoids

Using leaf ethanolic extracts, chlorophyll a (C_a), chlorophyll b (C_b) and total carotenoids (C_{x-C}) were quantified (µg/mL) with the Jeffrey and Humphrey (1975) methodology, using the following equations:

$$C_a = 15.65_{A666} - 7.34_{A653} \quad \text{Equation 1}$$

$$C_b = 27.05_{A653} - 11.21_{A666} \quad \text{Equation 2}$$

$$C_{x-c} = \frac{(1000_{A470} - 2.86C_a - 129.2C_b)}{221} \quad \text{Equation 3}$$

Where A is the absorbance.

Antioxidant capacity

An antioxidant capacity assay was carried out in ethanolic and ethyl acetate extracts of zaya. The evaluated tests were 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 1,1-diphenyl-2picrylhydrazil (DPPH) and Ferric Reducing Antioxidant Power (FRAP).

ABTS

An ABTS solution was prepared (7.04 mM) and mixed with sodium persulfate (2.75 mM). The solution was left to rest for 24 h in darkness at room temperature. The absorbance was read at 734 nm (Re *et al*., 1999). One mL of the cationic radical was taken and approximately 88 mL of ethanol were added. This should be adjusted to 0.7 ± 0.02 of absorbance at 734 nm. An amount of 270 µL of the adjusted cationic radical solution was taken and 20 µL of samples were added. After 30 min of rest, the samples were read at 734. Results were expressed as % of inhibition following the next equation:

$$\%\ of\ inhibition = \frac{Aintial - Afinal}{Ainitial}\ x\ 100 \quad \text{Equation 4:}$$

Where A is the absorbance.

DPPH

One milligram of DPPH free radical was dissolved in 15 mL of methanol, resulting in a concentration of 1.69×10^{-4} M. Samples (20 µL) were added to 200 µL of the DPPH solution and incubated in the dark for 30 minutes. Absorbance was then measured at 515 nm (Brand-Williams *et al*., 1995). Results were expressed as a percentage of inhibition using the ABTS equation.

FRAP

FRAP was prepared with acetate buffer (300 mM, pH 3.6), $FeCl_3$ (20 mM) and 2,4,6-tripyridyl-s-triazine (TPTZ, 10 mM) with HCl (40 mM). FRAP solution was made in a 10:1:1 proportion. A volume of 280 µL of FRAP was placed in a microplate reader with the 20 µL of the sample. Absorbance was read at 638 nm after 30 minutes in darkness (Benzie and Strain, 1996). A Trolox curve was prepared (0-1 mg/mL) and the results were expressed as micromole of Trolox equivalent per gram of sample (µmol TE/g).

Erythroprotective effect on ABO-Rh positive types groups

Red blood cells (RBC) to obtain A, B and O groups with Rh positive, were obtained by venipuncture from healthy adults of 21-45 years (with the informed consent of the participants). Ethical approval for the study was obtained (CI 2023-47). Samples were collected using sterile materials and tubes containing EDTA as anticoagulant. The RBC concentration was quantified using an automated hematology analyzer (BC-6000, Mindray), and the samples were adjusted to a concentration range of 4.7 to 6.1 × 10^6 cells/µL. A 10% erythrocytes suspension was prepared by washing three times with PBS (phosphate-buffered saline; 0.15 M; pH 7.4), removing the total plasma by centrifugation (2000 × *g* for 10 min) and recovering the globular package. Samples were prepared at a 1:9 proportion of DMSO/PBS to prevent erythrocyte hemolysis. The erythroprotective effect was determined by an inhibition hemolysis assay (Lu *et al*., 2010) with some modifications. AAPH (2,2 -azobis-[2-methylpropionam idine]) was used in this assay to induce a free radical in membrane erythrocytes. The reaction system was carried out as follows: 100 µL of AAPH + 100 µL of erythrocyte suspension + 100 µL of blood suspension. Also, a negative and positive control were prepared by using 200 µL of physiologic buffered saline solution and Triton X-100 1%, respectively, plus 100 µL of blood suspension. Results were expressed as percentages of

hemolysis inhibition and were calculated using the following

$$\%\ hemolysis\ inhibition\ \frac{AAPH - HS}{APPH}\ x\ 100 \qquad \text{Equation 5}$$

where AAPH represents the optical density of hemolysis induced by AAPH, and HS represents the optical density of hemolysis inhibition. The optical density was measured using a microplate reader at a wavelength of 540 nm, which is commonly used for hemoglobin release assays.

Cytotoxicity assay

This methodology was carried out as described by Agarwal *et al*. (2019) with some modifications. In Falcon tubes, 100 µL of each Zaya sample (LE, LEA, RE, REA, SE, SEA) + 100 µL of physiological solution + 100 µL of each blood sample (A, B, and O Rh +) were added. Concentrations of 10 mg/mL and 5 mg/mL were used for Zaya extract samples in different tubes. They were incubated for 2 h at 37 °C, with agitation (45 rpm). Then, 1 mL of physiological solution was added. Samples were centrifuged for 10 minutes at 3200 *x g* and 300 µL were read at 560 nm on a microplate. Also, a negative and positive control were prepared by using 200 µL of physiological buffered saline solution and Triton X-100 1%, respectively, + 100 µL of blood suspension. Results were reported as % of hemolysis according to the next equation:

$$\%\ of\ hemolysis\ \frac{A_{sample} - A_{PBS}}{A_{Triton - A_{PBS}}}\ x\ 100 \qquad \text{Equation 6}$$

Where: A_{sample}: Absorbance of the sample containing treated red blood cells. APBS: Absorbance of the negative control (PBS). A_{Triton}: Absorbance of the positive control (Triton X-100).

IC_{50}

The half-maximal inhibitory concentration (IC_{50}) was calculated from the dose-response curves generated in the DPPH and ABTS assays. In the first one, a linear regression model was applied due to the linear relationship between concentration and radical inhibition within the studied range (2–10 mg/mL). For ABTS, a non-linear regression model was used to estimate the IC_{50}, as the data distribution and absence of intermediate points between 2 and 10 mg/mL indicated a non-linear trend. The IC_{50} values represent the extract concentration required to inhibit 50% of the radical activity.

Statistical analysis

Tukey model was used for comparing means (with $p < 0.05$) to carry out the statistical analysis with analysis of variance (ANOVA). Standard deviation of the results is also presented. All data were analyzed using the statistical program JMP software v16. The study was carried out under controlled conditions with a minimum of three repetitions ($n \geq 3$) for each analysis.

RESULTS AND DISCUSSION

Qualitative phytochemical profile

In the qualitative phytochemical profile, secondary metabolites such as tannins, flavonoids, phenols, and carotenoids were found in the leaves, stem, and roots (Table 1). In the case of leaves, chlorophylls 'a' and 'b' were detected in both ethanol and ethyl acetate solvents. Since chlorophyll 'b' is more polar than chlorophyll 'a', ethyl acetate —a less polar solvent— showed higher efficiency in extracting chlorophyll 'a' from leaves. In roots and stem samples, it was not possible to detect chlorophyll. In roots, the absence of this compound is normal, since it is a part of the plant that is completely absent of light, therefore it lacks chloroplasts (organelles where chlorophyll is located). On the other hand, chlorophyll could be found in the stem, however, when the zaya stems were dried, they had a brownish color, which means the chlorophyll could have been oxidized and it was not possible to be detected (Hörtensteiner, 2006). No saponins or coumarins were found in any of the samples studied or with any of the solvents used. Saponins are generally considered toxic, so it is an advantage that this plant does not contain them. Flavonoid-type phenols are found in greater abundance than tannins (hydrolyzable type) in leaves. According to the results, phenols are catechol-type (excellent antioxidants). Carotenoids were found in greater amounts in ethyl acetate since they are generally considered non-polar type structures. The reason for finding or not finding certain secondary metabolites, as well as their amount in plants, may be due to different causes such as soil conditions, interchange of CO_2, temperature, ozone, light, and UV stress among others (Pant, 1990; Pant *et at*., 2021). In the present study, the Zaya sample was approximately one year old and cultivated under controlled irrigation conditions. As previously described, the plants were grown outdoors in sandy loam soil with a weekly drip irrigation rate of 1.0 cm, adjusted based on rainfall (suspended during weeks with precipitation exceeding 20 mm). The age of the plant and its cultivation conditions (e.g., irrigation, soil type, and environmental factors) can significantly influence the production and composition of secondary metabolites (Sui *et al*., 2012).

Table 1. Identification of secondary metabolites in ethanolic and ethyl acetate extracts from leaf, stem, and root of zaya **(*Amoreuxia palmatifida*)**
Tabla 1. Identificación de metabolitos secundarios en extractos etanólicos y de acetato de etilo de hoja, tallo y raíz de zaya (*Amoreuxia palmatifida*)

Metabolite	LE	LEA	RE	REA	SE	SEA
Tannins	+	+	+	+	+	+
Saponins	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-
Flavonoids	+++	++	+	+	+	+
Phenols	+++	+	+	+	++	+
Carotenoids	++	+++	+	+	+	+
Chlorophyll a	+++	++	-	-	-	-
Chlorophyll b	++	+	-	-	-	-

LE: Leaf ethanolic extract, LEA: Leaf ethyl acetate extract, RE: Root ethanolic extract, REA: Root ethyl acetate extract, SE: Stem ethanolic extract, SEA: Stem ethyl acetate extract; +: low; ++: moderate; +++: abundant; -: absent.

Quantitative phytochemical profile

Phenols

According to the results (Figure 1), LE and SE showed higher amounts of phenols than the rest of the samples. The literature is scarce regarding the phytochemical profile of zaya, however, the genus *Amoreuxia* to which the zaya belongs, is similar to the genus *Cochlospermum*, both belonging to the *Cochlospermaceae* family; studies such as that by Ahmad *et al*. (2021) have identified phenols in ethanolic extracts, which aligns with our findings through the use of the same solvent (ethanol) for extraction. The qualitative identification of catechol-type phenols could indicate that these compounds could be present in the ethanol solvent due to polarity. However, further studies related with their identification (e.g. High-performance liquid chromatography) on these metabolites in Zaya are needed.

Phenols play a series of different functions in the plant such as: metabolic, growth, reproduction and protection against pathogenic organisms, predators, and environmental conditions. That is why they are almost always found in greater quantities in the leaves, as demonstrated in our research. The antioxidant activity observed in our extracts could be attributed to the presence of phenolic compounds, which have been previously characterized in *Cochlospermum* species. For example, ethanolic extracts of *C. planchonii* showed a strong correlation between their high phenol content (476 mg GAE/g) and their ability to neutralize free radicals and chelate metals, effects mediated by hydroxyl groups in their structure (Oumar *et al*., 2014). These findings support the potential of the phenols identified in our study to modulate oxidative stress associated with chronic pathologies.

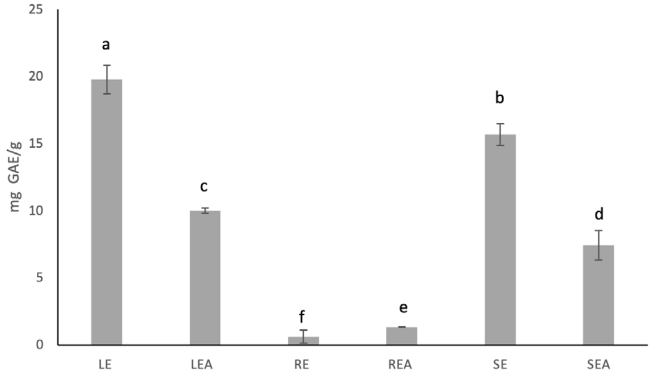

Figure 1. Quantification of total phenols in milligrams equivalent to gallic acid per gram of sample (mg GAE/g). LE: Leaf ethanolic extract, LEA: Leaf ethyl acetate extract, RE: Root ethanolic extract, REA: Root ethyl acetate extract, SE: Stem ethanolic extract, SEA: Stem ethyl acetate extract. One-way analysis of variance (ANOVA) with post-hoc analysis (Tukey's test) was performed. Different letters indicate significant differences between samples ($p<0.05$).

Figura 1. Cuantificción de fenoles totales en miligramos de equivalentes de ácido gálico por gramo de muestra (mg GAE/g). LE: extracto etanólico de hoja, LEA: extracto de acetato de etilo de hoja, RE: extracto etanólico de raíz, REA: extracto de acetato de etilo de raíz, SE: extracto etanólico de tallo, SEA: extracto de acetato de etilo de tallo. Se realizó análisis de varianza de una vía (ANOVA) con análisis post-hoc (Tukey's test). Letras diferentes indican diferencias significativas entre las muestras ($p<0.05$).

Flavonoids

The presence of flavonoids was found in both ethanolic and ethyl acetate extracts. Higher amounts of these metabolites are in Zaya's leaf in ethanolic extract (Figure 2). These compounds are characterized by one or more aromatic or benzene rings with hydroxyl groups, which confer antioxidant properties. As a result, phenols can help prevent oxidative damage, which is closely linked to the development of various diseases, particularly chronic degenerative conditions. Among phenolic compounds, flavonoids are one of the most common and diverse groups, playing key roles in plant defense and adaptation. Therefore, their quantification is essential for understanding their contribution to plant physiology and potential health benefits (Zhang *et al*., 2022). LE has approximately three times the amount of flavonoids than LEA. There is limited information available on flavonoids in Zaya, but in species from the same family, such as *Cochlospermum planchonii*, flavonoids have been found in the roots (Oumar *et al*., 2014). However, in our results, the amount of flavonoids in roots (RE and REA) and stems (SE and SEA) was significantly lower compared to leaves.

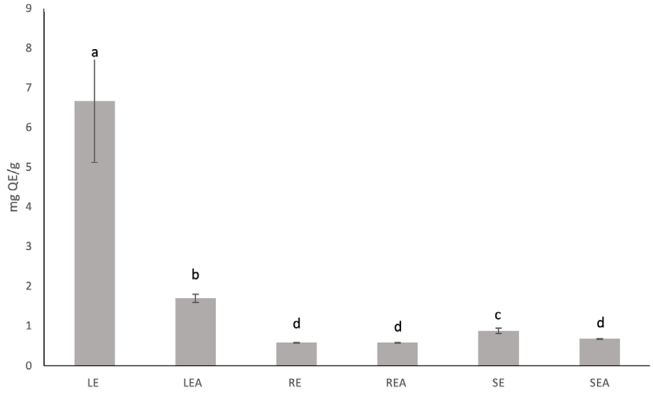

Figure 2. Quantification of flavonoids in milligrams equivalent to quercetin per gram of sample (mg GAE/g). LE: Leaf ethanolic extract, LEA: Leaf ethyl acetate extract, RE: Root ethanolic extract, REA: Root ethyl acetate extract, SE: Stem ethanolic extract, SEA: Stem ethyl acetate extract. One-way analysis of variance (ANOVA) with post-hoc analysis (Tukey's test) were performed. Different letters indicate significant differences between samples ($p<0.05$).

Figura 2. Cuantificción de flavonoides en miligramos de equivalentes de quercetina por gramo de muestra (mg GAE/g). LE: extracto etanólico de hoja, LEA: extracto de acetato de etilo de hoja, RE: extracto etanólico de raíz, REA: extracto de acetato de etilo de raíz, SE: extracto etanólico de tallo, SEA: extracto de acetato de etilo de tallo. Se realizó análisis de varianza de una vía (ANOVA) con análisis post-hoc (Tukey's test). Letras diferentes indican diferencias significativas entre las muestras ($p<0.05$).

Chlorophyll

The quantification of chlorophyll could only be determined in the leaf extracts, since they were undetectable in the stems, perhaps because they could have been oxidized during the drying of the plant, as mentioned in the qualitative determination part. Leaf in ethanolic extract (LE) shows better type "a" chlorophyll content (Figure 3). The higher content of chlorophylls in polar solvents may be due to coordination bonds in -OH groups (Lee *et al*., 2021). Currently, information about chlorophyll in this genus or species is scarce. In plants,

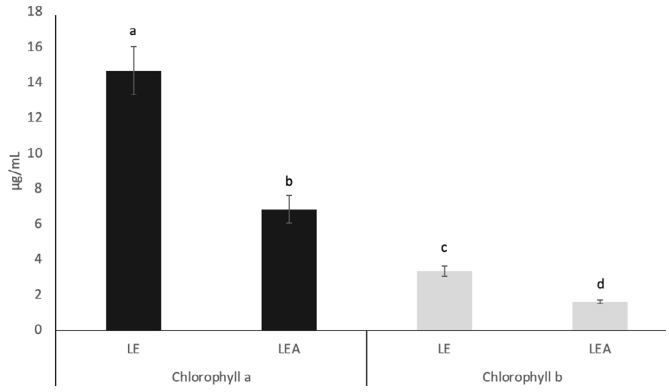

Figure 3. Quantification of chlorophyll type "a" and "b". LE: Leaf ethanolic extract, LEA: Leaf ethyl acetate extract, RE: Root ethanolic extract, REA: Root ethyl acetate extract, SE: Stem ethanolic extract, SEA: Stem ethyl acetate extract. One-way analysis of variance (ANOVA) with post-hoc analysis (Tukey's test) were performed. Different letters indicate significant differences between samples ($p<0.05$).

Figura 3. Cuantificción de clorofila "a" y "b". LE: extracto etanólico de hoja, LEA: extracto de acetato de etilo de hoja, RE: extracto etanólico de raíz, REA: extracto de acetato de etilo de raíz, SE: extracto etanólico de tallo, SEA: extracto de acetato de etilo de tallo. Se realizó análisis de varianza de una vía (ANOVA) con análisis post-hoc (Tukey's test). Letras diferentes indican diferencias significativas entre las muestras ($p<0.05$).

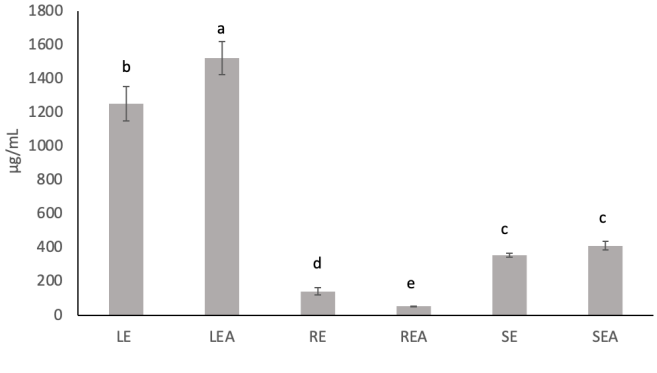

Figure 4. Carotenoids quantification in zaya. LE: Leaf ethanolic extract, LEA: Leaf ethyl acetate extract, RE: Root ethanolic extract, REA: Root ethyl acetate extract, SE: Stem ethanolic extract, SEA: Stem ethyl acetate extract. One-way analysis of variance (ANOVA) with post-hoc analysis (Tukey's test) were performed. Different letters indicate significant differences between samples ($p<0.05$).

Figura 4. Cuantificción de carotenoides en zaya. LE: extracto etanólico de hoja, LEA: extracto de acetato de etilo de hoja, RE: extracto etanólico de raíz, REA: extracto de acetato de etilo de raíz, SE: extracto etanólico de tallo, SEA: extracto de acetato de etilo de tallo. Se realizó análisis de varianza de una vía (ANOVA) con análisis post-hoc (Tukey's test). Letras diferentes indican diferencias significativas entre las muestras ($p<0.05$).

chlorophyll "a" is the main pigment that transforms light energy into chemical energy, while chlorophyll "b" transfers absorbed light to chlorophyll "a". For this reason, these compounds are substances that have antioxidant properties and can impact the prevention of oxidative damage, closely related to the onset of various diseases, principally degenerative chronic diseases (Martins *et al*., 2023).

Carotenoids

Carotenoids are natural yellow, orange or red pigments. LEA shows more carotenoid content (Figure 4). Carotenoids are considered non polar compounds, which means ethyl acetate is less polar than ethanol. For this reason LEA presented higher amounts of these compounds. However, leaves contain more carotenoids in zaya, obtained with both solvents. The rest of the samples showed very low carotenoid content. There is limited information available on the amount of carotenoids found in the genus *Amoreuxia* or the *Cochlospermaceae* family. However, literature mentions that carotenoids are found in the photosynthetic parts of plants, alongside chlorophyll, in this case, the leaf (Swapnil *et al*., 2021). The quantity of these carotenoids depends on the conditions to which the plant is exposed, particularly solar exposure (Isaksson, 2009). Carotenoids are a group of bioactive compounds known for their dual role as antioxidants and provitamins. Notably, certain carotenoids, such as beta-carotene, are converted into vitamin A within the body, playing a critical role in maintaining vision, immune function, and cellular health. Studies suggest that their antioxidant activity may help mitigate oxidative stress, a key factor in the development of chronic diseases, including cancer. This dual functionality —acting as both antioxidants and precursors to essential nutrients— makes carotenoids a compelling subject for further investigation, particularly in the context of disease prevention and health promotion (Kabir *et al*., 2022; Thirunavukarasu *et al*., 2022; Bohn *et al*., 2021).

Antioxidant capacity

The antioxidant activity of Zaya (Table 2) shows better results in the ethanolic extracts. LE presented higher antioxidant capacity with DPPH, FRAP, and ABTS. The IC_{50} was calculated in DPPH and ABTS, at 8.011 mg/mL (Figure 5) and 8.2465 mg/mL (Figure 6), respectively. The absence of intermediate data points in the ABTS assay (between 2 and 10 mg/mL) may introduce uncertainty in the IC_{50} estimation. However, the non-linear model provided a robust approximation under these constraints. The higher antioxidant activity may be due to the high content of chlorophylls, carotenoids, phenols

Table 2. Antioxidant activity of Zaya extracts from leaves, roots and stems in ethanol and ethyl acetate.

Tabla 2. Actividad antioxidante de extractos de Zaya de hojas, raíces y tallos en etanol y acetate de etilo.

	DPPH	ABTS	FRAP
	Inhibition (%)		µmol ET/g
LE	61.27 ± 1.03	58.21 ± 0.48	132.44 ±5.23
LEA	5.23 ± 0.21	32.09 ± 3.96	33.70 ±0.33
RE	10.03 ± 5.95	11.83 ± 0.21	37.08 ±0.56
REA	3.92 ± 5.50	6.78 ± 0.10	32.89 ±0.06
SE	25.41 ± 3.58	46.17 ± 3.61	42.53 ±0.96
SEA	5.54 ± 3.43	0.57 ± 0.87	32.92 ±0.45

The values are presented as mean ± standard deviation (SD) of three repetitions in extracts of Zaya. LE = Leaf with ethanol; LEA = Leaf with ethyl acetate; RE = Root with ethanol; REA = Root sith ethyl acetate; SE = Stem sith ethanol; SEA = Stem with ethyl acetate. Initial concentration of samples 28.5 mg/mL.

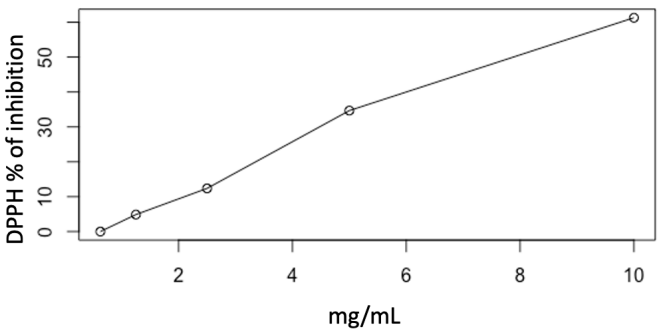

Figure 5. Comparision of different concentrations in mg/mL of ethanolic leaf extract of Zaya with DPPH assay. IC_{50}= 8.011 mg/mL.
Figura 5. Comparación de diferentes concentraciones en mg/mL de extracto etanólico de hoja de Zaya con el ensayo de DPPH. IC_{50}= 8.011 mg/mL.

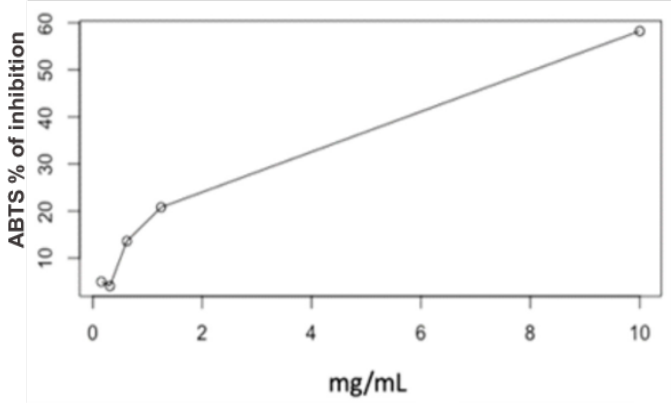

Figure 6. Comparision of different concentrations in mg/mL of ethanolic leaf extract of Zaya with ABTS assay. IC_{50}= 8.2465 mg/mL.
Figura 6. Comparación de diferentes concentraciones en mg/mL de extracto etanólico de hoja de Zaya con el ensayo de ABTS. IC_{50}= 8.2465 mg/mL.

and flavonoids in Zaya. It has been demonstrated that these compounds can exert this function. Due to their chemical structure, these compounds can donate electrons to stabilize the free radicals (Roshanak *et al*., 2016).

In the case of FRAP, an equivalent of 132.44 ±5.23 µmol ET/g was obtained. The difference between DPPH and ABTS could be explained by the selectivity of the former with free radicals containing hydroxyl groups, while the second one, can interact with a wider variety of antioxidant compounds, even those that do not contain the hydroxyl functional group (Parcheta *et al*., 2021). Leaf demonstrate significant amounts of phenols compared with root and stem, so we can attribute the high percentage of inhibition in DPPH to these compounds and others not studied, such as ascorbic acid. As for ABTS, the percentage of inhibition is slightly lower, even though it is an assay where the range of antioxidants that can neutralize this radical is greater, but it is possible that carotenoids are more effective in ABTS than in DPPH, as Müller *et al*. (2011) mention. The FRAP method works by reducing the ferric ion to ferrous, indicating the reducing potential although it is not able to measure the antioxidant activity of compounds that act through different mechanisms (Benzie and Strain, 1996), and the higher amount of phenols and flavonoids could explain the high result compared to the other parts of the plant.

Erythroprotective effect of Zaya extracts on ABO-positive types of groups

The erythroprotective effect was determined by an inhibition hemolysis assay (Figure 7). Zaya is a plant where all its parts are commonly consumed, according to Celaya-Michel *et al*. (2017), however, there is limited information about its health

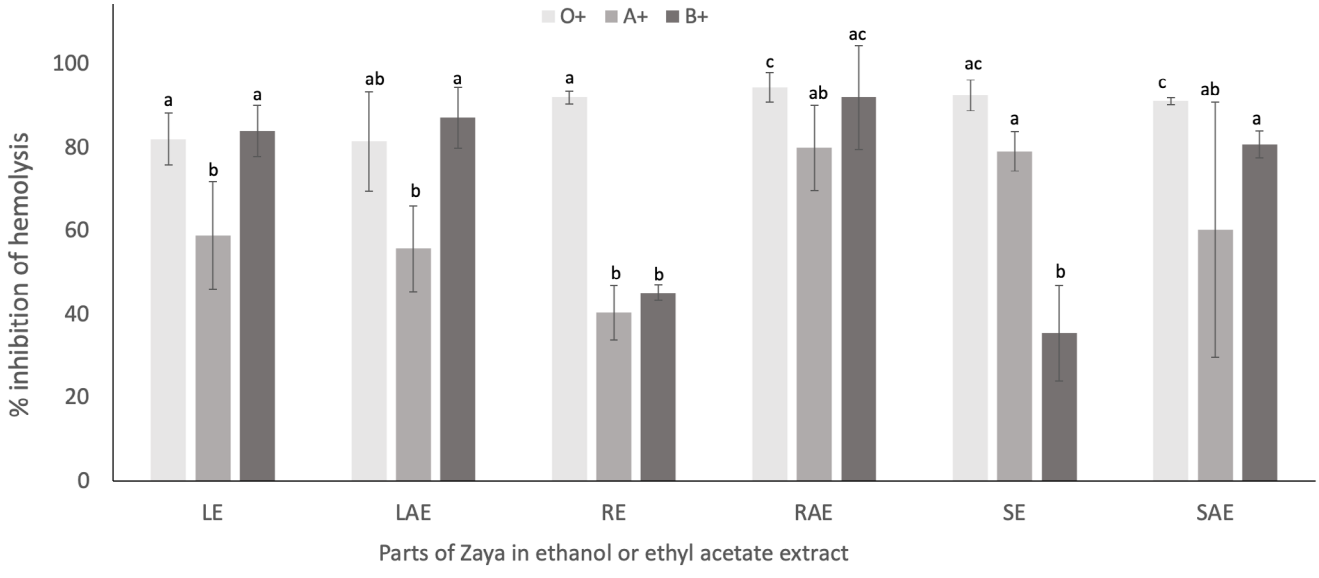

Figure 7. Effect of erythroprotective activity against oxidative damage of Zaya (HE = Leaf with ethanol; HEA = Leaf with ethyl acetate; RE = Root with ethanol; REA = Root with ethyl acetate; TA = Stem with ethanol; TEA = Stem with ethyl acetate). One-way analysis of variance (ANOVA) with post-hoc analysis (Tukey's test) were performed. Different letters indicate significant differences between samples ($p<0.05$).
Figure 7. Efecto de la actividad eritroprotectora contra el daño oxidataivo de Zaya. HE = Hoja con etanol,; HEA = Hoja con etil acetato; RE = Raíz con etanol; REA = Raíz con etil acetato; TA = Tallo con etanol; TEA = Tallo con etil acetato. Se realizó análisis de varianza de una vía (ANOVA) con análisis post-hoc (Tukey's test). Letras diferentes indican diferencias significativas entre las muestras ($p<0.05$).

effects. Once Zaya is digested, the compounds it contains are metabolized and sent to the bloodstream. In this process, there could be an interaction with antigens depending on the blood type. For this reason, it's interesting to study the selectivity of the Zaya extracts with these antigens to know if the erythroprotective effect is influenced by some of them. That's why the percentage inhibition of hemolysis was evaluated when interacting with different extracts of *Amoreuxia Palmatifida*. In these results, all extracts had erythroprotective effect on O+ (80 - 87 %), followed by B+ (22 - 85 %) and then with A+ (38 - 60 %). The presence of antioxidant compounds in Zaya samples (tannins, phenols, flavonoids, carotenoids, and chlorophylls) protected the erythrocyte from cellular damage, as these structures have the ability to neutralize free radicals through either the transfer of electrons (-OH groups) or protons (NH_2 groups) (Parcheta *et al*., 2021).

Cytoxicity effect of Zaya extracts on ABO-positive blood types

The cytotoxicity effect is presented in figure 8 where 5 mg/mL (Figure 8A) and 10 mg/mL (Figure 8B) of Zaya extracts were studied. The highest percentage of hemolysis (41 - 50

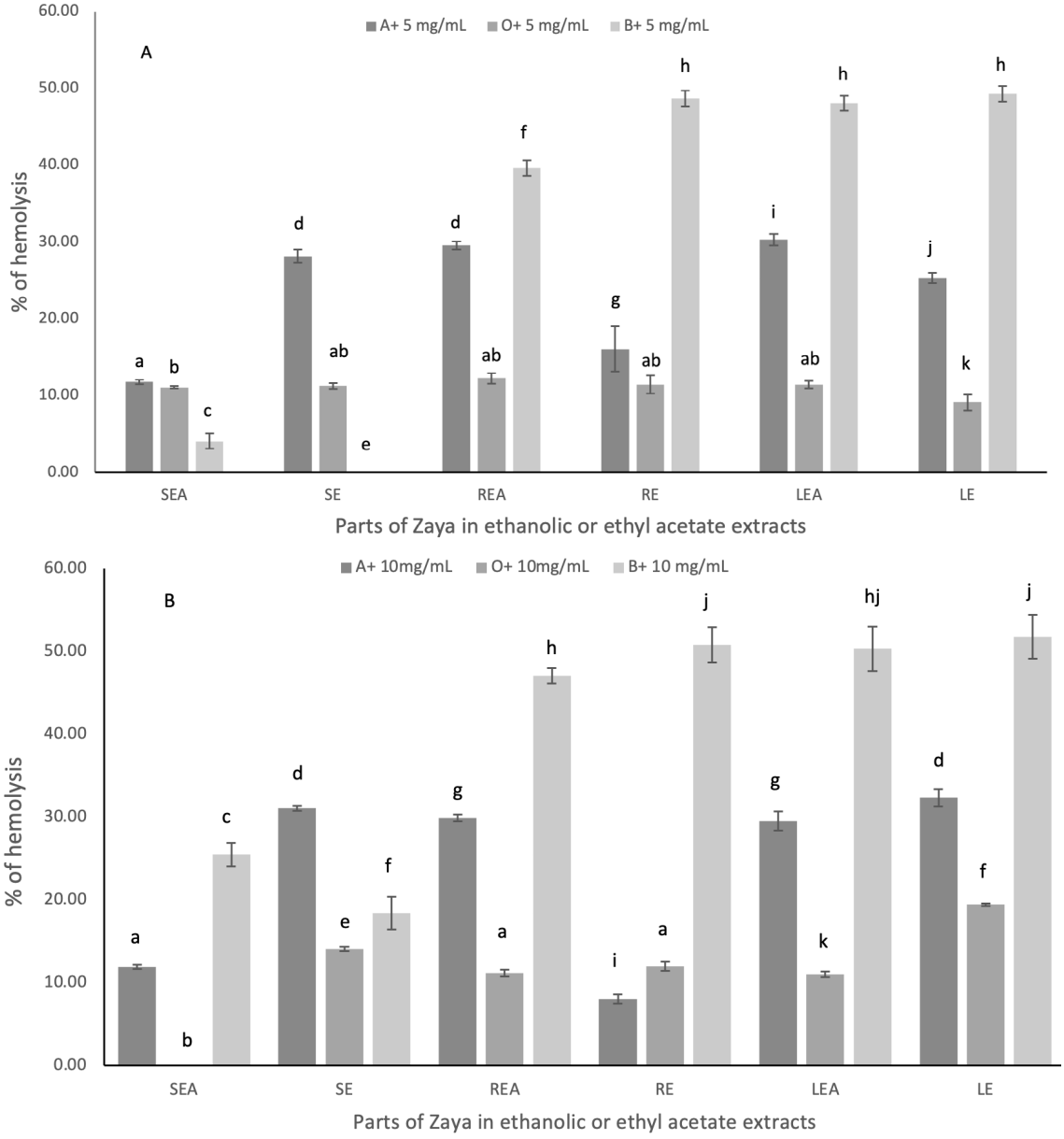

Figure 8. Cytotoxicity of Zaya samples at 5 mg/mL (A) and 10 mg/mL (B) represented by percentage of hemolysis of blood types A+, B+, and O+. HE = Leaf with ethanol; HEA = Leaf with ethyl acetate; RE = Root with ethanol; REA = Root with ethyl acetate; TA = Stem with ethanol; TEA = Stem with ethyl acetate. One-way analysis of variance (ANOVA) with post-hoc analysis (Tukey's test) were performed. Different letters indicate significant differences between samples ($p<0.05$).

Figure 8. Citotoxicidad de muestras de Zaya a 5 mg/mL (A) y 10 mg/mL (B) representado por el porcentaje de hemólisis de sangre del tipo A+, B+, and O+. HE = Hoja con etanol,; HEA = Hoja con etil acetato; RE = Raíz con etanol; REA = Raíz con etil acetato; TA = Tallo con etanol; TEA = Tallo con etil acetato. Se realizó análisis de varianza de una vía (ANOVA) con análisis post-hoc (Tukey's test). Letras diferentes indican diferencias significativas entre las muestras ($p<0.05$).

%) occurred in blood type B+ with REA, RE, LEA and LE, while the lowest was in blood type O+. This is in accordance with the results of the erythroprotective effect. Generally, in blood type O+, there is not as much cell damage compared to other blood types, and at the highest concentration in the polar leaf extract, it is not affected, while it slightly increases in the non-polar extract (Figure 8B). However, with only two concentrations tested, the results are not extensive. It is possible that at lower concentrations, certain parts of the Zaya, such as the stem, may have a lesser effect on blood types like B+, as seen in the significant reduction of hemolysis in SEA. Further research is needed to explore the effects on a wider range of blood types, including Rh negative and less common groups, as well as on different cell types. Additionally, studying other parts of Zaya, such as its seeds or flowers, could provide valuable insights.

CONCLUSIONS

Polar compounds of Zaya, particularly in leaves, showed better results in antioxidant activity. This could be attributed in part to the presence of chlorophylls, phenols, flavonoids, and carotenoids. This specific part of the plant appears to have greater potential for further investigation. Further extensive studies are needed to assess the erythroprotective effect and cytotoxicity of Zaya, involving a wider range of blood types and samples. This will provide a more comprehensive biological evaluation of the effects of Zaya. This study represents a preliminary approach to Zaya, a plant that has received limited research despite being consumed for years. It is expected that further investigation will be conducted in the future to understand its potential effects on health, as well as to obtain a more comprehensive phytochemical profile.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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