Botanical Sciences 104 (2): 512-530. 2026
DOI: 10.17129/botsci.3788

Received: August 18, 2025, Accepted: January 13, 2026
On line first: February 25, 2026

Phytochemistry / Fitoquímica

Metabolomic profile of *Amaranthus palmeri* S. Watson (Amaranthoideae, Amaranthaceae): Effect on enzymes related to metabolic syndrome

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Abstract
Background: *Amaranthus palmeri* is recognized as one of the most harmful weeds worldwide due to its aggressive spread and resistance to herbicides, complicating its control in agricultural systems. However, it may contain phytochemicals similar to those identified in other *Amaranthus*.
Questions: What are the main phytochemical components in the polar extracts from leaves and inflorescences of *A. palmeri*? Do these polar organic extracts have the potential to inhibit enzymes associated with metabolic syndrome?
Studied species: *Amaranthus palmeri*.
Study site and dates: Greenhouses at the Centro de Investigación Científica de Yucatán, Mérida, Yucatán, Mexico, in 2024.
Methods: Betalains and polyphenols were extracted from the leaves and inflorescences of *A. palmeri* using MSPD. Betalain profiling was performed using LC-MS/MS, and polyphenols were quantified using HPLC-UV-DAD. Additionally, antioxidant capacity was assessed by DPPH assay and enzyme inhibition assays were conducted against α-glucosidase, α-amylase, and ACE.
Results: Nine betalains were found with higher accumulation in inflorescences than in leaves, while 13 polyphenols were found in leaves, with flavonoids such as rutin and quercetin being the most prevalent. The extracts showed a significant amount of antioxidant capacity and enzyme inhibitory activity, with the leaf extracts showing greater bioactivity than inflorescence extracts.
Conclusions: *A. palmeri* is a promising source of bioactive phytochemicals due to its antioxidant potential and its inhibitory effect on key enzymes involved in metabolic syndrome. These findings demonstrate that this species has added value beyond its conventional view of being harmful to agriculture and has potential applications in pharmacology and biotechnology.
Keywords: Angiotensin-converting enzyme (ACE), betalains, flavonoids, α-glucosidase, Palmer amaranth, polyphenols.

Resumen
Antecedentes: *Amaranthus palmeri* está reconocida como una de las malezas más nocivas del mundo por su agresiva propagación y resistencia a los herbicidas, lo que complica su control en los sistemas agrícolas. Sin embargo, puede contener fitoquímicos similares a los identificados en otras especies de *Amaranthus*.
Preguntas: ¿Cuáles son los principales fitoquímicos en los extractos polares de hojas e inflorescencias de *A. palmeri*? ¿Pueden estos extractos inhibir las enzimas asociadas al síndrome metabólico?
Especies de estudio: *Amaranthus palmeri*.
Sitio y años de estudio: Centro de Investigación Científica de Yucatán, Mérida, Yucatán, México en el año 2024.
Métodos: Se extrajeron betalaínas y polifenoles de hojas e inflorescencias de *A. palmeri* utilizando MSPD. El perfil de betalaínas se obtuvo mediante LC-MS/MS y los polifenoles se cuantificaron utilizando HPLC-UV-DAD. Además, se evaluó la capacidad antioxidante mediante el ensayo de DPPH y se realizaron ensayos de inhibición enzimática contra α-glucosidasa, α-amilasa y ECA.
Resultados: Encontramos nueve betalaínas con mayor acumulación en inflorescencias que en hojas, trece polifenoles foliares, con flavonoides como rutina y quercetina los más prevalentes. Los extractos mostraron capacidad antioxidante y actividad inhibitoria enzimática, teniendo los extractos foliares mayor bioactividad que los de las inflorescencias.
Conclusiones: *A. palmeri* es una fuente prometedora de fitoquímicos bioactivos debido a su potencial antioxidante y su efecto inhibitorio sobre enzimas clave involucradas en el síndrome metabólico. Estos hallazgos demuestran que esta especie posee un valor añadido más allá de su percepción como perjudicial para la agricultura, con aplicaciones potenciales en la farmacología y la biotecnología.
Palabras clave: Amaranto Palmer, betalaínas, Enzima Convertidora de Angiotensina (ECA), flavonoides, α-glucosidasas, polifenoles.

Plants with bioactive molecules are of high significance because they are essential in the novel drug discovery programs. Uncontrolled weed growth can have a negative impact on crop yield in most, if not all, cases, as well as on crop health (Linde *et al*. 2016, Colbach *et al*. 2020, Dentika *et al*. 2021). However, there is evidence for possible advantages of weeds as natural sources for discovering new bioactive metabolites and beneficial microorganisms (Ilic 2023), among other potential benefits (Norris & Kogan 2005, von Redwitz *et al*. 2025). Most of the research on weeds as sources of phytochemicals has focused on metabolites with herbicide and insecticidal activities because weeds produce allelopathic compounds that affect target organisms in a concentration- and species-specific fashion (Rob *et al*. 2021, Tlak Gajger & Dar 2021, Duke *et al*. 2022). The high phytochemical content of weeds has led to the discovery of various pharmaceuticals, most of which are secondary metabolites. For instance, *Catharanthus roseus* (L.) G. Don is known for producing vinblastine and vincristine, which are utilized in cancer treatments, and *Datura metel* L. produces scopolamine, which can be used to prevent postoperative nausea, vomiting, and motion sickness, among other potential plant species sources that have great therapeutic potential (Stepp 2004).

The genus *Amaranthus*, given its diversity, with nearly 75 species, of which closely 20 behaved as weeds (Rapoport *et al.* 2009, Hernández-Ledesma *et al*. 2015), could be a significant plant source of diverse bioactive compounds with nutritional and pharmaceutical properties. Compounds associated with amaranth include betalains, which are red-violet pigments, and flavonoids (Gandhi *et al*. 2021). While the impact of red-violet pigments and polyphenols as antioxidants (Cai *et al.* 2003, Araujo-León *et al.* 2024a), antidiabetics (Sarker & Oba 2019a, Araujo-León *et al*. 2023, 2024a), antihypertensive (Sarker & Oba 2019a, Araujo-León *et al.* 2023, 2024a), antilipidemic, anticancer, and as antimicrobials in cultivated amaranths is becoming clear (Sarker & Oba 2020a, Sarker *et al*. 2022a, Araujo-León *et al.* 2024a), the impact of weedy species such as *Amaranthus viridis* L., *Amaranthus spinosus* L., *A. blitum* L., *Amaranthus crispus* (Lesp. & Thévenau) A.Braun ex J.M.Coult. & S.Watson, *Amaranthus hybridus* L., as sources of bioactive compounds is emerging (Sarker & Oba 2019a,b, 2020b, Sarker *et al*. 2020, Bang *et al.* 2021, 2024, Cuervo *et al.* 2025). There are many promising features to notice for weeds of the genus *Amaranthus*, besides the features that have negative impacts and challenges the intensive agriculture, including abiotic tolerance, nutrient richness, and betalains in leaves.

Given that *A. palmeri* species outperforms even other *Amaranthus* weeds in growth (Horak & Loughin 2000, Sellers *et al.* 2003, Massinga *et al.* 2001, Norsworthy *et al*. 2014, Gaines *et al.* 2020), *A. palmeri* can be harmful to crops yield and native amaranths through hybridization (Sellers *et al.* 2003). The biology of *A. palmeri* has been partially understood through studying its growth rates against to other amaranth weeds, herbicide resistance (Gossett *et al*. 1992, Culpepper *et al.* 2006, Jhala *et al*. 2014, Shimono *et al.* 2020, Sandhu *et al*. 2023, Zulet-Gonzalez *et al.* 2023, Manicardi *et al.* 2025), interaction with crops (Culpepper *et al*. 2006, Ward *et al*. 2013), variation in growth rate on treatments with climate changes variables (Shilling *et al.* 2023), the C4 photosynthesis metabolism (Wang *et al.* 1992, Borgato *et al.* 2025), and stress tolerance (Maroli *et al*. 2015, Sandhu *et al.* 2023, Zulet-Gonzalez *et al.* 2023). *A. palmeri* may, like other invasive weeds amaranths (Sarker & Oba 2019a, 2020a, Sarker *et al*. 2022b), have the potential to be an inexpensive source of bioactive compounds such as chlorophylls, β-carotene, betacyanins, betaxanthins, vitamin C, polyphenols, among other bioactive compounds.

The metabolic syndrome is characterized by the clustering of multiple risk causes in one individual, including abdominal obesity, hypertension, hyperglycemia, dyslipidemia, and insulin resistance, which all together enlarges the risk conditions for cardiovascular diseases and type 2 diabetes (Pigeot & Ahrens 2025). Although it is unknown the global population count for metabolic syndrome, multiple organizations and analyses have converged in the conclusion that it is increasing constantly (Islam *et al.* 2024, Zila-Velasque *et al.* 2024, Pigeot & Ahrens 2025). For instance, according to the International Diabetes Federation (IDF), 20–25 % of the adult population worldwide is estimated to have metabolic syndrome (Jamali *et al.* 2024). In Mexico, the highest incidence of metabolic syndrome is found in adults aged 40 years and older (Campos-Nonato *et al.* 2025). Within the diagnostic markers and therapeutic targets for type 2 diabetes and hypertension, the α-amylase (luminal starch breakdown), α-glucosidase (brush-border release of absorbable glucose), and angiotensin-converting enzyme (ACE) are crucial (Mirabito Colafella *et*

al. 2019, Tundis *et al.* 2010). Antidiabetic and antihypertensive drugs acting on those enzyme therapeutic targets are valuable by reducing their activity. Due to the distinctive side effects associated with certain drugs and the prevalence of metabolic syndrome, it is essential to find sources of bioactive compounds that act on multiple targets and are safe. The aim of this study was to examine ethanolic extracts from *A. palmeri* for their content of betalains and polyphenols, as well as their antioxidant activity and inhibition potential of enzymes associated with metabolic syndrome. This data insight into the phytochemistry of *A. palmeri,* showing that it may be used in pharmacological applications as a source of bioactive compounds.

Materials and methods

Plant material. The seeds of *Amaranthus palmeri* S. Watson collection took place in Mérida, Yucatán, Mexico, where Dr. Ivonne Sánchez-del Pino conducted taxonomic identification (Sánchez-del Pino *et al.* 2019). A voucher specimen was deposited at the Yucatan Center for Scientific Research (CICY) Herbarium (determination, *A. palmeri* S. Watson; collector-voucher number, I. Sánchez-del-Pino-593; region, México, Yucatán, Mérida City; locality, Gran Santa Fe II; coordinates, 21° 1' 40.0872" N; 89° 41' 33.1002" W). The plants were grown in both experimental plots and greenhouses at CICY. Leaves and inflorescences were harvested from plants that were 60-day-old. The plant tissue was immediately frozen with liquid nitrogen, and then it was lyophilized at –51 °C for 72 h. The dried tissue was ground with a blade mill and stored in darkness at –20 °C until later analysis.

Chemicals and reagents. Methanol (MeOH) and water (H_2O) that are HPLC grade and supplied by Tedia (Fairfield, CT, USA) were employed as solvents for extracting the phytochemicals. For performing the chromatographic analysis by HPLC-UV-DAD, HPLC-grade MeOH, acetonitrile (MeCN), and H_2O (Tedia, Fairfield, CT, USA) were utilized. The mobile phase was acidified using HPLC-grade acetic acid (Fisher Scientific, Waltham, MA, USA). A commercial beetroot extract (Sigma-Aldrich, Cat. # 901266, St. Louis, MO, USA) was served as the reference standard. The matrix solid-phase dispersion (MSPD) method was carried out using BondElut-C18 as the sorbent (Agilent Technologies, CA, USA), which has a particle size of 40 µm. Analytical standards (≥ 99 % purity) were obtained from Sigma-Aldrich for quantifying flavonoids and organic acids using HPLC-UV-DAD. Within the analytical standards, there were rutin, hesperidin, hesperetin, naringin, naringenin, quercetin, catechin, kaempferol, vanillic acid, 4-hydroxybenzoic acid, *p*-coumaric acid, caffeic acid, and ferulic acid. Betanin obtained from beetroot extract was used as the standard for quantifying betalains.

Extraction of phytochemicals using matrix solid-phase dispersion (MSPD). Betalains, organic acids, and flavonoids were extracted by using the MSPD technique described by Araujo-León *et al*. (2023, 2024a). To prepare each sample, 100 mg of freeze-dried plant tissue was finely ground at room temperature with 400 mg of BondElut-C18 sorbent using a mortar and pestle. The homogenized material was packed into solid-phase extraction cartridges. The phytochemicals were eluted using a Visiprep vacuum manifold system (SUPELCO, Bellefonte, PA, USA), with 9 mL of water acidified with 0.1 % acetic acid for betalains and with a 9 mL solution of water acidulated (0.1 % acetic acid) and methanol at 1:1 (v/v) for organic acids and flavonoids. Applying a vacuum of −15 mmHg. The extracts were combined, evaporated to dryness, and stored until needed for analysis.

Characterization of betalain profile using LC-MS/MS. The betalains were analyzed using the LC-MS method that was previously described (Araujo-León *et al.* 2023). The Ultimate 3000 UHPLC system (Thermo Scientific, Waltham, MA, USA), coupled to a UV/Vis detector (Ultimate 3000 UV/VIS, Dionex), and an LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a Heated Electrospray Ionization source (HESI-II, Thermo Fisher Scientific, Waltham, MA, USA), were used for LC-MS analysis. A 20 µL aliquot of each sample was injected, and we used a Hypersil GOLD C18 analytical column (100 × 2.1 mm, 1.9 µm particle size, Thermo Scientific, San Jose, CA, USA) at a flow rate of 300 µL/min. A binary gradient comprised of water acidulated

with 0.1 % acetic acid (solvent A) and MeOH acidulated with 0.1 % acetic acid (solvent B) was used to perform elution under the following conditions: 5 to 100 % B for 30 min, 100 % B for 10 min, and then re-equilibration at 5 % B for another 10 min. UV/Vis absorbance was taken at 540 nm. Mass spectrometry data were acquired in positive ionization mode, with MS1 scans at a resolution of 60,000 and a scan range of 100–1,500 *m/z*, while MS2 scans were set at a resolution of 60,000 within a 100–600 *m/z* range. Fragmentation was achieved by higher-energy collisional dissociation (HCD) with 50 eV. The quantitative determinations were based on the peak areas that were obtained from extracted ion chromatograms (EICs) in MS1 mode by using Xcalibur v. 4.1 software (Thermo Scientific, USA). Graphical data were generated using GraphPad Prism v. 9.0 (GraphPad, San Diego, CA, USA).

Extraction of phytochemicals for in vitro assays. Briefly, 25 g of plant tissue (leaves or inflorescences) were placed in a 100 mL Erlenmeyer flask and exhaustively macerated with 100 mL de methanol:water (80:20, v/v) acidified to 0.1 % (v/v). After concentration under reduced pressure using a rotatory evaporator, the extract was immediately frozen under liquid nitrogen temperature and lyophilized for 48 h to obtain a dry extract. The dried extracts of leaves or inflorescences were transferred to amber vials and stored at –80 °C until *in vitro* bioactive assay evaluation.

Quantification of betacyanins and betaxanthins using spectrophotometric methods. The concentration of betacyanins and betaxanthins was performed using the spectrophotometric method described by Cai *et al*. (1998). For extracting metabolites, 100 mg of lyophilized plant tissue were mixed with 25 mL of an aqueous methanol solution (H_2O:MeOH, 80:20 v/v) acidified with 0.1 % acetic acid. After mixing the plant material for 10 min, the mixture was centrifuged at 9,000 rpm at 20 °C, and the supernatant was collected and transferred to amber vials for spectrophotometric analysis.

The concentrations of betacyanins and betaxanthins were calculated using the following equations:

$$\text{Betacyanins (mg/g)} = (A_{536} \times MW \times V \times DF) / (\varepsilon \times L \times W)$$
$$\text{Betaxanthins (mg/g)} = (A_{480} \times MW \times V \times DF) / (\varepsilon \times L \times W)$$

Where A_{536} and A_{480} are the absorbance values at 536 and 480 nm, respectively, which correspond to betacyanins and betaxanthins. The molecular weight (MW) and molar extinction coefficient (ε) values for amaranthin (betacyanins) were 726.6 g/mol and 5.66×10^4 L mol^{-1} cm^{-1}, respectively, while for indicaxanthin (betaxanthins) were 308 g/mol and 4.81×10^4 L mol^{-1} cm^{-1}, respectively. V is the extraction volume in milliliters. DF is the diluting factor. L is the length of edge of the path (typically 1 cm). W is the dry weight of the plant tissue in grams.

Quantification of polyphenols by HPLC-UV-DAD. The dried extracts were reconstituted into an acetic acid-water methanol mixture (1:1, v/v) and placed in an amber vial. The organic acids and flavonoids were quantified using the validated method reported by Araujo-León *et al.* (2024a). Elution was carried out using a ternary gradient system consisting of water acidulated with 0.1 % acetic acid (solvent A), MeOH acidulated with 0.1 % acetic acid (solvent B), and MeCN acidulated with 0.1 % acetic acid (solvent C), with a column flow rate of 350 µL/min. The gradient profile began with 100 % solvent A for the first 2 min, followed by a linear increase from 2 to 16 min to reach a final composition of 80 % solvent B and 20 % solvent C. A subsequent purge step of 8 min and a re-equilibration phase to the initial conditions were performed to stabilize the system. UV/Vis detection was monitored at 230 nm. Chromatographic peak areas were integrated using Chromeleon 7.1 software (Thermo Scientific, Waltham, MA, USA), and graphical representations were created with GraphPad Prism version 9.0 (GraphPad, San Diego, CA, USA).

DPPH free radical scavenging assay. The antioxidant activity of the extracts was determined based on their ability to scavenge the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl), following the procedure described by Shimada *et al*. (1992). A total of 150 µL of either leaf or inflorescence extract was mixed with a 1,350 µL of a 0.1 mM DPPH solution that was made in ethanol. Mixing and keeping the reaction mixtures in amber vials at 25 °C for 30 min was done to prevent light-induced degradation. Afterward, the absorbance was recorded at 517 nm by using

a UV-Vis spectrophotometer (Evolution 220, Thermo Scientific, Waltham, MA, USA). At 180 µg/mL, ascorbic acid served as a positive control because of its well-known antioxidant properties. All the measurements were performed in triplicate.

The radical scavenging activity was expressed as a percentage, calculated according to the following equation:

$$\% \text{ Scavenging activity} = 100 \times [(AC - AE) / AC]$$

Where AC represents the absorbance of the control (DPPH without extract) and AE corresponds to the absorbance in the presence of the extract.

In vitro α-glucosidase inhibition assay. The inhibitory potential of the aqueous-methanolic extracts from leaves and inflorescences of *A. palmeri* against α-glucosidase activity was assessed following a modified *in vitro* assay (Tamil *et al.* 2010). The enzyme α-glucosidase from *Saccharomyces cerevisiae* Meyen ex E.C bought from Sigma-Aldrich (2 U/mL) was made in phosphate buffer (50 mM, pH 6.8) and then pre-incubated for 5 min at 37 °C. Afterward, 100 µL of *p*-nitrophenyl-α-D-glucopyranoside, which was previously dissolved in the same buffer, was added to start the enzymatic reaction. The mixture was incubated for one hour at 37 °C. To stop the reaction, 250 µL of 1.0 M sodium carbonate was added to each tube. After centrifuging at 4,500 rpm for 10 min at 4 °C, the resulting supernatant was collected and measured using spectrophotometry at 405 nm. Acarbose (100 µg/mL; 154.89 µM) was used as a positive control, with a reported IC_{50} value of 83.33 µg/mL (129.07 µM) (Tamil *et al.* 2010). All assays were performed in triplicate with extract concentrations of 1,000 µg/mL.

In vitro α-amylase inhibition assay. The inhibitory effect of the aqueous-methanolic extracts from leaves and inflorescences of *A. palmeri* on α-amylase activity (from *Bacillus licheniformis* from Sigma-Aldrich) was assessed using an adjusted protocol based on Tamil *et al.* (2010). In microtubes (200 µL capacity), 2 mg of soluble starch were suspended in 0.5 M Tris-HCl buffer (pH 6.9) supplemented with 0.01 M $CaCl_2$. The suspension was boiled for 5 min and later kept at 37 °C for another 5 min. Next, 200 µL of 50 % (v/v) dimethyl sulfoxide, 200 µL of plant extracts (1,000 µg/mL), 200 µL of porcine pancreatic α-amylase solution (2 U/mL), and 500 µL of 0.1 % 3,5-dinitrosalicylic acid (DNS) were added sequentially to each tube. The reaction was initiated at 37 °C for 10 min and ended after adding 0.5 mL of 50 % acetic acid was added. After centrifuging at 4,500 rpm for 10 min at 4 °C, the supernatant was extracted for analysis.

Absorbance measurements were taken at 540 nm using a UV-Vis spectrophotometer. Acarbose, at a concentration of 100 µg/mL (154.89 µM), was used as a positive control, with a reported IC_{50} value of 83.33 µg/mL (129.07 µM) under these conditions (Tamil *et al.* 2010). All the assays were performed in triplicate.

The percentage of enzyme inhibition was determined using the following formula:

$$\text{Inhibition (\%)} = [(Ac+) - (Ac-) - (As - Ab)] / [(Ac+) - (Ac-)] \times 100$$

Where Ac+ is the absorbance corresponding to full enzyme activity (enzyme + solvent), Ac− represents the absorbance with enzyme inactivation (solvent without enzyme), As is the absorbance in the presence of the sample extract, and Ab is the absorbance of blanks.

In vitro angiotensin-converting enzyme (ACE) inhibition assay. The capacity of aqueous-methanolic extracts from *A. palmeri* leaves and inflorescences to inhibit ACE (from rabbit lung from Sigma-Aldrich) was assessed at a concentration of 1 mg/mL. The procedure used was reported by Hayakari *et al*. (1978), which involves the enzymatic hydrolysis of hippuryl-L-histidyl-L-leucine (HHL) into hippuric acid and His-Leu by ACE, followed by a colorimetric reaction between hippuric acid and 2,4,6-trichloro-s-triazine (TT) (Tehreem *et al.* 2021). 20 µL of ACE (100 mU/mL) and 40 µL of plant extract were mixed and kept at 37 °C for 5 min in a thermostatic water bath (VWR® Heating circulator

model 1130-2S, VWR International, Radnor, PA, USA). Following this, each reaction tube received 100 µL of 0.3 % HHL substrate, which was made in a buffer containing 40 µmol potassium phosphate and 300 µmol sodium chloride (pH 8.3). Next, the mixtures were kept at 37 °C for 45 min. The reaction was stopped by adding 360 µL of TT (3 % w/v in dioxane) and 720 µL of 0.2 M phosphate buffer (pH 8.3). After centrifuging at 10,000 rpm for 10 min, the absorbance of the supernatant was measured at 382 nm using a UV-Vis spectrophotometer (Thermo Scientific Evolution 220, Thermo Scientific, Waltham, MA, USA). Captopril was employed as a positive control at 50 µg/mL (230.11 µM), with an IC_{50} of 28.7 nM under these conditions (Hayakari *et al*. 1978). All the assays were performed in triplicate.

Statistical analysis. The spectrophotometric, spectroscopic, and *in vitro* biological measurements were performed three times each. The summaries of data are presented as mean ± standard deviation (SD), with error bars showing SD. Pairwise comparisons were made using two-tailed Student's t-tests with a significance threshold of $p < 0.05$ (GraphPad Prism v. 9.0). Welch's correction was used if equal variances were not met. Raw data were recorded in Microsoft Excel and later exported to GraphPad Prism v. 9.0 for statistical analyses and figure generation.

Results

Betalain profile characterization by LC-MS/MS. A total of nine betalains were identified in leaf and inflorescence extracts of *A. palmeri* using LC-MS/MS. The profile included six betacyanins, consisting of three amaranthin-type betacyanins and three betanin-type betacyanins, and three betaxanthins (Figure 1). When peak areas representing the relative abundance of betacyanins were examined in extracts from leaves and inflorescences, amaranthin and its epimer isoamaranthin were found to be the most abundant, with a 1:4 peak area ratio between leaves and inflorescences, respectively. Isobetanin, which is the epimer of betanin, exhibited a peak area ratio of 1:10 ratio. Leaves and inflorescences displayed betaxanthins that include proline-(betaxanthin)bx, tryptophan-bx, and threonine-bx, with proline-bx being the most abundant. These results confirm that inflorescences of *A. palmeri* accumulate a greater amount of these compounds. Despite the quantitative differences, the qualitative betalain profile remained similar between both tissues.

Further, the betalains were characterized by their chromatographic behavior and mass spectrometry fragmentation patterns (Table 1). The retention times of betalains ranged from 1.65 to 8.51 min (Figure 2A and B), and mass measurements were accurate with an error less than 5 ppm. Both amaranthin- and betanin-type betacyanins displayed typical fragment ions at *m/z* 389.0979, 343.0925, and 194.0448 (Figure 2C and D), supporting their identification. Betaxanthins such as proline-bx, tryptophan-bx, and threonine-bx showed characteristic fragment ions at *m/z* 211.0713 and 194.0448 (Araujo-León *et al*. 2023, 2024b).

Quantification of betacyanins and betaxanthins using the spectrophotometric method. The quantifying data on of betalains revealed significant differences between leaves and inflorescences. Total betacyanin content was significantly higher in inflorescences, with a ratio of roughly 3:1 compared with leaves (Figure 3). Similarly, the total betaxanthins abundance showed a marked increase in inflorescences, with concentrations of nearly two times greater than in leaves. These results confirm that inflorescences accumulate higher levels of both betacyanins and betaxanthins than leaves ($p \leq 0.05$) in *A. palmeri*.

Polyphenols quantification by HPLC-UV-DAD. A total of 13 polyphenolic compounds were analyzed, which included eight flavonoids, two of the benzoic acid-type, and three of the cinnamic acid-type. A higher overall concentration and diversity of flavonoids were found in leaves than inflorescences. Rutin and its aglycone quercetin were the main flavonoids in both tissues. The abundance of rutin in leaves was 2.5 times higher than in inflorescences, while quercetin had a 5:1 ratio with a significantly higher concentration in leaves (Figure 4A**)**. The total flavonoids content was higher in leaves, with a ratio of roughly 3:1 compared to inflorescences (Figure 4B). The total content of benzoic acids or cinnamic acids in both tissues remained unchanged (Figure 4B).

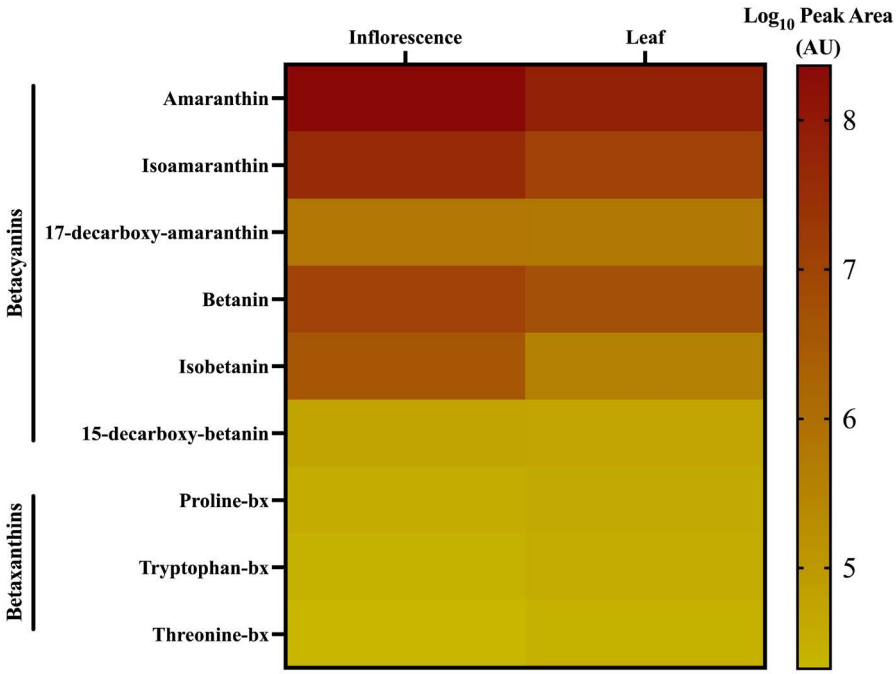


Figure 1. Relative abundances of the main betalains profile in *A. palmeri*. Heatmaps of relative abundances calculated using $\log_{10}$ for the betalains areas in the chromatograms.

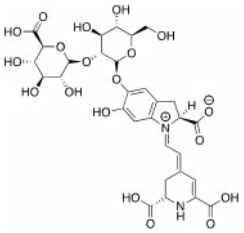
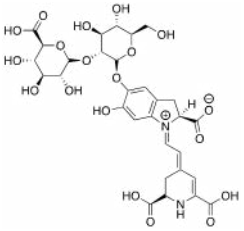
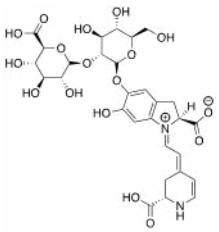
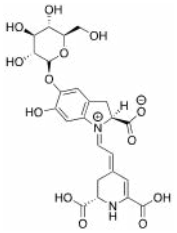
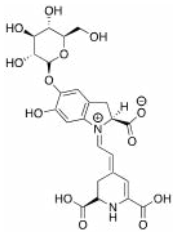
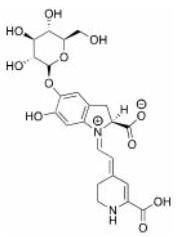
The most abundant flavonoid detected in both inflorescence and leaf samples was rutin, which is evident at peak 8 in Figure 5A and 5B. The compound was characterized by mass spectrometry at high resolution in negative ionization mode and fragmentation of higher-energy collisional dissociation (HCD) at 80 eV. The mass spectrum of rutin showed the deprotonated molecular ion $[M - H]^-$ with a *m/z* of 609.1489 and a mass error of 4.59 ppm. Additionally, the $[2M - H]^-$ adduct was also detected. During fragmenting of the $[M - H]^-$ ion at 80 eV, product ions at *m/z* 301.0354, 300.0276, and 151.0037 were observed (Figure 5C, D). The fragment ions reported for rutin in the mzCloud database agree with those found in this study.

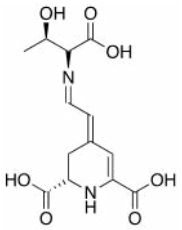
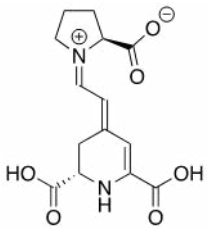
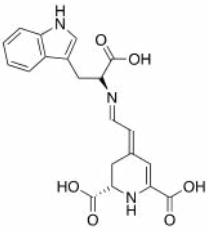
DPPH free radical scavenging assay. The extracts of the leaf and inflorescence were assayed at 1,000 µg/mL. The methanolic aqueous extract from leaves of *A. palmeri* showed the highest antioxidant capacity, reaching 85 % DPPH radical scavenging activity. Compared with the leaves, the extract from the inflorescences showed a significantly lower scavenging effect ($p < 0.05$) and a 60 % inhibition. The ascorbic acid was used as a positive control at 180 µg/mL and showed 95 % of radical scavenging (Figure 6A).

In vitro α-glucosidase inhibition assay. The extracts of the leaf and inflorescence were assayed at 1,000 µg/mL. There were no significant differences between the leaf and inflorescence extracts of *A. palmeri* ($p \geq 0.05$) for their inhibitory against α-glucosidase, but there was a tendency to decrease the activity. The inhibition rate displayed by the leaf extract was 62 %, whereas the inflorescence was 68 %. The acarbose which served as a positive control at 100 µg/mL and showed 98 % inhibition (Figure 6B).

In vitro α-amylase inhibition assay. The extracts of the leaf and inflorescence were assayed at 1,000 µg/mL. The α-amylase inhibition assay showed significant differences in the plant tissues analyzed ($p \leq 0.05$). The leaf extract of *A. palmeri* had an inhibitory rate of 78 % for α-amylase, which was significantly higher than the 66 % inhibition rate seen for the inflorescence extract. Acarbose was used as a positive control at 100 µg/mL and showed inhibition of 98 % (Figure 6C).

Table 1. Chromatographic and mass spectrometry data for betalains from *A. palmeri* in leaves and inflorescences.

Compound	Retention time (Rt) min	Chemical Formula	Theorical *m/z* [M+H]	Observed *m/z* [M+H]	Error (ppm)	Fragments	Chemical structure
Amaranthin-type							
Amaranthin	2.93	$C_{30}H_{34}N_2O_{19}$	727.1826	727.1804	3.03	389.0979, 345.1081, 343.0925, 194.0448, 150.0550, 132.0444, 106.0651	
Isoamaranthin	4.64	$C_{30}H_{34}N_2O_{19}$	727.1826	727.1808	2.48	389.0979, 345.1081, 343.0925, 194.0448, 150.0550, 132.0444, 106.0651	
17-Decarboxy-amaranthin	5.12	$C_{29}H_{34}N_2O_{17}$	683.1930	683.1938	-1.17	345.1081, 194.0448, 150.0550, 132.0444, 106.0651	
Betanin-type							
Betanin	4.02	$C_{24}H_{26}N_2O_{13}$	551.1508	551.1518	-1.81	389.0979, 345.1081, 343.0925, 194.0448, 150.0550, 132.0444, 106.0651	
Isobetanin	6.87	$C_{24}H_{26}N_2O_{13}$	551.1508	551.1522	-2.54	389.0979, 345.1081, 343.0925, 194.0448, 150.0550, 132.0444, 106.0651	
15-Decarboxy-betanin	8.51	$C_{23}H_{26}N_2O_{11}$	507.1609	507.1621	-2.37	345.1081, 194.0448, 150.0550, 132.0444, 106.0651	

Compound	Retention time (Rt) min	Chemical Formula	Theorical *m/z* [M+H]	Observed *m/z* [M+H]	Error (ppm)	Fragments	Chemical structure
Betaxanthins							
Threonine-bx	1.65	$C_{13}H_{16}N_2O_7$	313.103	313.1021	2.87	211.0713, 194.0448, 150.0550, 132.0444, 106.0651	
Proline-bx	2.81	$C_{14}H_{16}N_2O_6$	309.1081	309.1071	3.24	211.0713, 194.0448, 150.0550, 132.0444, 106.0651	
Tryptophan-bx	3.19	$C_{20}H_{19}N_3O_6$	398.1347	398.1334	3.27	211.0713, 194.0448, 150.0550, 132.0444, 106.0651	

In vitro angiotensin-converting enzyme (ACE) inhibition assay. The extracts of the leaf and inflorescence were assayed at 1,000 µg/mL. The ACE inhibition assay showed that the leaf extract of *A. palmer*i inhibited 77 % of ACE activity, which was significantly higher than the 65 % inhibition obtained with the inflorescence extract ($p \leq 0.05$). Captopril was used as a positive control at 50 µg/mL and showed an inhibitory effect of 97 % (Figure 6D).

Discussion

This study aimed to characterize the main phytochemicals present in the leaves and inflorescences of *A. palmeri* and to evaluate if *A. palmeri*-derived extracts can inhibit key enzymes associated with metabolic syndrome. Our study showed that this weed species of Amaranthaceae has valuable bioactive properties that are supported by its main phytochemicals, which include polyphenols and betalains.

The HPLC and HPLC-MS analysis revealed the presence of polyphenols and betalains in the leaves and inflorescences of *Amaranthus palmeri*. These results are consistent with the previous studies that have reported flavonoids, organic acids and betalains in species of weedy amaranths related to *A. palmeri,* such as *A. spinosus* and other cultivated amaranth species, including *A. cruentus* L. and *A. hypochondriacus* L. In *A. spinosus* L., which is closely related to *A. palmeri* by genetic and morphologic features (Sánchez-del Pino *et al*. 2019), besides flavonoids and betalains the phytoconstituents include phytosterols (β-sitosterol), fatty acids, and hydroxycinnamates and glycosides of quercetin and kaempferol (Stintzing *et al.* 2004, Suryavanshi *et al.* 2008, Mondal *et al.* 2015).

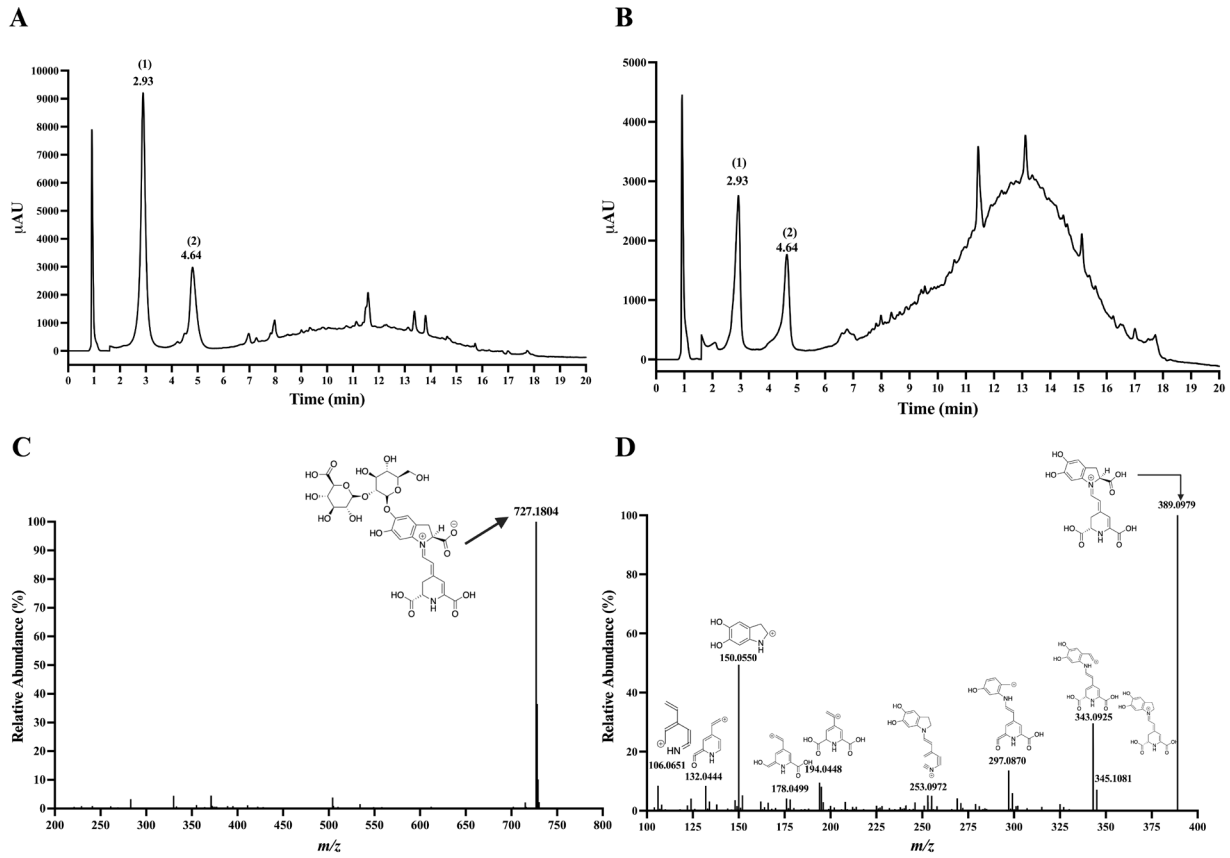


Figure 2. Chromatograms at 540 nm of inflorescence (A) and leaves (B) of *A. palmeri* and the MS1 of amaranthin (C) and their product ions of MS2 at 50 eV in HCD (D). The chromatograms show the annotations (1) and (2), which represent amaranthin and isoamaranthin, respectively.

Our findings on the variation in the concentration of polyphenols among plant tissue are consistent with previous studies. For example, an analysis of the whole plant of *A. spinosus* L. in Mumbai, India, revealed the presence of rutin (quercetin-3-*O*-rutinoside), with concentrations ranging from 15.65 to 20.13 µg/mL (Suryavanshi *et al.* 2008). In *Amaranthus paniculatus* L. (synonym with *A. cruentus* L.), the rutin levels are 3,620 µg/g (Kraujalis *et al.* 2015). In *A. hybridus* L., the leaves of two accessions from Lithuania displayed the highest levels of rutin levels with 4,072 µg/g when compared with aqueous methanolic extracts of inflorescences, stems, and seeds (Kraujalis *et al*. 2013). However, variations in concentrations, which are genotype- or development-dependent, can affect the ratio of specific polyphenols. Karamać *et al.* (2019) noted a gradual increase in rutin levels in *A. caudatus* L. from early vegetative stages (15,000 µg/g) to flowering stages (36,200 µg/g). Our findings showed that *A. palmeri* extract showed a remarkable ratio of rutin to quercetin values of 18:1 for leaves and 35:1 for inflorescence. A high ratio of rutin to quercetin has also been found in *Amaranthus retroflexus* L., with 332 µg/g rutin and 0.56 µg/g quercetin (Sărăcin *et al.* 2023). Netshimbupfe *et al.* (2023) found a similar trend in an analysis of *Amaranthus* species in Africa, with more rutin than quercetin in the foliar tissues of *A. caudatus* L. (550 µg/g rutin / 437 µg/g quercetin), *A. hypochondriacus* L. (449 / 412 µg/g), *A. cruentus* L. (363 / 204 µg/g), and *A. spinosus* (397 / 88 µg/g). Kalinová & Dadáková (2009) determined the levels of rutin and quercetin at the flowering stage of several species of *Amaranthus* in the Czech Republic, where *A. hybridus* L. (K-526) contained the highest concentration (15,600 and 16,913 µg/g, respectively), followed by *Amaranthus hypochondriacus* L. (Kois) [7,375 µg/g / 7,704 µg/g], *Amaranthus caudatus* L. (Oscar Blanco) [6,695 µg/g / 7,755 µg/g], and *Amaranthus tricolor* L. [1,395 µg/g / 1,217 µg/g], with an almost 1:1 ratio between the two flavonoids. According to our findings, *A. palmeri* is one of the species with the highest levels of rutin accumulation described so far, with 25,299 µg/g in leaves and 9,809 in inflorescences.

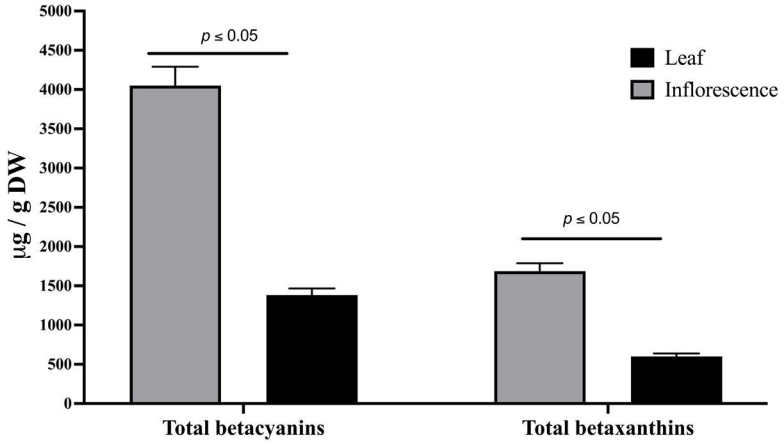


Figure 3. Total content of betacyanins and betaxanthins in *A. palmeri* leaves and inflorescences. Spectrophotometric analysis was used to quantify betacyanins and betaxanthins. Mean ± SD (n = 3). Leaf–inflorescence contrasts by two-tailed Student's *t*-test, α = 0.05.

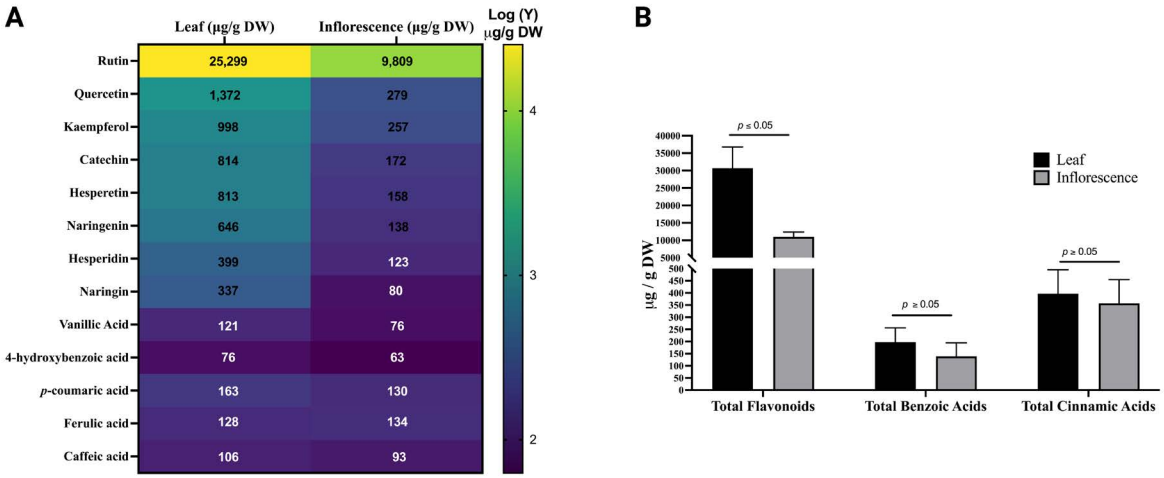


Figure 4. Polyphenol profiles in *A. palmeri* leaves and inflorescences. (A) Heatmap showing the distribution of polyphenol concentrations. (B) Quantification of the total polyphenols, which are classified as flavonoids, benzoic acid derivatives, and cinnamic acid derivatives. Mean ± SD (n = 3). Leaf–inflorescence contrasts by two-tailed Student's *t*-test, α = 0.05.

Besides flavonoids, betalains are included in the phytochemical profile of *A. palmeri*. The presence of betacyanins such as amaranthin and isoamaranthin, and betaxanthins such as proline-(betaxanthin)bx is consistent with the previously suggested resiliency to oxidative stress within reproductive tissues and cellular processes (Sawicki *et al.* 2016, Pari *et al*. 2024). Although *A. palmeri* has green colored inflorescences and dioecious species are not typically associated with betalain production, this study has documented the first complete betalain profile in both leaves and inflorescences using LC-MS/MS (Figure 1 and Table 1). The presence of characteristic fragment ions for betacyanins (*m/z* 389.0979, 343.0925, 194.0448, 150.0550, 132.0444, 106.0651) and betaxanthins (*m/z* 211.0713, 194.0448, 150.0550, 132.0444, 106.0651) confirmed the identify of these pigments, which are consistent with the typical fingerprinting for betalains described by Araujo-León *et al.* (2024b). It is not surprising that *A. palmeri* contains a low concentration of betalains compared to other species of *Amaranthus* with green inflorescences, such as *A. hybridus* L. and *Amaranthus lividus* L. (Khanam *et al.* 2012, Araujo-León *et al*. 2024a, Sarker *et al.* 2020a),

but it strongly suggests that *A. palmeri* is provided with a pathway for betalain biosynthesis. Analytical standards, with the current betanin, will allow for absolute quantification of betalains in *A. palmeri* and other plants containing betalains in the future.

The impact of polyphenols and betalains from species of the genus *Amaranthus* as antioxidants and against enzymes related to metabolic syndrome has gained attention. For instance, inhibiting α-glucosidase and α-amylase enzymes that is a straightforward approach to antidiabetic treatment by aqueous leaf extracts and petroleum ether leaf extracts fraction of *A. viridis* L. and aqueous extract of *A. lividus* L. (Jin *et al.* 2013, Hasbal-Çelikok *et al.* 2022). *A. spinosus* L. along with wild edible plants from the Himalayas have displayed strong antioxidant activity and inhibitory activity against α-glucosidase (Gupta *et al.* 2024). Antidiabetic activity has also been found in *A. tricolor* L. extracts and is thought to be due to phenolic compounds such as flavonoids and tannins, but the specific mechanisms of action still need further research (Zanzabil *et al.* 2023). Besides the leaf extracts, the inflorescence extracts of *A. cruentus* L. have been linked to reducing the α-glucosidase enzyme rate and have shown positive effects on postprandial glycemia regulation in normoglycemic murine models (Araujo-León *et al.* 2024a). Metabolomic analyses identified flavonoids such as quercetin, kaempferol, catechin, hesperetin, rutin, and naringenin, and cinnamic acid derivatives as potential contributors to this activity (Araujo-León *et al.* 2024a). The ratio of betalains to flavonoids content being two orders of magnitude in the inflorescence when compared to the leaves suggests that betalains may also play a role in the postprandial glycemia response in normoglycemic murine models (Araujo-León *et al*. 2024a). Despite the ratio of betalains to flavonoids found in *A. palmeri* was one order of magnitude in the inflorescence when

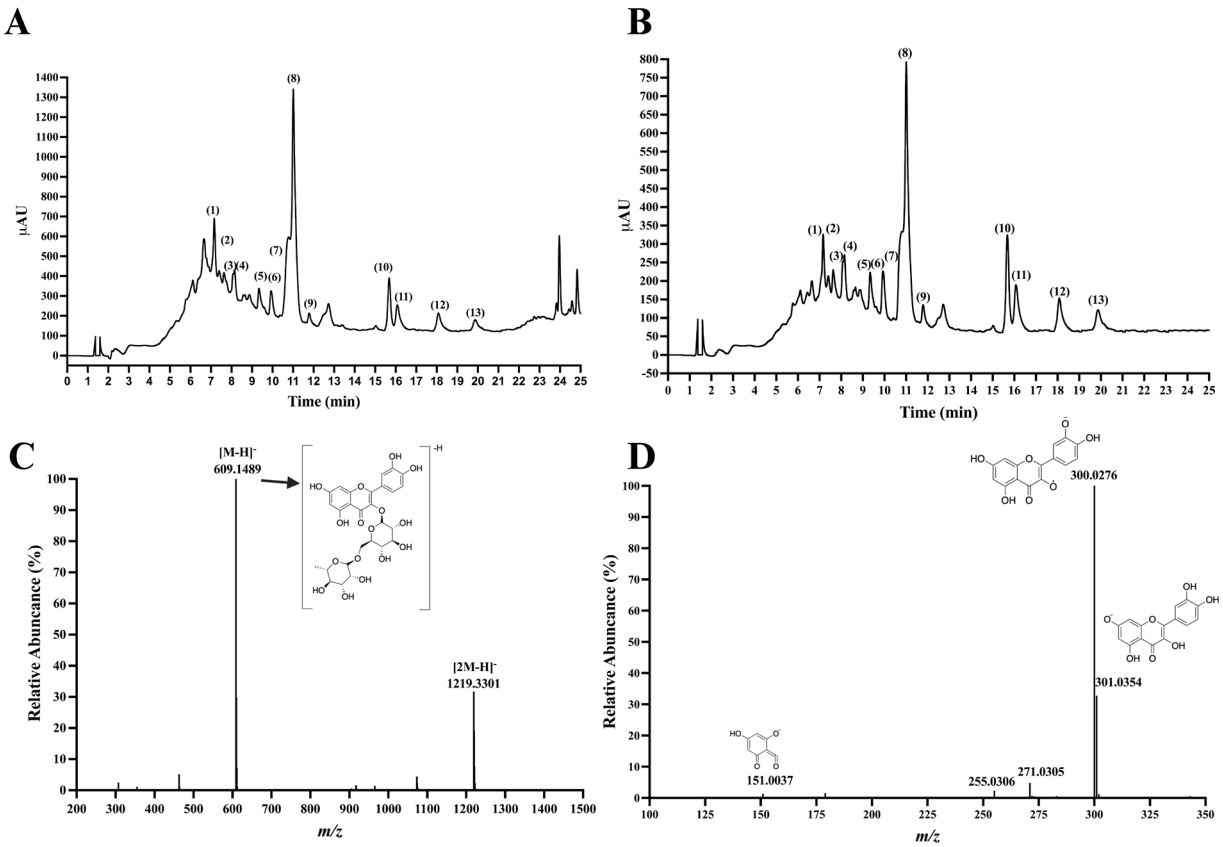


Figure 5. Chromatograms at 230 nm of inflorescence (A) and leaves (B) of *A. palmeri.* MS1 mass spectrum of rutin (C). MS2 mass spectrum of rutin shows product ions produced 80 eV in HCD (D). The annotations in the chromatograms are (1) catechin, (2) 4-hydroxybenzoic acid, (3) caffeic acid, (4) vanillic acid, (5) *p*-coumaric acid, (6) ferulic acid, (7) naringin, (8) rutin, (9) hesperidin, (10) quercetin, (11) naringenin, (12) hesperetin and (13) kaempferol.

compared to the leaves, the profile of metabolites was characterized by a lower abundance of betalains and high abundance of the flavonoid rutin 25,299 µg/g in leaves and 9,809 in inflorescences. Under our evaluated conditions for the activity of extracts at 1 mg/mL, we found that *A. palmeri* extracts explain nearly 90 % of value obtained for the ascorbic acid in the DPPH assay and ~60-78 % of the value obtained for acarbose in the α-glucosidase and α-amylase enzymes assay. An analysis of leaf and inflorescence extracts has been shown that relaxing *ex vivo* aortic mice specimens, which indicates a possible vasodilatory effect, is accompanied by inhibiting of ACE by the presence of flavonoids such as catechin, rutin, and quercetin, with high affinity of catechin for the ACE active (Araujo-León *et al*. 2024a). Under our evaluated conditions for the activity of extracts at 1 mg/mL, we found that *A. palmeri* extracts explain ~65–77 % of the values for captopril in the ACE enzyme assay (Henda *et al*. 2013). Despite the unknown effective molarity for *A. palmeri* extracts, our findings are in line with the predicted affinity of flavonoids and betalains with α-glucosidase and α-amylase enzymes (Araujo-León *et al*. 2024a). To support these hypotheses, it is necessary to conduct more detailed studies (Visvanathan *et al*. 2024).

Outside genus *Amaranthus*, the phytoconstituents associated with antioxidant activity and to target enzymes related to metabolic syndrome include rutin, quercetin, chlorogenic acid, and catechin in Brazilian native fruits (Gonçalves *et al.* 2010), rutin, gallic acid, and protocatechuic acid in *Tithonia diversifolia* (Hemsl) A.Gray., *Parkia biglobosa* Benth, and *Crossopteryx febrifuga* Benth (Tamfu *et al.* 2022), rutin and 8-prenylquercetin in *Desmodium caudatum* DC. (Zhang *et al.* 2022), gallic acid, neohesperidin, hydroxybenzoic acid, resveratrol, rutin, trans-cinnamic acid, quercetin, and flavones in poppy vinegar (Yıkmış *et al.* 2024), and up to 25 flavonoids including rutin

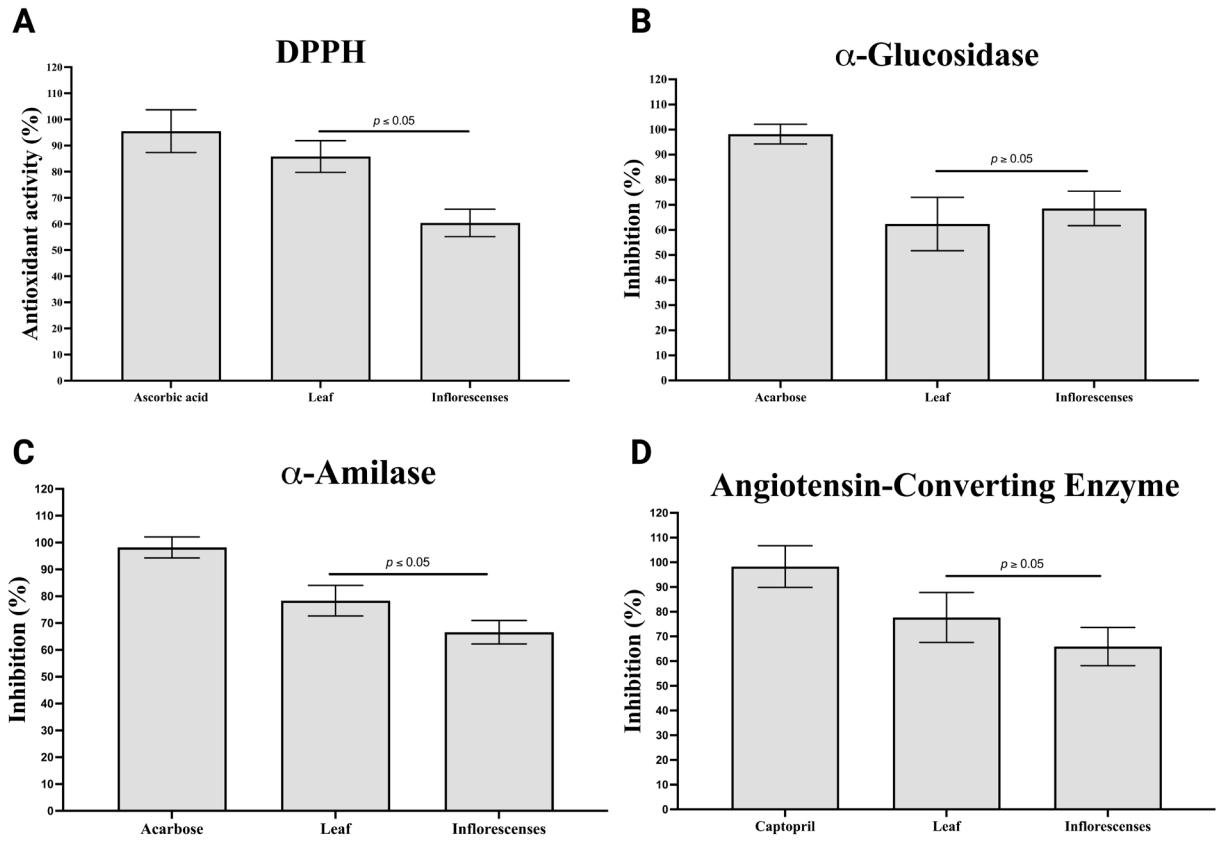


Figure 6. Bioactivity of *A. palmeri* leaf and inflorescence methanolic aqueous extracts. (A) DPPH radical scavenging activity; (B) α-glucosidase inhibition assay; (C) α-amylase inhibition assay; and (D) angiotensin-converting enzyme (ACE) inhibition assay. The extracts of the leaf and inflorescence were assayed at 1,000 µg/mL. Mean ± SD (n = 3). Leaf–inflorescence contrasts by two-tailed Student's *t*-test, α = 0.05.

and quercetin are able to decrease the rate of α-glucosidase, pancreatic lipase, and ACE enzymes (Pham *et al.* 2014, Wang *et al.* 2024).

Amaranthus palmeri has an inherent resistance to herbicides and this is explained in part for the presence of the diverse phytochemical constituents. An analysis of the metabolites present in resistant and sensible biotypes of *A. palmeri* after treatment with the herbicide glyphosate has shown high antioxidant activity linked to the presence of compounds derived from shikimic acid, amino acids, organic acids, and sugars (Maroli *et al*. 2015). Besides accumulating phenylpropanoids, which allows for the high antioxidant potential of *A. palmeri*, obviously stress-specific metabolites induced by glyphosate lead to the accumulation of flavonoids, including glycosides of dihydroxylated and methoxylated flavanols and ferulic acid derivatives (Sandhu *et al.* 2023, Zulet-Gonzalez *et al*. 2023).

Our results on the accumulation of flavonoids and organic acids in *A. palmeri* confirm that this species is a great source of metabolites, which can be further exploited for biotechnological purposes and considered for their use in the benefit of human health. Particularly, because it might be possible to have a dual impact on the antioxidant potential and enzymes related to metabolic syndrome by the flavonoids rutin and quercetin of *A. palmeri*.

Acknowledgements

Sánchez-del Pino I., Peraza-Sánchez S.R. and Aguilar-Hernández V. thank CONAHCYT for support under the project FORDECYT-PRONACES-15319/2020. Araujo-León J. A. thanks CONAHCYT for PhD scholarship 800585.

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Associate editor: Elihú Bautista

Author contributions: JAAL, conceptualization, formal analysis, investigation, methodology, and writing original draft. ISP, conceptualization, funding acquisition, and writing-review & editing. ROA, methodology, resources, and investigation. ACL, methodology, resources, and investigation. LGBA, methodology, and validation. SRPS, investigation, and data curation. AXL, methodology, investigation, and data curation. VAH, conceptualization, formal analysis, resources, investigation, supervision, and writing-review & editing. All authors have read and agreed to the published version of the manuscript.

Supporting Agencies: This research was funded by CONAHCYT/SECIHTI, grant FORDECYT-PRONACES-15319/2020.

Conflict of interest: The authors declare that there is no conflict of interest, financial or personal, in the information, presentation of data, and results of this article.