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Research Article

Biophenolic compounds and metal ions associated with the antioxidant and antibacterial activity of the ethanolic extract of *Heliotropium arborescens* L. leaves from the Andean region of Ayacucho-Peru

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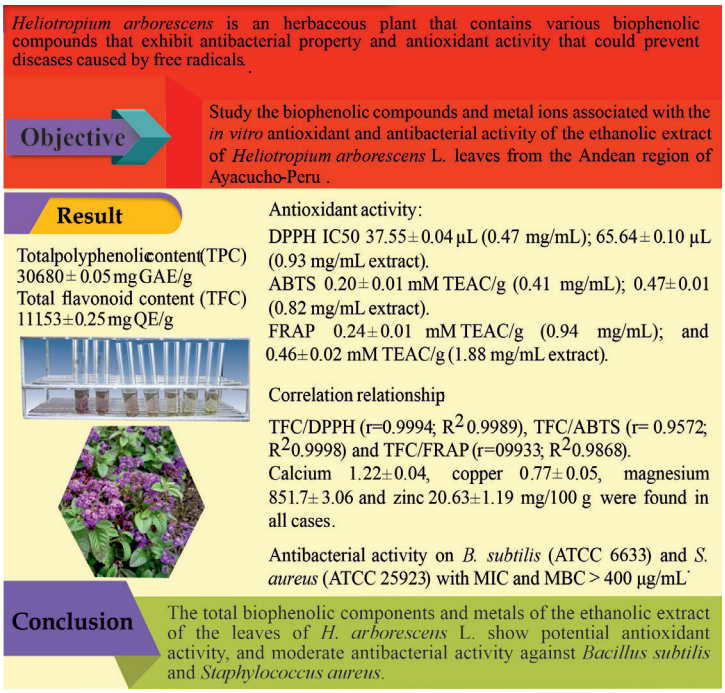
Abstract

Heliotropium arborescens (Galán de Mera et al. 2022) is a herbaceous plant that contains various biophenolic compounds that exhibit antibacterial property and antioxidant activity that could prevent diseases caused by free radicals. The objective was to study the biophenolic compounds and metal ions associated with the *in vitro* antioxidant and antibacterial activity of the ethanolic extract of *Heliotropium arborescens* L. leaves from the Andean region of Ayacucho-Peru. The total polyphenolic content (TPC) was determined by the Folin-Ciocalteu method; Total flavonoid content (TFC) was determined by spectrophotometry at 510 nm, TFC was expressed in mg quercetin/g sample (mg QE/g). Antioxidant activity by three *in vitro* methods; determination of metals by flame atomic absorption spectrophotometry (FAAS) and antibacterial activity by agar diffusion method. The values obtained from the TPC were 306.80±0.05 mg GAE/g, TFC 111.53±0.25 mg QE/g. Antioxidant activity: DPPH IC_{50} 37.55±0.04 µL and 65.64±0.10 µL, at 0.47 and 0.93 mg/mL extract, respectively; ABTS 0.20±0.01 mM TEAC/g (0.41 mg/mL extract) and 0.47±0.01 (0.82 mg/mL extract); FRAP 0.24±0.01 mM TEAC/g (0.94 mg/mL extract) and 0.46±0.02 mM TEAC/g (1.88 mg/mL extract). Correlation relationship TFC/DPPH (r=0.9994; R^2 0.9989), TFC/ABTS (r = 0.9572; R^2 0.9998) and TFC/FRAP (r = 09933; R^2 0.9868). Calcium 1.22±0.04, copper 0.77±0.05, magnesium 851.7±3.06 and zinc 20.63±1.19 mg/100 g were found in all cases. Antibacterial activity on *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923) with a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) > 400 µg/mL. It is concluded that the total biophenolic components and metals of the ethanolic extract of the leaves of *H. arborescens* L. show potential antioxidant activity, and moderate antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*.

Graphical abstract:



Keywords

Heliotropium arborescens, flavonoids, biophenols, antioxidant, antibacterial

Introduction

The genus *Heliotropium* (helium in Greek means "sun", tropium comes from the Greek word "tropein" which means to turn) belongs to the family Boraginaceae and subfamily Heliotropioideae (Fayed et al. 2022; Willocx et al. 2022; Ozntamar-Pouloglou et al. 2023; Rueangsawang and Chantaranothai 2023), and comprises more than 450 species distributed in tropical, subtropical, and temperate areas of the world (Rueangsawang and Chantaranothai 2023). This genus contains various polyphenols such as flavanones (naringenin, 4'-acetyl-5-hydroxy-7-methoxyflavanone, 5,3'-dihydroxy-7,4'-dimethoxyflavanone, 5,4'-dihydroxy-7-methoxyflavanone, 7- O-methyleryodictyol); flavonols (quercetin, taxifolin or dihydroquercetin, 3-O-methylgalangin), filifolinol (3 H-spiro[1-benzofuran-2,1'-cyclohexane]), filifolinoic acid, filifolinone, filifolinyl senecionate ester, 4-methoxy-3-[(2)-7'-methyl-3'-hydroxymethyl-2',6'-octadienyl]phenol and 3-oxo-2-arylbenzofuran (Modak et al. 2009; Modak et al. 2010; Goyal and Sharma 2014; Valdés et al. 2018; Fayed 2021). The species *Heliotropium arborescens* popularly known as heliotrope, curutmi or cayaraja (Galán de Mera et al. 2022), whose correct name is *Heliotropium corymbosum* Ruiz & Pav., and native to Peru (Luebert et al. 2010; Luebert 2020), is distributed throughout the Andean region of Peru (Ayacucho, Arequipa, and Moquegua) and northern Chile (Arica and Parinacota Region), between 200 and 3300 meters above sea level (Luebert 2020). It is a densely branched shrub 100 cm high, with pubescent juvenile branches, small blue sessile flowers that emit a pleasant sweet smell similar to vanilla with an admixture of caramel (Fayed 2021); calyx persistent, divided to the base; sepals linear to lanceolate, strigose-hairy outside, glabrous inside; corolla infundibuliform, white with a yellow center, turning blue or violet at maturity; lobes rounded; tube villous outside and glabrous inside; stamens included; anthers linear with papillose margin; rounded and corded base. Ovary glabrous, with nectariferous disc developed at the base; style longer than stigmatic column; stigmatic column conical and finely papillose on the edges. Dry fruits, glabrous and dark brown. Leaves alternate, sessile or petiolate, pubescent, strigose on the adaxial surface, woolly-tomentose on the abaxial surface; elliptical blade, with well-marked nerves on both sides, entire margin, attenuated base, acute or rarely rounded apex. Dichotomously branched terminal inflorescences (Luebert 2020). Aromatic aldehydes (benzaldehyde, anisic aldehyde or 4-anisaldehyde), aromatic ester (benzyl acetate), and 3,4-methylenedioxybenzaldehyde or heliotropin (piperonal) have been characterized and isolated from the essential oil of *Heliotropium arborescens* (Fayed 2021); fatty acids such as linoleic acid (30%), stearic acid (22%), palmitic acid (18.3%), oleic acid (12.7%), α-linolenic acid (5.4%), γ-linolenic acid (2.4%) and stearidon-

ic acid (1.5%); and γ-tocopherol (Velasco and Goffman 1999).

In several studies, it has been proven that the biophenolic compounds of *Heliotropium* exhibit cardiotonic, antiplatelet, healing, anti-infectious (antiviral, antifungal, antibacterial) properties and antioxidant activity (Baroi et al. 2022; Ozntamar-Pouloglou et al. 2023). Due to this activity, they could reduce inflammatory processes and prevent diseases caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS); Among ROS, the superoxide anion ($O_2^{\bullet-}$), hydroperoxyl ($HO_2^{\bullet}$), hydroxyl ($^{\bullet}OH$), and peroxyl radical ($ROO^{\bullet}$) have been described; and among RNS, nitric oxide ($^{\bullet}NO$), nitrogen dioxide (NO_2), nitrate radical (NO_3^-) and peroxynitrite ($ONOO^-$) have been reported (Sharma et al. 2009; Noumedem et al. 2013; Carvajal 2019; Baroi et al. 2022; Surco-Laos et al. 2022a, 2022b, 2023); they could also prevent several types of cancer that have an environmental component and overexpression of genes *CYP1A1*, *CYP2E1*, *CYP2C19*, *CYP2D6* and *CYP17* that encode their respective isoenzymes that biotransform procarcinogenic agents (benzopyrene, arylamines, N-nitrosamines, and dioxins) into carcinogenic metabolites that bind to guanine in DNA (Wongpratate et al. 2020; Alvarado et al. 2021; Sung et al. 2021).

Various molecules such as nuclear factor kappa B (FN kB) and activating protein 1 (AP-1) can stimulate the mitochondrial superoxide dismutase (SOD2) genes, overexpressing the synthesis of their respective enzyme, forming excess hydrogen peroxide (H_2O_2); at the same time, the enzymatic system that metabolizes H_2O_2 is inactivated due to depletion of the NADPH enzymatic cofactors that are used in reactions that generate ROS (Carvajal 2019). Fig. 1 proposes the conversion process of H_2O_2 into hydroxyl radical ($^{\bullet}OH$) through the Fenton reaction pathway with the participation of the ferrous ion (Fe^{2+}) that is converted into ferric ion (Fe^{3+}). $^{\bullet}OH$, generates lipid oxidation of the membranes by subtracting a hydrogen from the CH_2 group of the side chain of a polyunsaturated fatty acid (PUFA), generating a lipid carbon radical, which reacts with molecular oxygen (O_2) to give rise to lipid peroxyl radical ($LOO^{\bullet}$) that undergoes rearrangements forming endoperoxides, and subsequently malondialdehyde (MDA) and 4-hydroxyl-nonenal (4-HNA) are synthesized, responsible for the oxidation of guanine (8-hydroxyguanosine) in DNA and damage to membrane proteins (Avello and Suwalsky 2006; Carvajal 2019). Likewise, it is observed that oxidized glutathione (GSSG) is activated into reduced glutathione (GSH) by glutathione reductase (GR); GR and aldose reductase (AR) use NADPH as an enzymatic cofactor, and at the same time this cofactor is oxidized to $NADP^+$; AR participates in hyperglycemia by converting excess glucose into sorbitol, which is responsible for overhydrating the lens of the eye, causing cataract; another percentage of sorbitol is converted into fructose, and in this reaction, NADH is produced from the oxidized NAD^+, and when NADH is formed in excess, superoxide anion ($O_2^{\bullet-}$) is generated, catalyzed by NADH oxidase (Macedo-Márquez 2012; Carvajal 2019; Wongpratate et al. 2020; Alvarado et al. 2021; Surco-Laos et al. 2023).

The SciELO, PubMed-NCBI and ScienceDirect database was searched for published research on the species *H. arborescens* (heliotrope, curutmi or cayaraja), and it is evident that studies on this species are scarce, in this sense it is justified to carry out studies chemical preliminaries, *in vitro* and preclinical. Therefore, the objective was to study the biophenolic compounds and metal ions associated with the *in vitro* antioxidant and antibacterial activity of the ethanolic extract of *Heliotropium arborescens* L. leaves from the Andean region of Ayacucho-Peru.

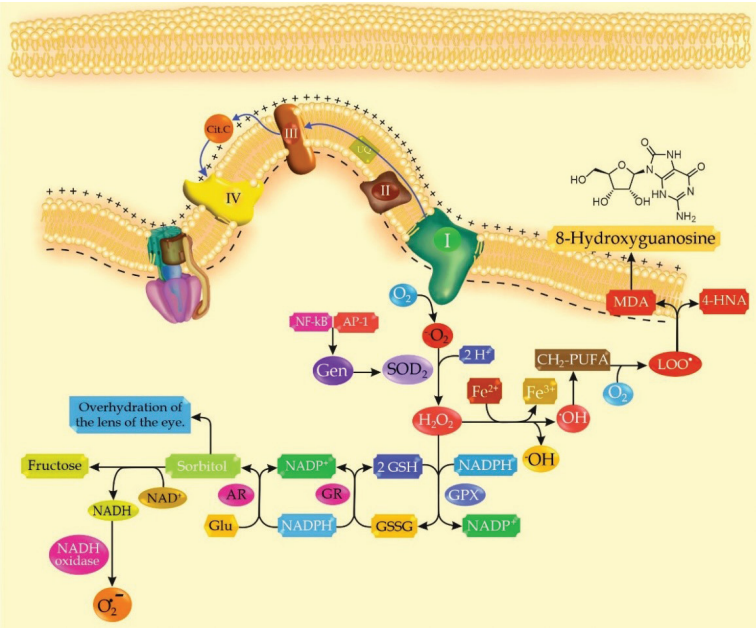


Figure 1. Fenton reaction pathway with the participation of the ferrous ion (Fe^{2+}), liperoxidation of the inner membrane of the mitochondria and oxidation of the guanine of DNA. Figure made by the authors.

Materials and methods

Plant materials

The samples were the leaves of the bush *Heliotropium arborescens* L. "heliotrope, curutmi or cayaraja" that was collected between February and March 2023 in the district of Pauza (Geographically located between 15°16'44"S, 73°20'50"W), altitude 2524 m.a.s.l, province of Paucar del Sara Sara, region of Ayacucho, zone of the Peruvian Andes. The climate is cold to temperate, rainy between December and March with a rainfall of 575 mm; from May to September it is dry and October to November in between; the temperature varies between 8 to 22 °C, in the capital of the department the average temperature is 17.5 °C (De la Cruz-Arango et al. 2020). Agricultural land represents 3% of the province's territory, 92% is under irrigation by river water, and 8% is dry and depends on the rains; natural pastures represent 9.53%, being mostly mountainous, rugged lands and rugged topography. The sample was identified by a botanist from the Herbarium of the Museum of Natural History of the National University of San Marcos, Lima, Peru (certificate No. 103-USM2011).

Extraction and isolation

The *H. arborescens* L. plants were transported to the Instrumental Analysis laboratory of the Faculty of Pharmacy, Universidad Nacional San Luis Gonzaga de Ica, where they were dried under shade for 15 days, then the leaves were separated manually, and immediately ground in a manual mill (IVYMEN JP SELECTA, YCW-010E, Spain) to obtain a fine powder. Subsequently, 500 g of the fine powder was weighed (Sartorius model ED224S, Germany) and placed in an amber glass bottle, 96% ethanol was immediately added (sample: solvent 1:3) and to extract the bioactive compounds, it was shaken for 30 min daily for 15 days; after maceration, the liquid extract was filtered, and the solvent was evaporated in a rotary evaporator (Heidolph model LABOROTA 4000, Germany) at reduced pressure and 38±2 °C; the semisolid extract was placed in an oven (Binder series 05-75803, Germany), obtaining a dry extract that was stored in an amber bottle at 4 °C until analysis (Rojas et al. 2012; Carrasco et al. 2013; Merino et al. 2015; Chávez et al. 2021).

Phytochemical study

Through coloring and/or precipitation reactions, polyphenol-type secondary metabolites were identified in solvents of different polarities. In fraction A, flavonoids, phenols and tannins (A1) were identified; a 1% HCl solution was added to fraction A2, it was stirred, filtered, and an insoluble part and an acid solution were obtained; the insoluble part was washed with distilled water until neutral pH, then dissolved with 5 mL of dichloromethane, anhydrous sodium sulfate was added and filtered to obtain fraction B (with it, triterpenoids and/or steroids were identified). Subsequently, the acid solution was filtered, 25% ammonium hydroxide and dichloromethane were added to the filtered liquid, generating two phases (dichloromethane and aqueous). The dichloromethane phase was washed with distilled water, anhydrous sodium sulfate was added, filtered and fraction C was obtained (triterpenoids and/or steroids were identified); the aqueous phase was saturated with 5 g of anhydrous sodium sulfate, and a mixture of dichloromethane: ethanol (3:2) for extraction, forming an organic and an aqueous phase. The organic phase was washed with anhydrous sodium sulfate solution and saturated with 1 g of anhydrous sodium sulfate, filtered to obtain fraction D (flavonoids, triterpenoids and/or steroids, and alkaloids were identified); the aqueous residue was added to the aqueous phase (extraction result dichloromethane: ethanol), generating fraction E and with-it flavonoids and leucoanthocyanidins and/or catechins were identified (Chávez et al. 2021; Surco-Laos et al. 2022b).

Total polyphenol content

A calibration curve of the gallic acid standard (Sigma-Aldrich) in 70% alcohol was performed in the range of 1–7.5 µg/mL. 0.1 mL (900 µg/mL) of the ethanolic extract was reacted with 0.45 mL of Folin-Ciocalteu solution (Merck, ratio 1 reagent: 2 HPLC grade water), homogenized and allowed to react for 5 min; after that time, 0.45 mL of 20% sodium carbonate (Na_2CO_3) and HPLC grade water sufficient quantity for (sqf) 3 mL were added, and it was shaken vigorously to homogenize. It was reacted for 30 min under protection from light and at laboratory temperature. The absorbances of the samples and the blank were read in triplicate at a wavelength of 760 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). The total polyphenolic content was expressed in mg of gallic acid (mg GAE)/g of ethanolic extract (Surco-Laos et al. 2022a; Surco-Laos et al. 2023).

Determination flavonoids

In a 2 mL tube, 200 µL of the ethanolic extract and 1000 µL of distilled water were added and gently homogenized; then, 75 µL of 5% sodium nitrite ($NaNO_2$) was added, mixed, and allowed to react for 5 min. After that time, 75 µL of 10% aluminum chloride ($AlCl_3$) was added, gently homogenized, and allowed to react for 6 min, subsequently, 500 µL of 1 M sodium hydroxide (NaOH) was added. The mixture was allowed to stand for 5 min. A quercetin calibration curve of 50–500 µg/mL was prepared and evaluated simultaneously with the ethanolic extract. The absorbances of the samples were read in triplicate at a wavelength of 510 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). The total flavonoid content was expressed in mg of quercetin/g of sample (Ramos-Escudero et al. 2012; Vega et al. 2017).

Antioxidant assay

DPPH radical scavenging assay

The 100 Mm DPPH (Sigma) solution was prepared in 80% methanol (Analytical grade, Beaker) and the absorbance was established between 0.9–1.1 at a wavelength of 517 nm (Spectrophotometer Peak Instrumental, model C- 7100 USA). Various 3 mL tubes were marked, then 0.1 mL of each of the dilutions of the ethanolic extract, plus 2.9 mL of DPPH solution, were added and homogenized. The homogenized mixture (ethanolic extract + DPPH) reacted for 30 min protected from light and at laboratory temperature; after the reaction time, the homogenized mixture and the blank (methanol) were measured in triplicate at a wavelength of 517 nm (Spectrophotometer Peak Instrumental, model C-7100 USA); the inhibition percentage (% Inh) was determined using the following formula:

$$\%\text{Inh.} = [(\text{blank abs} - \text{sample abs}) / (\text{blank abs})] \times 100$$

The IC_{50} was determined from a curve of % inhibition vs µL of ethanolic extract (Ramos-Escudero et al. 2012; Surco-Laos et al. 2023).

Ferric Reducing Antioxidant Power (FRAP)

3 mL of freshly prepared reagent was added to a quartz cuvette and read at a wavelength of 593 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). Subsequently, 0.1 mL of each of the ethanolic extracts plus 1.5 mL of the FRAP solution was added to marked tubes, homogenized in a vortex for 30 sec, then it was allowed to react for 6 min at laboratory temperature. The study sample (ethanolic extract + FRAP solution) was measured in triplicate at a wavelength of 593 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). Trolox at a concentration of 0.0312–1.0 mM was used as a reference standard. The final absorbance was obtained by subtracting the value from the initial absorbance of the FRAP solution. A quantification curve of mM of Trolox/g of ethanolic extract was carried out. Previously, the FRAP solution was prepared, 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ solution (2,4,6-tripyridyl-s-triazine) 10 mM diluted in HCl 40 mM and 2.5 mL of 20 mM ferric chloride ($FeCl_3.6H_2O$) solution (Ramos-Escudero et al. 2012; Surco-Laos et al. 2023).

ABTS radical scavenging activity

ABTS reagent (2,2'-azino-bis-(3-ethyl benzothiazolin-6-sulfonate ammonium) was reacted with potassium persulfate ($K_2S_2O_8$) for 12 h and under the protection of light. 10 µL of the ethanolic extract and 990 µL of $ABTS^{\bullet+}$ radical solution was incorporated into a 3 mL tube, allowing the reaction to occur at 37 °C for 4 min. After that time, the homogenized mixture and the initial ABTS reagent were measured in triplicate at a wavelength of 734 nm (Spectrophotometer Peak Instrumental, model C-7100 USA). The results were expressed as values of mM of Trolox equivalent antioxidant capacity (TEAC)/g of dry extract (Trolox equivalent) by constructing a Trolox standard curve (Rezanejad et al. 2020; Surco-Laos et al. 2022a).

Determination of metals

In a porcelain crucible, 1 g of dry ethanolic extract was accurately weighed and immediately calcined on an electric kitchenette, subsequently, the calcined sample was incorporated into the muffle furnace at 500 °C for 4 h until gray ashes were obtained. Said ashes were allowed to cool to room temperature, then 10 mL of 10% HCl was added, brought to a boil, and allowed to cool. The acidified liquid was filtered through Whatman 2 paper into a 25 mL volumetric flask, and sufficient distilled water for 25 mL was immediately added. For the determination of calcium and magnesium, a 1:100 dilution was carried out, adding 10 mL of 1% lanthanum chloride to the final solution (to avoid interferences).

The determination of calcium, copper, magnesium, and zinc was carried out by flame atomic absorption spectrophotometry (FAAS: Perkin Elmer AA-800 spectrophotometer); said equipment was previously calibrated for each metal according to the manufacturer's instructions (Sharma et al. 2020; Surco-Laos et al. 2022a).

Antibacterial activity by agar diffusion method

The strains *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomona aeruginosa* (ATCC 28573) were activated in Mueller Hinton Broth (MHB) at 37 °C in a water bath with shaking for 24 h.

After activation, each strain was adjusted to a concentration of 10^8 CFU/mL compared to the standard 0.5 of the McFarland scale reading at 580 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). The study mixture consisted of 9.95 mL of 1% sulfuric acid (H_2SO_4) and 0.05 mL of 1% $BaCl_2$. The experiment was carried out by adding 20 mL of Mueller-Hinton Agar (MHA) to Petri dishes and allowing it to melt, then the bacterial cultures (1000 µL) were sown, distributing it evenly with a Drigalsky spatula. The plates were allowed to dry for 5 min, then six 6 mm diameter wells were made using a sterile punch.

50 µL of negative control (50% alcohol) was added to each plate, four sample concentrations of the plant extract (50, 100, 250 and 500 mg/mL), the concentrations were defined by previous tests, and oxacillin (5 µg/mL) was used as a positive control. All plates were refrigerated for 30 min, after which time they were incubated at 37 °C for 24 h. Antibacterial activity was detected by measuring the zone of inhibition (including the diameter of the wells) in mm using a calibrated Vernier. Tests were performed in triplicate for each strain (CLSI M07 2018; De Zoysa et al. 2019; Surco et al. 2022b).

Identification of the minimum inhibitory concentration (MIC)

The wells of the microplate were identified as columns to carry out the experiment: 200 µL of negative sterility control (190 µL MHB + 10 µL DMSO) was added to the well of column 1; in the following columns, the extract samples were incorporated (50 µL of extract sample at concentrations of 500, 250, 100 and 50 mg/mL + 50 µL of MHB + 100 µL of bacterial inoculum at a concentration of 10^8 CFU/mL according to the McFarland scale), and in the last column 200 µL of positive control (100 µL of MHB + 100 µL of inoculum) was incorporated. Oxacillin was dissolved with MHB and 200 µL was added to each well at concentrations 64, 49, 24.50, 12.25, 6.12, 3.06, 1.53, 0.76 and 0.38 µg/mL.

A negative sterility control (200 µL of MHB) and a positive control (100 µL of MHB and 100 µl of bacterial inoculum) were considered. The final volume of all wells was 200 µL.

All plates were incubated at 37 °C for 24 h. To evaluate bacterial growth (the variation from blue purple to pinkish color was observed). The MIC is measured visually as the lowest extract concentration at which the initial blue-purple coloration was maintained (no visible growth). All tests were in triplicate and the average of three values was reported as MIC and expressed in µg/mL (CLSI M07 2018; Surco et al. 2022b).

Identification of the minimum bactericidal concentration (MBC)

The microplate wells that maintained the blue-purple color (indicates no visible growth of the bacteria) were considered for the identification of MBC. From these wells, 100 µL of sample was measured and seeded in Petri dishes with MHB. 100 µL of bacterial inoculum and oxacillin was used as a positive control, and MHB without inoculum and without antimicrobial was used as a negative control. The plates were incubated at 37 °C for 24 hours. The MBC was determined by observing those plates where the ethanolic extract sample was able to eliminate bacterial development, comparing it with the positive control. The average of three values was reported as MBC (Tenorio-Abreu et al. 2015; CLSI M07 2018; Surco et al. 2022b).

Results and discussion

Secondary metabolites of the ethanolic extract of *Heliotropium arborescens* L leaves were identified by the coloration and precipitation method. The most representative being flavonoids, phenols and tannins, triterpenoids and/or steroids, leucoanthocyanidins and/or catechins, and alkaloids (Table 1). There are no reported studies on *H. arborescens* L., but there are publications on other species, such as the study by Modak et al. 2002 who indicated the presence of flavonoids in H. sinuatum; and recently Singh et al. 2017 identified flavonoids in *H. subulatum*. In the present study, the presence of flavonoids has been identified through different reactions, which have also been quantified (Table 2), and could be responsible for the antioxidant and antibacterial activity.

Table 2 reports the total polyphenol content (TPC) and total flavonoid content (TFC) evaluated at a concentration of 1.12 mg/mL and 1.17 mg/mL of ethanolic extract of *H. arborescens* L leaves, respectively. TPC was determined in mg gallic acid equivalents (mg GAE)/g dry ethanolic extract, while TFC was quantified in mg quercetin equivalents (mg QE)/g dry ethanolic extract. The potential antioxidant activity of said ethanolic sample analyzed at two concentrations (mg/mL) and by three *in vitro* methods is also reported.

The reduction capacity of the DPPH radical was determined by the decrease in absorbance at 515 nm, which indicates that the ethanolic extract has the capacity to capture reactive oxygen species (ROS) (Mendoza Isaza et al. 2020), and whose mechanism of action is due to the transfer of a hydrogen atom from the hydroxyl group of the biophenolic compound to DPPH (HAT) to generate a stable compound (Andzi Barhe´ and Feuya Tchouya 2016). The percentage of inhibition was determined, and with these data the concentration at which 50% of the DPPH free radicals are

Table 1. Presence of secondary metabolites in the ethanolic extract of Heliotropium arborescens L leaves.

Fractions	Secondary metabolites	Assay	Results	Observations
A	Flavonoids	Shinoda	+	Red coloring.
	Phenols and tannins	Ferric chloride	+	Bluish green.
B	Triterpenoids and/or steroids	Lieberman Burchard	+	Green coloring.
C	Triterpenoids and/or steroids	Lieberman Burchard	+	Green coloring.
D	Flavonoids	Shinoda	+	Red coloring.
	Triterpenoids and/or steroids	Lieberman Burchard	+	Green coloring.
	Alkaloids	Dragendorff	+	Slight orange precipitate.
		Mayer	+	White or light-yellow precipitate.
		Hager	+	Brown precipitate.
E	Flavonoids	Shinoda	+	Red coloring.
	Leucoanthocyanidins and/or catechins	Rosenheim	+	Brown coloration.

Table 2. Estimation of the content of total polyphenols, flavonoids, and antioxidant activity of the ethanolic extract of *Heliotropium arborescens* L (curutmi) leaves.

Assay	Concentration of ethanolic extract (mg/mL)	Mean±SD	95%CI	*Reference compounds
TPC (mg GAE/g)	1.12	306.80±0.05	0.0566	Gallic acid
TFC (mg QE/g)	1.17	111.53±0.25	0.2848	Quercetin
DPPH (IC_{50}) µL	0.47	37.55±0.04	0.0397	Trolox
	0.93	65.64±0.10	0.1161	Trolox
ABTS (mM TEAC/g)	0.41	0.20±0.01	0.0095	Trolox
	0.82	0.47±0.01	0.0099	Trolox
FRAP (mM TEAC/g)	0.94	0.24±0.01	0.0058	Trolox
	1.88	0.46±0.02	0.0174	Trolox

TPC: total polyphenol content in mg gallic acid equivalent (mg GAE/g); TFC: total flavonoid content in mg quercetin equivalent (mg QE/g); TEAC: mM Trolox equivalent antioxidant capacity/g; 95% CI: 95% confidence interval.
*Calibration curve values.

neutralized (IC_{50}) was found; it is observed that the DPPH radical scavenging activity of the ethanolic extract of the leaves of *H. arborescens* L at the concentration of 0.93 mg/mL was higher (65.64±0.10 µL) compared to the extract concentration of 0.47 mg/mL. Using the ABTS method, the plant ethanolic extract was evaluated at two concentrations, of which the 0.82 mg/mL concentration extinguished the $ABTS^{\bullet+}$ cationic radical (due to discoloration of the solution) with a reduction in absorbance at 730 nm. The $ABTS^{\bullet+}$ cationic uptake capacity of the ethanolic extract of curutmi leaves was 0.47±0.01 mM TEAC/g of dry sample.

The third method applied to evaluate the antioxidant activity of the ethanolic extract of the leaves of *H. arborescens* L was FRAP, for this, two concentrations of the ethanolic extract were also used, observing that the bioactive compounds at the concentration of 1.88 mg/mL have the greater capacity to transfer an electron to the FRAP radical (SET) by reducing the ferric ion (Fe^{3+}) to a ferrous ion (Fe^{2+}), which is visualized by changing the color of the solution to intense blue. It has been determined that a high absorbance value is an indication of a strong reducing power that translates into a high antioxidant capacity of the bioactive compounds (Prior et al. 2005). For these last two methods, a calibration curve was carried out with the Trolox standard, to establish the antioxidant activity as Trolox equivalent (mM TEAC/g of dry sample).

In a study carried out by Surco et al. 2022b on the ethanolic extract of the leaves of *S. nutans Sch*. Bip., reported that low IC_{50} values indicate high antioxidant power, in this case the ethanolic extract of *H. arborescens* L leaves has good antioxidant activity due to a low IC_{50} (37.55±0.04 µg/mL), and said activity should be to its biophenolic components. Fayed 2021, found that the species *H. arborescens* L. contains aromatic aldehydes (benzaldehyde, 4-anisaldehyde or anisic aldehyde), aromatic ester (benzyl acetate) and piperonal or heliotropin (3,4-methylenedioxybenzaldehyde. In other species of the same genus such as *H. indicum*, aromatic aldehydes have been identified such as phenylacetaldehyde, (E)-2-nonenal and (E, Z)-2-nonadienal; additionally, hexahydrofarnesylacetone (Ogunbinu et al. 2009). *H. stenophyllum* contains β-epi-bisabolol, α-cedrene epoxide, junenol, longiborneol, (E, Z)-geranyl linalool and seline-3,11-dien-6-α-ol (Urzúa et al. 2013); and in *H. europaeum* phytol acetate, cis-linolenic acid methyl ester, phytol, geranyl acetone and (E)-β-ionone have been identified (Saeedi and Morteza-Semnani 2009). The ethyl acetate, aqueous, chloroformic and methanolic extracts of this genus present an important antioxidant activity, which is attributed to its biophenolic components (Murugesh et al. 2006). In another study, it has been reported that biophenolic compounds are responsible for antioxidant activity, and could prevent Alzheimer's disease, atherosclerosis, diabetes, and different types of cancer (Ahmad et al. 2014). In this sense, the potential antioxidant activity of the ethanolic extract of *H. arborescens* L leaves would be due to its biophenolic aromatic compounds, especially flavonoids, as has been established in studies of the relationship between total flavonoid content TFC/DPPH, TFC /ABTS and TFC/FRAP.

Fig. 2A shows the relationship of total polyphenolic content (TPC: mg GAE/g) and DPPH (IC_{50}) at the concentration of 0.47 mg/mL of ethanolic extract, whose correlation is moderate and negative (r = -0.4271) and its coefficient of determination (R^2 = 0.1824) which indicates a 18.24% relationship at a linear level of both variables, this indicates that when one of the variables increases, the other decreases. Fig. 2B describes the relationship between TFC (mg QE/g) and DPPH assay (IC_{50}) µL at the concentration of 0.47 mg/mL of extract, whose correlation is strong and positive (r = 0.9994; R^2 = 0.9989) with a 99.89% relationship at a linear level of both variables, that is, by increasing the amount of flavonoids, the antioxidant capacity increases.

Fig. 3A details the relationship between TPC (mg GAE/g) and ABTS assay (mM TEAC/g) at the concentration of 0.82 mg/mL of extract, whose correlation is weak (r = -0.1147; R^2 = 0.0132), not correlation was found at the concentration of 0.41 mg/mL of ethanolic extract. Fig. 3B shows a strong and positive correlation (r = 0.9572) between TFC (mg QE/g) and ABTS (mM TEAC/g) at a concentration of 0.82 mg/mL, and its coefficient of determination (R^2 = 0.9998) indicates a 99.98% relationship at a linear level of both variables.

Fig. 4A indicates the inverse and moderate correlation (r = -0.5; R^2 = 0.25) of the TPC (mg GAE/g) and the FRAP as-

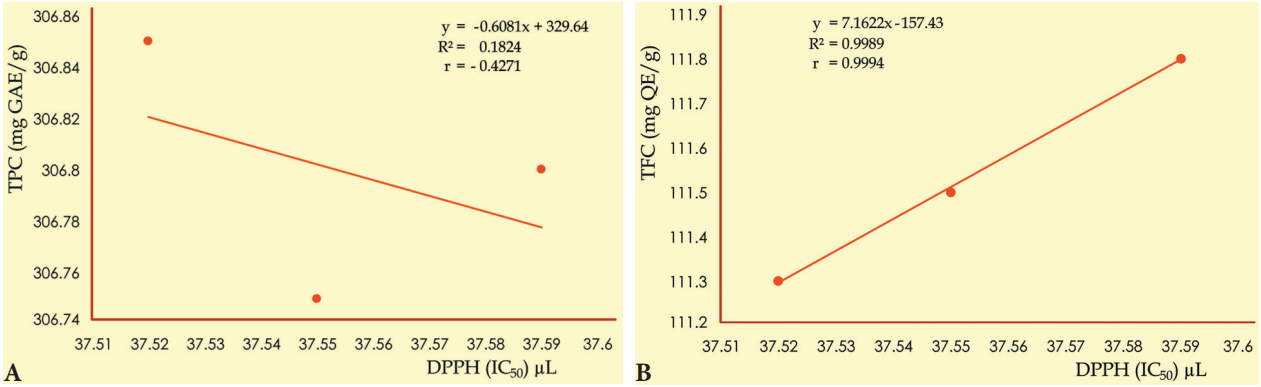


Figure 2. Pearson correlation coefficient of total polyphenol content (TPC)/antioxidant activity of DPPH (**A**), and total flavonoid content (TFC)/DPPH (**B**).

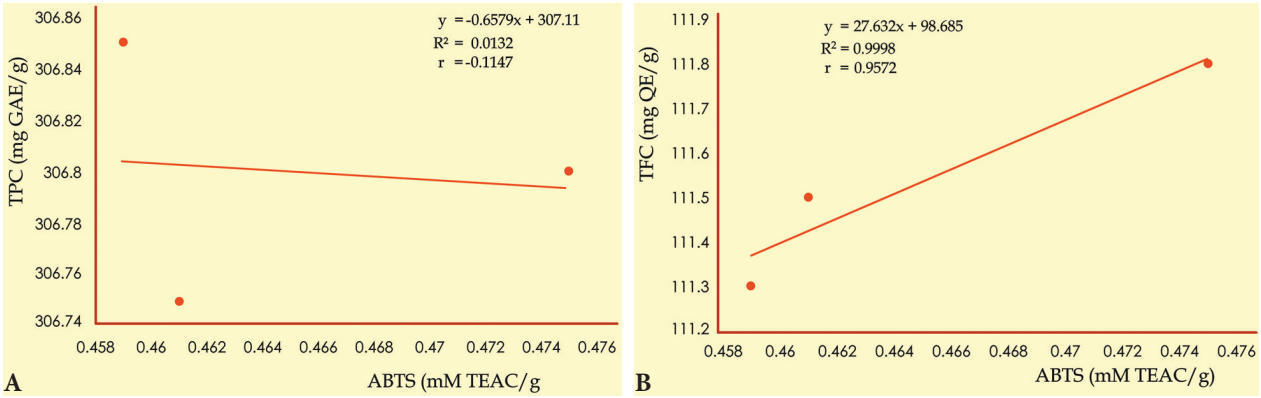


Figure 3. Pearson correlation coefficient of total polyphenol content (TPC)/antioxidant activity of ABTS (**A**), and total flavonoid content (TFC)/DPPH (**B**).

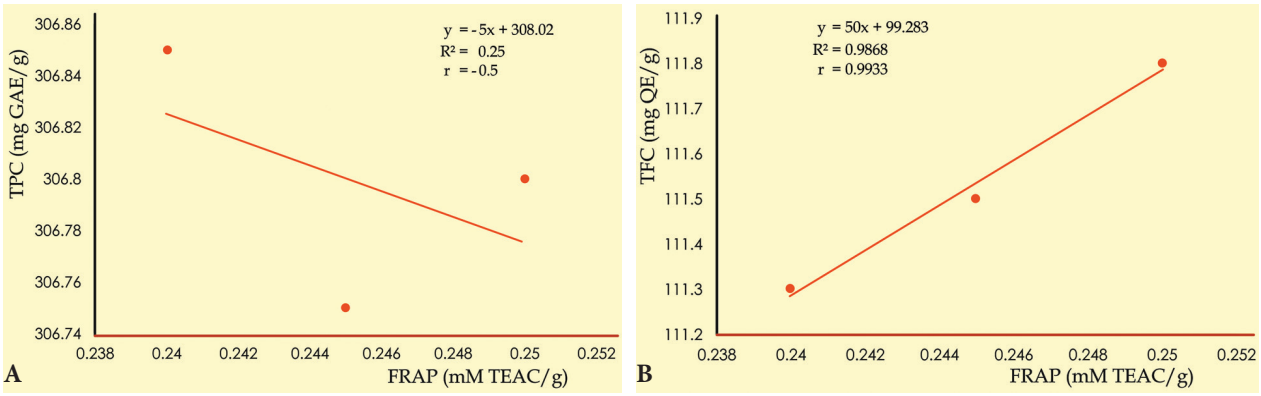


Figure 4. Pearson correlation coefficient of total polyphenol content (TPC)/antioxidant activity of FRAP (**A**), and total flavonoid content (TFC)/DPPH (**B**).

say (mM TEAC/g); additionally, the relationship of TFC (mg QE/g)/FRAP (mM TEAC/g) was studied, obtaining a strong and positive correlation (r = 0.9933; R^2 = 0.9868) (Fig. 4B); both studies were carried out at a concentration of 0.9373 mg/mL of ethanolic extract. These results indicate a strong correlation of antioxidant activity with flavonoid content.

Plant biophenolic compounds could sequester or form stable complexes of ROS preventing liperoxidation (González-Torres et al. 2000); They can stimulate the synthesis and functional activity of the antioxidant enzyme system, as proposed in Fig. 5: peroxiredoxin III (PRX III) biotransforms H_2O_2 into water molecules (H_2O); Catalase (CAT) converts two molecules of H_2O_2 into two molecules of $H_2O + O_2$; Glutathione peroxidase (GPx) metabolizes hydrogen peroxide into two H_2O molecules. This enzyme requires the cofactor selenium, NADPH, and reduced glutathione (GSH, which is activated by sulfur) (Shetty 2004; Avello and Suwalsky 2006; Ramos-Escudero et al. 2012; Carvajal 2019; Alvarado et al. 2023).

Table 3 describes the concentration of the metals found in the ethanolic extract of *H. arborescens* L, with magnesium (Mg) being observed as the main macromineral, followed by the micromineral zinc (Zn) and in a lower concentration copper (Cu).

In various studies it has been shown that these trace elements or microminerals are enzymatic cofactors, such as the study by Shetty 2004 and Surco-Laos et al. 2022a to those who indicate that metals in natural products could participate in activating enzymatic proteins, such as Cu and Zn that activate cytosolic superoxide dismutase (SOD1), Mn activates mitochondrial SOD2 and extracellular SOD3, maintaining a balance in the synthesis of hydrogen peroxide, for its subsequent neutralization. In other studies, it has been described that Cu, Zn, Se, and Mn are antioxidant metals, and when deficient selenium (Se) activity is generated, the prevalence of colorectal cancer increases (Sánchez-Valle and Méndez-Sánchez 2013; Surco-Laos et al. 2022a). While sulfur (S) deficiency inactivates the protein glutathione-S-transferase (GST), increasing the activity of CYP1A1 that biotransforms food procarcinogens into carcinogens (Alvarado et al. 2021; Surco-Laos et al. 2022a). Meanwhile, magnesium is the macromineral responsible for activating the various enzymes of the glycolytic pathway in a homeostatic state (Surco-Laos et al. 2022a).

The potential antibacterial activity of the ethanol extract of *H. arborescens* L. leaves was evaluated at concentrations of 5%, 10%, 25% and 50% on strains of *Bacillus*

Table 3. Determination of metals from the ethanolic extract of *H. arborescens* L leaves.

Sample	Calcium	Copper	Magnesium	Zinc
	Mean±SD (mg/100 g)	Mean±SD (mg/100 g)	Mean±SD (mg/100 g)	Mean±SD (mg/100 g)
Ethanolic extract of *H. arborescens* L.	1.22±0.04	0.77±0.05	851.7±3.06	20.63±1.19

SD: standard deviation.

subtilis, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomona aeruginosa*. Antibacterial activity was observed against *B. subtilis* and *S. aureus*, at concentrations of 250 mg/mL (25%) and 500 mg/mL (50%), using a volume of 50 µL, with minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) > 400 µg/mL. However, no concentration showed activity against *E. coli* and *P. aeruginosa*. Oxacillin (5 µg/mL) was used as an antibacterial positive control (Table 4).

In a study on plants used in Brazilian popular medicine for the treatment of infectious diseases Holetz et al. 2002 indicates that the antimicrobial activity of plant extracts is evaluated based on MIC: MIC < 100 µg/mL indicates good activity, 100 to 500 µg/mL indicates moderate activity, 500–1000 µg/mL indicates weak activity, and when it is greater than 1000 µg/mL the extract is considered inactive. In the present study, the ethanolic extract of *H. arborescens* L. leaves showed moderate antibacterial activity (>400 µg/mL), being one of the first studies in this species. There are studies in other species, such as the study by Osungunna and Adedeji 2011 who evaluated the antibacterial activity of the methanolic extract of *H. indicum* leaves (6.25, 12.5, 25, 50, 100 and 200 mg/mL) against a gram-positive bacteria (*S. aureus*) and four gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. mirabilis* and *Klebsiella*); activity was observed at MIC of 3 mg/mL against *S. aureus* and *Klebsiella*, MIC of 10 mg/mL against *P. mirabilis* and *P. aeruginosa*, and MIC 20 mg/mL against *E. Coli*. Hussain et al. 2010 evaluated the antimicrobial activity of *H. strigosum*, reporting that the MIC of 6 and 8 mg/mL exhibited antibacterial activity against *P. aeruginosa*, *S. epidermidis*, methicillin-resistant *S. aureus* and *B. subtillus*, but without activity against *E. coli* and *K. pneumonia*. Rahimifard et al. 2014 reported that the aqueous extract of *H. bacciferum* has greater activity against other solvents. Being active against *S. enteritidis*, *S. aureus* and *B. cereus* with MIC of 7.81 µg/mL, while *P. aeruginosa* and *E. coli* are inhibited with MIC of 15.62 µg/mL.

The limitations of the research are that only the ethanolic extract of the leaves of *H. arborescens* L. was studied, and not having quantified, isolated, and elucidated the chemical structure of the biophenolic compounds responsible for the biological activity. Without prejudice to the above, we consider that this study is relevant, because it constitutes solid scientific evidence of this plant species, given that no reports of the antioxidant activity of leaf, stem or root extracts were found. Therefore, our research group is considering continuing to study this plant species in a second stage, to isolate the bioactive components, carry out preclinical studies in animal models, determine the toxicity of the therapeutic dose and evaluate the pharmacological effects.

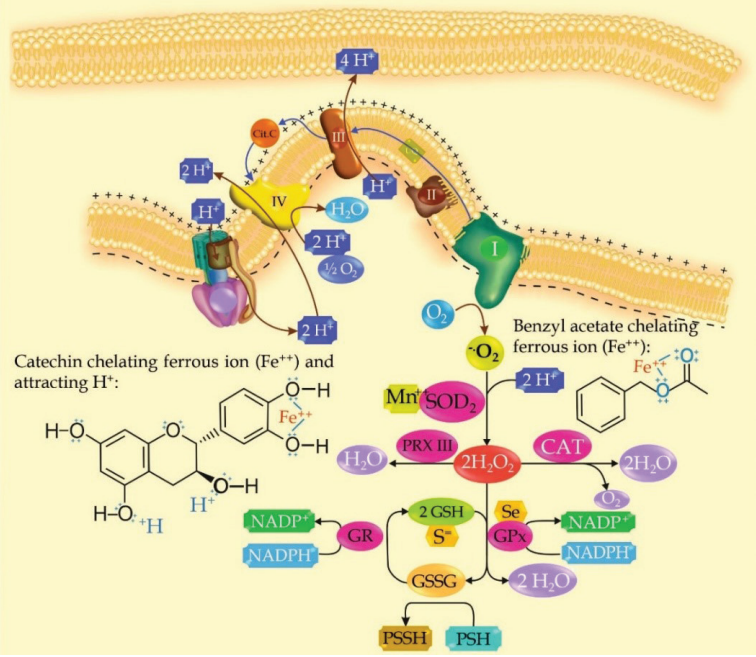


Figure 5. Chelation of reactive oxygen species by quercetin from medicinal plants and activation of the antioxidant enzyme system. Figure made by the authors.

Table 4. Percentage, inhibition diameter, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanolic extract of *H. arborescens* L leaves.

Microorganism	Type	Extract concentration (mg/mL)	Extract inhibition		MIC	MBC	Oxacillin*	
			(%) (Mean±SD)	Diameter (Mean±SD mm)	(µg/mL)	(µg/mL)	%	Diameter (mm)
Bacillus subtilis ATCC 6633	Gram+	250	69.76±1.80	69.76±1.80	>400	>400	100	14.33
		500	77.86±2.58	77.86±2.58	>400	>400	100	14.33
Staphylococcus aureus ATCC 25923	Gram+	250	34.92±1.37	34.92±1.37	>400	>400	100	35.33
		500	41.53±1.28	41.53±1.28	>400	>400	100	35.33

* Standard antibiotic; SD: standard deviation; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

Conclusions

Based on the results, it is concluded that the biophenolic components and metals of the ethanolic extract of the leaves of *H. arborescens* L. show potential antioxidant activity, and moderate antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*.

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References

Ahmad S, Ahmad S, Bibi A, Ishaq MS, Afridi MS, Kanwal F, Zakir M, Fatima F (2014) Phytochemical analysis, antioxidant activity, fatty acids composition, and functional group analysis of Heliotropium bacciferum. The Scientific World Journal 2014: 829076. https://doi.org/10.1155/2014/829076

Alvarado AT, Muñoz AM, Tasayco-Yataco N, Gamarra-Castillo F, Ybañez-Julca RO, Bendezú MR, Chávez H, García JA, Surco-Laos F, Melgar-Merino EJ, Cuba-Garcia PA, Castillo-Romero P, Vega-Ramos N, Loja-Herrera B, Pineda-Pérez M, Bolarte-Arteaga M (2023) In vitro antioxidant and in vivo hypoglycemic activity of biophenols and polyunsaturated fatty acids from *Vitis vinifera* L. muscat and quebranta seeds from the Valley of Ica-Peru. Pharmacia 70: 733–744. https://doi.org/10.3897/pharmacia.70.e109129

Alvarado AT, Muñoz AM, Bartra MS, Valderrama-Wong M, González D, Quiñones LA, Varela N, Bendezú MR, García JA, Loja-Herrera B (2021) Frequency of CYP1A1*2A polymorphisms and deletion of the GSMT1 gene in a Peruvian mestizo population. Pharmacia 68: 747–754. https://doi.org/10.3897/pharmacia.68.e71621

Andzi Barhe´ T, Feuya Tchouya GR (2016) Comparative study of the anti-oxidant activity of the total polyphenols extracted from Hibiscus Sabdariffa L., Glycine max L. Merr., yellow tea and red wine through reaction with DPPH free radicals. Arabian Journal of Chemistry 9: 1–8. https://doi.org/10.1016/j.arabjc.2014.11.048

Avello M, Suwalsky M (2006) Radicales libres, antioxidantes naturales y mecanismos de protección. Atenea 494: 161–172. https://doi.org/10.4067/S0718-04622006000200010

Baroi A, Popiteu M, Fierascu I, Sardarescu I, Fierascu R (2022) Grapevine Wastes: A Rich Source of Antioxidants and Other Biologically Active Compounds. Antioxidants 11: 393. https://doi.org/10.3390/antiox11020393

Carrasco Rueda JM, Guerrero AF, Chávez ÁS, Reyes JL, Quiroz AP, Castilla Candela LC, Bravo RN, Giraldi KO, Yarasca ÁA, Berta Loja Herrera, Granara AS (2013) Effect of the alkaloid extract from Jatropha curcas L. seeds on the intestinal motility [Efecto sobre la motilidad intestinal del extracto de alcaloides de semilla de Jatropha curcas L]. Revista Cubana de Plantas Medicinales 18: 84–91.

Carvajal C (2019) Especies reactivas del oxígeno: formación, función y estrés oxidativo. Revista Medicina Legal de Costa Rica 36: 91–100.

Chávez H, Palomino F, Angelino J, Torres E, Bendezú MR, García JA, Loja B, Muñoz AM, Alvarado AT (2021) In vivo bronchodilator evaluation of the ethanolic extract of the stems of Jatropha macrantha Müll.Arg. Journal of Pharmacy & Pharmacognosy Research 9: 937–946. https://doi.org/10.56499/jppres21.1109_9.6.937

CLSI M07 [Clinical and Laboratory Standards Institute] (2018) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th edn. document M07. Wayne, PA: National Committee for Clinical Laboratory Standard. https://clsi.org/media/1928/m07ed11_sample.pdf

De la Cruz-Arango J, Gómez-Carrión J, Chanco-Estela M, Carrillo-Fuentes EP, Aucasime-Medina L (2020) Flora y vegetación de la provincia de Huamanga (Ayacucho-Perú). Journal of the Selva Andina Biosphere 8: 3–18. https://doi.org/10.36610/j.jsab.2020.080100003

De Zoysa MHN, Rathnayake H, Hewawasam RP, Wijayaratne WMD (2019) Determination of In Vitro Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. International Journal of Microbiology 2019:7431439. https://doi.org/10.1155/2019/7431439

Fayed MAA (2021) Heliotropium; a genus rich in pyrrolizidine alkaloids: A systematic review following its phytochemistry and Pharmacology. Phytomedicine Plus 1: 100036. https://doi.org/10.1016/j.phyplu.2021.100036

Fayed MAA, Abouelela ME, Refaey MS (2022) Heliotropium ramosissimum metabolic profiling, in silico and in vitro evaluation with potent selective cytotoxicity against colorectal carcinoma. Scientific reports 12: 12539. https://doi.org/10.1038/s41598-022-16552-1

Galán de Mera A, Linares Perea E, Montoya Quino J, Vicente Orellana JA (2022) Nuevos datos sobre la vegetación del sur del Perú: adiciones a los departamentos de Arequipa, Cusco y Puno [New data on the vegetation of Southern Peru: Additions to the departments of Arequipa, Cusco and Puno]. Arnaldoa 29: 9–30.

González-Torres MC, Betancourt-Rule M, Ortiz-Muñiz R (2000) Daño Oxidativo y Antioxidantes. Bioquimia 25: 3–9.

Goyal N, Sharma S (2014) Bioactive phytoconstituents and plant extracts from genus Heliotropium. International Journal of Green Pharmacy 8: 217–225.

Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Filho BP (2002) Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Memorias do Instituto Oswaldo Cruz 97: 1027–1031. https://doi.org/10.1590/s0074-02762002000700017

Hussain S, Jamil M, Ullah F, Khan A, Ullah F, Arfan M, Ahmad S, Khatoon L (2010) Antimicrobial and antioxidant activities of the plant heliotropium strigosum. African Journal of Biotechnology 9: 7738–7743.

Luebert F, Weigend M, Hilger HH (2010) Epitypification of Heliotropium arborescens L. (Heliotropiaceae). Taxon 59: 1263–1266. https://doi.org/10.1002/tax.594023

Luebert F (2020) Revisión de Heliotropium sects. Heliothamnus, Heliotrophytum, Hypsogenia, Plagiomeris y Platygine (Heliotropiaceae) en Chile. Gayana Botanica 77: 115–138. https://doi.org/10.4067/S0717-66432020000200115

Macedo-Márquez A (2012) La producción de especies reactivas de oxígeno (EROs) en las mitocondrias de Saccharomyces cerevisiae. TIP Revista especializada en ciencias químico-biológicas 15: 97–103.

Mendoza Isaza NA, Hoyos-Arbeláez JA, Peláez-Jaramillo CA (2020) Capacidad antioxidante y contenido de polifenoles totales de extractos

de tallo de Stevia rebaudiana en varios modelos in vitro. Revista EIA 17: 1–9. https://doi.org/10.24050/reia.v17i34.1282

Merino FJZ, Oliveira VB, Paula CS, Cansian FC, Souza AM, Zuchetto M, Hirota BCK, Duarte AFS, Kulik JD, Miguel MD, Miguel OG (2015) Análise fitoquímica, potencial antioxidante e toxicidade do extrato bruto etanólico e das frações da espécie Senecio westermanii Dusén frente à artemia salina. Revista Brasileira de Plantas Medicinais 17: 1031–1040. https://doi.org/10.1590/1983-084X/14_137

Modak B, Arrieta A, Torres R, Urzua A (2002) Actividad antibacteriana de flavonoides aislados del exudado resinoso de Heliotropium sinuatum: efecto del tipo de estructura. Boletín de la Sociedad Chilena de Química 47. https://doi.org/10.4067/S0366-16442002000100005

Modak B, Salina M, Rodilla J, Torres R (2009) Study of the chemical composition of the resinous exudate isolated from *Heliotropium Sclerocarpum* and evaluation of the antioxidant properties of the phenolic compounds and the resin. Molecules 14: 4625–4633. https://doi.org/10.3390/molecules14114625

Modak B, Sandino AM, Arata L, Cárdenas-Jirón G, Torres R (2010) Inhibitory effect of aromatic geranyl derivatives isolated from *Heliotropium filifolium* on infectious pancreatic necrosis virus replication. Veterinary Microbiology 141: 53–58. https://doi.org/10.1016/j.vetmic.2009.09.005

Murugesh K, Yeligar V, Dash DK, Sengupta P, Maiti BC, Maity TK (2006) Antidiabetic, antioxidant and antihyperlipidemic status of *Heliotropium zeylanicum* extract on streptozotocin-induced diabetes in rats. Biological and Pharmaceutical Bulletin 29: 2202–2205. https://doi.org/10.1248/bpb.29.2202

Noumedem JA, Mihasan M, Lacmata ST, Stefan M, Kuiate JR, Kuete V (2013) Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria. BMC Complementary Medicine and Therapies 13: 1–9. https://doi.org/10.1186/1472-6882-13-26

Ogunbinu AO, Flamini G, Cioni PL, Adebayo MA, Ogunwande IA (2009) Constituents of *Cajanus cajan* (L.) Millsp., *Moringa oleifera* Lam., *Heliotropium indicum* L. and *Bidens pilosa* L. from Nigeria. Natural product communications 4: 573–578. https://doi.org/10.1177/1934578X0900400427

Osungunna MO, Adedeji KA (2011) Phytochemical and antimicrobial screening of methanol extract of Heliotropium indicum leaf. Journal of Microbiology and Antimicrobials 3: 213–216.

Ozntamar-Pouloglou KM, Cheilari A, Zengin G, Graikou K, Ganos C, Karikas GA, Chinou I (2023) Heliotropium procubens Mill: Taxonomic Significance and Characterization of Phenolic Compounds via UHPLC-HRMS- In Vitro Antioxidant and Enzyme Inhibitory Activities. Molecules 28: 1008. https://doi.org/10.3390/molecules28031008

Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53: 4290–4302. https://doi.org/10.1021/jf0502698

Rahimyar N, Bagheri E, Asgarpanah G, Kabiri Balajadeh B, Yazdi H (2014) Antibacterial activity of total extract, petroleum ether, chloroform, ethyl acetate and aqueous fractions of aerial parts of *Heliotropium bacciferum*. Journal of Medicinal Plants 13: 122–135.

Ramos-Escudero F, Muñoz AM, Alvarado-Ortíz C, Alvarado A, Yáñez JA (2012) Purple corn (*Zea mays* L.) phenolic compounds profile and its assessment as an agent against oxidative stress in isolated mouse organs. Journal of Medicinal Food 15: 206–215. https://doi.org/10.1089/jmf.2010.0342

Rezanejad R, Heidarieh M, Ojagh SM, Rezaei M, Raeisi M, Alishahi A (2020) Values of antioxidant activities (ABTS and DPPH) and ferric reducing and chelating powers of gamma-irradiated rosemary extract". Radiochimica Acta 108: 477–482. https://doi.org/10.1515/ract-2019-3113

Rojas JJH, Aguilar ESC, Rojas MAQ, Paredes AML, Escobar SEL, Bocángel AAP, Villacorta FJR, Herrera BL, Yarasca AA, Calderón JM, Granara AS (2012) Efectos sobre la temperatura, frecuencia respiratoria, frecuencia cardiaca y electrocardiograma de Maytenus macrocarpa (Ruiz & Pav.) Briq. (chuchuhuasi). Revista Cubana de Plantas Medicinales 17: 233–243.

Rueangsawang K, Chantaranothai P (2023) A taxonomic synopsis of Heliotropiaceae and new combinations in Heliotropium from Thailand. PhytoKeys 232: 189–210. https://doi.org/10.3897/phytokeys.232.103647

Saeedi M, Morteza-Semnani K (2009) Chemical composition and antimicrobial activity of the essential oil of Heliotropium europaeum. Chemistry of Natural Compounds 45: 98–99. https://doi.org/10.1007/s10600-009-9239-8

Sánchez-Valle V, Méndez-Sánchez N (2013) Estrés oxidativo, antioxidantes y enfermedad. Revista de Investigación Médica Sur México 20: 161–168.

Sarkar C, Mondal M, Khanom B, Hossain MM, Hossain MS, Sureda A, Islam MT, Martorell M, Kumar M, Sharifi-Rad J, Al-Harrasi A, Al-Rawahi A (2021) Heliotropium indicum L.: From Farm to a Source of Bioactive Compounds with Therapeutic Activity. Evidence-Based Complementary and Alternative Medicine 2021: 9965481. https://doi.org/10.1155/2021/9965481

Sharma P, Tripathi S, Chandra R (2020) Phytoremediation potential of heavy metal accumulator plants for waste management in the pulp and paper industry. Heliyon 6: e04559. https://doi.org/10.1016/j.heliyon.2020.e04559

Sharma RA, Singh B, Singh D, Chandrawat P (2009) Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. Journal of Medicinal Plants Research 3: 1153–1175.

Shetty K (2004) Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. Process Biochemistry 39: 789–803. https://doi.org/10.1016/S0032-9592(03)00088-8

Singh B, Sahu PM, Sharma RA (2017) Flavonoids from Heliotropium subulatum exudate and their evaluation for antioxidant, antineoplastic and cytotoxic activities II. Cytotechnology 69: 103–115. https://doi.org/10.1007/s10616-016-0041-8

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians 71: 209–249. https://doi.org/10.3322/caac.21660

Surco-Laos F, García JA, Bendezú MR, Alvarado AT, Laos D, Valle M, Panay J, Palomino JJ, Yarasca PE, Muñoz AM, Bolarte M, Pineda M, Loja B (2023) Characterization of polyunsaturated fatty acids and antioxidant activity of *Vitis vinifera* L. (grape) seeds from the Ica Valley, Peru. Journal of Pharmacy & Pharmacognosy Research 11: 270–280. https://doi.org/10.56499/jppres23.1575_11.2.270

Surco-Laos F, García J, Valle-Campos M, Panay-Centeno JF, Bonifaz-Hernandez M, Melgar EJ, Cuba-Garcia PA, Sullón-Dextre L, Alvarado AT (2022a) Compuestos bioactivos y actividad antioxidante in vitro del extracto etanólico e hidroalcohólico de *Ricinus communis* L. (Higuerilla). Revista Cubana de Farmacia 55: e840.

Surco F, García JA, Bendezú MR, Laos D, Panay JF, Valle M, Palomino JJ, Yarasca PE, Loja B, Alvarado AT (2022b) In vitro antioxidant properties and antimicrobial activity of the ethanolic extract of Senecio nutans Sch. Beep. (Asteraceae). Journal of Pharmacy & Pharmacognosy Research 10: 1026–1036. https://doi.org/10.56499/jppres22.1471_10.6.1026

Tenorio-Abreu A, Gil J, Bratos MA, de la Iglesia A, Borrás M, Ortiz R, Ávila A, Colomina J, Pérez JA, Saavedra JM, Márquez A, Domínguez A, de la Iglesia M (2015) Estudio multicéntrico sobre la actividad in vitro de ceftarolina frente a *Staphylococcus aureus* aislados en España [*In vitro* activity of ceftaroline against Spanish isolates of *Staphylococcus aureus*: a multicenter study]. Enfermedades Infecciosas y Microbiología Clínica 33: 101–104. https://doi.org/10.1016/j.eimc.2014.02.009

Urzúa A, Modak B, Santander R, Heit C, Echeverría J (2013) Insecticidal properties of Heliotropium stenophyllum essential oil on the House fly, *Musca domestica* L. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 12: 196–200.

Valdés C, Valenzuela B, Modak B (2018) Evaluation in vitro of proliferative activity of epithelial cells by flavonoid 3-O-methylgalangine and terpenenic derivative Filifolinone. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 17: 575–582.

Vega A, De León J, Reyes S (2017) Determinación del Contenido de Polifenoles Totales, Flavonoides y Actividad Antioxidante de 34 Cafés Comerciales de Panamá. Información Tecnológica 28: 29–38. https://doi.org/10.4067/S0718-07642017000400005

Velasco L, Goffman FD (1999) Chemotaxonomic significance of fatty acids and tocopherols in Boraginaceae. Phytochemistry 52: 423–426. https://doi.org/10.1016/S0031-9422(99)00203-4

Willocx M, Van der Beeten I, Asselman P, Delgat L, Baert W, Janssens SB, Leliaert F, Picron JF, Vanhee C (2022) Sorting out the plants responsible for a contamination with pyrrolizidine alkaloids in spice seeds by means of LC-MS/MS and DNA barcoding: Proof of principle with cumin and anise spice seeds. Food chemistry 4: 100070. https://doi.org/10.1016/j.fochms.2021.100070

Wongpratate M, Ishida W, Phuthong S, Natphopsuk S, Ishida T (2020) Genetic polymorphisms of the human cytochrome P450 1A1 (CYP1A1) and cervical cancer susceptibility among Northeast Thai women. Asian Pacific Journal of Cancer Prevention 21: 243–248. https://doi.org/10.31557/APJCP.2020.21.1.243