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Cahuiche (*Vaccinium leucanthum* Schltdl.): A berry with antioxidant, antihypertensive and antibacterial potential

*Javier Castro-Rosas[1], Carlos Alberto Gómez-Aldapa[1], Edna Zaranne Martinez-Ramirez[2], Netzahualcoyol Calcáneo-Martínez[1], Reyna Nallely Falfán-Cortés[1,3]**

[1]*Autonomous University of Hidalgo State (UAEH), Pachuca, Hidalgo, Mexico*

[2]*Technological Institute of the Western of the State of Hidalgo (ITSOEH), Mixquiahuala de Juárez, Hidalgo, Mexico*

[3]*Researcher for Mexico, National Council of Humanities, Sciences, and Technologies; (CONAHCYT), Mexico City, Mexico*

**Corresponding author: reyna_falfan@uaeh.edu.mx*

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Abstract: The aim of this research was to quantify the bioactive compounds; antioxidant, antibacterial, and antihypertensive capability of different cahuiche (V*accinium leucanthum* Schltdl.) extracts *in vitro*. Aqueous, ethanolic, and methanolic extracts were obtained from cahuiche berries (*Vaccinium leucanthum* Schltdl.). Total phenols, flavonoids (quercetin and catechin), and anthocyanins contents were determined, and antioxidant activity was also determined using ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid] and DPPH (2,2-diphenyl-1-picrylhydrazyl), antibacterial activity, and antihypertensive potential, using angiotensin I-converting enzyme (ACE) inhibitor activity (%) *in vitro*. Methanolic extract showed the highest total phenols (1187.88 ± 87.48 mg gallic acid equivalents), catechin (372.17 ± 35.23 mg catechin equivalents), anthocyanins (578.89 ± 38.5 mg cyanidin-3-glucoside equivalents), ABTS (98.26 ± 0.00%) and DPPH (44.06 ± 3.95%) antioxidant activity, and ACE inhibitor activity of 80.20 ± 2.46%. All extracts presented antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *Listeria monocytogenes* (ATCC 19115) with inhibitor zones of > 11 and 13 mm, respectively. Cahuiche berries are potentially an ideal food or functional ingredient due to their biological activities as natural antihypertensive and antibacterial agent, and high bioactive compound contents.

Keywords: *Vaccinium*; ACE inhibitors (%); antioxidant activity; antibacterial activity

According to Hancock et al. (2003), the *Ericaceae* family includes around 13 genera of shrubs and heaths, including *Vaccinium*, the most relevant genus, whose juicy berries are produced and consumed locally. *Vaccinium* includes 450 species, approximately, among which the most important are *Cyanococcus, Oxycoccus, Vitis-Idaea, Myrtillus*, and *Vaccinium.* In the 20th century, three *Vaccinium* fruit crops (blueberry, cranberry, and lingonberry) were domesticated, however, other *Vaccinium* species show a great potential as new crops (Song and Hancock 2010). *Vaccinium* fruits are considered to promote health due to their nutritional and therapeutic properties, marked by relatively high levels of phytonutrients and biologically active compounds, antioxidants, and anti-inflammatory capacity with possibility of success when obtaining herbal medications and nutritional supplements (Tundis et al. 2021). Polyphenolic compounds, mainly from *V. mirtillus* and *V. macrocarpon* fruits, have been widely studied from a pharmacological stand-

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point across species because of their antioxidant and antimicrobial effects in urinary infections. Proanthocyanidins (PAC) from these fruits have been proven to interfere with fimbrial adhesions of uropathogenic *Escherichia coli* (Abreu-Guirado et al. 2008; Tundis et al. 2021). The *Vaccinium leucanthum* Schltdl. tree (also known as cahuiche, huicapol, huicapola, and xoxocotzi) reaches 6 m in height and 20 cm in diameter (Lascurain et al. 2010). It grows in the wild, and its reddish-brown bark is finely fissured; its leaves are leathery, simple and alternate, sharp, and lanceolate-oblong to widely oval. They measure 2–4.5 cm in length and 12–16 mm in width, with crenated, serrated margin. Its flowers are white, grow in cluster, measure 2.5–3 mm in length and are jug-shaped. The fruits are round to globulous, measuring 5 mm in diameter, red to dark purple when ripe, and are found in Hidalgo, Puebla, Veracruz, Chiapas, Oaxaca, and Guerrero in Mexico (Lascurain et al. 2010; Sánchez-Franco et al. 2019). There is scarce research on cahuiche (*V. leucanthum* Schltdl.) reporting that the fruit shows functional properties due to its dietary fibre content, phenolic compounds, and antioxidant capacity (Sánchez-Franco et al. 2019; Bernal-Gallardo et al. 2024; García-Mateos et al. 2024). They conclude the berries constitute a relevant source of fibre, carbohydrates, phenolic compounds, and antioxidant activity, over *Vaccinium corymbosum* (blueberry), a more commercial fruit of the same species. No works have been found to report the antibacterial activity against food pathogens nor the antihypertensive action of *V. leucanthum* Schltdl. although the potential health benefits make these berries a key object of study. Therefore, the aim of this research was to quantify the bioactive compounds and antioxidant, antibacterial, and antihypertensive capacities of different *Vaccinium leucanthum* Schltdl. extracts through assays *in vitro*.

MATERIAL AND METHODS

Cahuiche (*Vaccinium leucanthum* Schltdl.) berries were purchased from the local market in Omitlán de Juárez, Hidalgo, Mexico (2 410 m a.s.l.) in February 2023. Although the fruit was not collected directly from the wild, its identification as *V. leucanthum* is consistent with previous taxonomic characterisations by Sánchez-Franco et al. (2019) and García-Mateos et al. (2024), the latter of which included a confirmed voucher specimen. The fruit is traditionally harvested from wild populations in Omitlán and surrounding municipalities such as Huasca de Ocampo and traded in local markets. While the precise origin of individual lots may vary, these areas share similar ecological conditions and are both recognised as native habitats of *V. leucanthum* (García-Mateos et al. 2024). For the purpose of this study, which focused on the biochemical characterisation of the species rather than geographic variability, market acquisition was considered a representative and valid approach.

Obtention of extracts. Berries maturity stages (S3) according to Shi et al. (2023) were dried in an air recirculating oven at 40 °C for 24 h, and then ground in a cyclone mill (BW-J20, Nutribullet, China) to obtain a fine, homogeneous powder that was sieved through an 80 mesh. The powder was used to obtain the extracts as follows: 1 g sample (cahuiche powder) was weighed in a 2-mL Eppendorf tube and 1 mL solvent (water, ethanol, and methanol, respectively) was added. The solution was mixed in a vortex mixer (LabDancer, IKA, Germany) for 30 s. The sample was centrifuged at 3 500 × g in a mini centrifuge (MiniSpin plus, Germany) for 15 min. The procedure was repeated three times for three consecutive extractions until reaching an approximate volume of 3 mL extract. The extract (3 mL) was placed in a volumetric flask, which was filled with 10 mL water, according to the method reported by Vargas-León et al. (2018). The flasks were capped, covered with aluminium foil, and stored in a fridge for later analysis.

Content total phenols. Total phenols were determined by a Folin-Ciocalteu's (Merck, Germany) assay, according to the method described by Pękal and Pyrzynska (2014), with modifications. Firstly, a gallic acid (Sigma, China) calibration curve from 0 to 100 ppm was prepared. Eppendorf tubes were filled with 250 µL extract and 625 µL of 10% Folin reagent; they were kept under stirring and left to stand for 3 min. Finally, 500 µL of 7.5% Na_2CO_3 was added. The tubes were stirred again and left standing at room temperature in the dark for 2 h. Absorbance was measured at 760 nm in a spectrophotometer (NanoDrop One UV/Vis, Thermo Fisher Scientific, USA). The corresponding linear regression was applied with the gallic acid curve per 100 g sample [mg GAE·(100 g)$^{-1}$ sample dry base (d.b.)].

Content of total flavonoids. The content of total flavonoids was identified using two aluminium complex formation assays, as reported by Pękal and Pyrzynska (2014) and Vargas-León et al. (2018). The first assay consisted in the reaction between 1 000 µL berry extract with 100 µL of 10% aluminium chloride,

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using a curve of quercetin from 0 to 50 ppm, and absorbance was read at 430 nm in a spectrophotometer (NanoDrop One UV/Vis, Thermo Fisher Scientific, USA). The results were expressed in mg of quercetin (Sigma, China) (R^2 = 0.998 6) equivalents per 100 g sample [mg QE·(100 g)$^{-1}$ sample]. The second assay consisted in the reaction of 1 000 µL berry extract and 100 µL aluminium chloride in alkaline medium (200 µL 1M NaOH), in the presence of 100 µL of 5% sodium nitrate. The mixture was left to react for 5 min in darkness to prevent photooxidation, and absorbance was read at 510 nm in a spectrophotometer, using a curve of catechin (Sigma, China) (R^2 = 0.990 9) from 0 to 50 ppm. The results were expressed as mg of catechin equivalents per 100 g sample [mg CE·(100 g)$^{-1}$ sample d.b.].

Content of total monomeric anthocyanins. Total monomeric anthocyanins were determined by following the method described by Del Carpio et al. (2009), with some modifications. Buffer solutions (1 mL) of potassium chloride, pH 1, and sodium acetate, pH 4.5, were added to two different Eppendorf tubes. Then, 250 µL extract was added to each tube; the mixtures were stirred and left to react for 15 min in complete darkness to prevent photooxidation. Absorbance was read in each solution at 510 (peak absorption wavelength for anthocyanins) and 700 nm in a spectrophotometer (NanoDrop One UV/Vis, Thermo Fisher Scientific, USA) using the corresponding buffer as target.

$$A = \left(A_{510nm} - A_{700nm}\right)_{pH1.0} - \left(A_{510nm} - A_{700nm}\right)_{pH4.5} \quad (1)$$

Final absorbance was calculated with Equation 1.

The value of the absorbance calculated was substituted in the equation below to obtain the concentration of total monomeric anthocyanins.

$$Monomeric\ anthocyanins\ \left(mg \cdot L^{-1}\right) = \\ = A \times MW \times DF \times 1\,000 / \varepsilon \times 1 \quad (2)$$

where: MW – molecular weight; ε – the molar absorptivity (269 000) of the predominant anthocyanin in the sample; DF – the dilution factor (total volume/extract volume).

The result obtained was expressed as milligrams of (anthocyanin) equivalents per 100 g sample d.b.

Antioxidant capacity by ABTS and DPPH. The antioxidant capacity by ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid] of the extract was determined following the method developed by Vargas-León et al. (2018) with modifications. The ABTS radical was obtained after the reaction of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration), which were incubated at room temperature (± 25 °C) in complete darkness for 16 h. Once formed, the radical was diluted with ethanol until the absorbance was 0.7–735 nm (peak absorption wavelength). A curve of Trolox (Sigma-Aldrich, USA, R^2 = 0.992 6) was used as reference antioxidant in concentrations from 0 to 150 ppm. Standard and extracts (50 µL each) were mixed with 1.050 mL ABTS radical (previously formed) in Eppendorf tubes and left to stand for 30 min in complete darkness to prevent radical degradation. Finally, absorbance was measured at 735 nm in a spectrophotometer (NanoDrop One UV/Vis, Thermo Fisher Scientific, USA). Concentration was determined by linear regression, and the results were expressed in mg of Trolox quivalents per 100 g sample [mg Trolox E·(100 g)$^{-1}$ sample d.b.]. Antioxidant capacity by DPPH (2,2-diphenyl-1-picrylhydrazyl) was carried out following the method developed according to the methodology by Brand-Willams et al. (1995) with modifications, which is based on absorbance reduction in radical DPPH to 510 nm by antioxidant substances. A solution of radical DPPH (100 µM) was prepared and dissolved in methanol aqueous (80% v/v). Absorbance was adjusted to 0.70 at a wavelength of 510 nm. A pattern curve of ascorbic acid (Sigma, China, R^2 = 0.992 6) was used with concentrations from 0 to 150 ppm. Standard and extract (50 µL each) were mixed with 1.05 mL DPPH radical in Eppendorf tubes, which were left standing for 30 min. Absorbance was read at 510 nm in a spectrophotometer (NanoDrop One UV/Vis, Thermo Fisher Scientific, USA). The radical scavenging capacity of the extracts was assessed through linear regression. Results were expressed in mg ascorbic acid equivalents per 100 g sample [mg AAE·(100 g)$^{-1}$ sample d.b.].

Antibacterial activity. To obtain extracts for the evaluation of antibacterial activity, 20 g cahuiche powder (d.b.) was weighed and added to 180 mL solvent (water, ethanol, methanol, respectively). The mixture was left standing for 24 h, and the solvent was removed in a rotary evaporator, and two grams of the extract were weighed and dissolved in 18 mL water. To evaluate the antibacterial activity, tubes containing 3 mL trypticase soy broth were inoculated with *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), *Escherichia coli* (O157:H7 E09), and *Salmonella typhymurium* (ATCC

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14028), respectively, and were incubated at 35 °C for 18 h. Cultures were washed twice in sterile isotonic saline solution (ISS) by centrifugation at 3500 × g for 20 min. Pellets were resuspended in sterile peptone water at a concentration of 10^8 colony forming units (CFU)·mL^{-1}. Two decimal dilutions of the cultures were conducted to obtain an approximate concentration of 10^6 CFU·mL^{-1}. Petri dishes were inoculated with 1 000 µL of each pathogen bacteria, and standard method agar was added by plate pouring and distributed evenly. Wells were formed (cavities measuring 5 mm in diameter), and 50-µL aliquots of each extract (aqueous, ethanolic, and methanolic) were placed in them. A negative target with water and a positive one with ethylenediaminetetraacetic acid (EDTA) (0.25 M) were added. Assays were done in triplicate per extract. Plates were incubated at 35 ± 1 °C for 24 h. The diameter of the resulting inhibition zones was measured in mm.

Antihypertensive activity of methanolic cahuiche extract. To measure the antihypertensive activity *in vitro*, angiotensin I-converting enzyme (ACE) inhibition was evaluated according to the method by Hayakari et al. (1978), with some modifications. The method is based on the spectrophotometric read of hippuric acid at 382 nm, formed after the reaction between the hippuryl-histidyl-leucine (HHL) substrate and ACE, both in the presence and absence of an inhibitor. Methanol was discarded from a volume of the extract by evaporations, and the extract was then recovered using deionised water to prevent the solvent effects on the enzymatic activity. The HHL substrate was used at 0.3% (w/v); it was dissolved in a 200 mM potassium phosphate buffer and 300 mM NaCl (pH 8.3). The enzyme (ACE) was prepared at 100 mU and dissolved in deionised water. A reaction was prepared between the enzyme (20 µL), 40 µL buffer, and substrate (100 µL) free of inhibitor, to observe the formation of the product (hippuric acid). The reaction using 40 µL cahuiche extract or control drug (Captopril) was done at the same time for 45 min at 37 °C. The enzyme became inactive once pH was lowered by adding 360 µL 2,4,6-trichloro-S-triazine (TT). The hippuric acid formed during the reaction was suspended with 720 µL phosphate buffers (200 mM). It was kept under stirring and absorbance was read at 382 nm in a spectrophotometer. Finally, inhibition (%) of each sample was determined using Equation 3.

$$\%\ ACE\ inhibitory\ activity = \left(ABScontrol - ABStarget - \frac{ABSsample - ABStargetsample}{ABScontrol - ABStarget} \right) \times 100 \quad (3)$$

where: *ABScontrol* – the absorbance of hippuric acid formed after ACE action on HHL without inhibitor; *ABStarget* – the absorbance of HHL added with TT; *ABSsample* – the absorbance of hippuric acid formed after ACE action on HHL in the presence of inhibitors (extract or captopril); *ABStargetsample* – the absorbance of ACE with HHL and sample previously added with TT.

Statistical analysis. Each determination was performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). The statistical analysis of the determination was carried out using ANOVA and a significance level of α = 0.05 (95%). When statistically significant differences were found, a Tukey's test (α = 0.05, 95%) was conducted. Analyses were done using Statistica v.10.

RESULTS AND DISCUSSION

Content of total phenols. Table 1 shows that the methanolic extract presented the highest phenol concentration as compared against the aqueous and ethanolic extracts (P < 0.05). Due to its intermediate polarity between water and ethanol, methanol is considered an effective solvent, capable of disrupting plant cell structures and facilitating the extraction of a higher yield of phenolic compounds. According to the polyphenol classification proposed by Vasco et al. (2008), when using low [< 100 mg GAE·(100 g)$^{-1}$], medium [100–500 mg GAE·(100 g)$^{-1}$] and high [> 500 mg GAE·(100 g)$^{-1}$] concentrations, cahuiche (*Vaccinium leucanthum* Schltdl.) is an excellent source of phenolic compounds. The results obtained from the methanolic extract are higher than those reported by Sánchez-Franco et al. (2019) and García-Mateos et al. (2024), however Bernal-Gallardo et al. (2024), reported 1 675 mg GAE·g^{-1} d.b. Cranberries (*Vaccinium corymbosum* L.) are considered one of the richest sources of phenolic compounds with reported values between 264.06 ± 20.75 mg GAE·(100 g)$^{-1}$ and 528.15 ± 22.37 mg GAE·(100 g)$^{-1}$ sample, as found by Dragović-Uzelac et al. (2010). On the other hand, according to the results obtained in this research work, cahuiche berries are an excellent source of phenolic compounds and are a promising crop because the plant is affordable and found in the wild. The consumption of cahuiche berries may constitute a health ben-

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Table 1. Composition of cahuiche (*Vaccinium leucanthum* Schltdl.) bioactive compounds per 100 g extract (dry base)

Type of extract	Total phenols (mg gallic acid equivalents)	Flavonoids (mg quercetin equivalents)	Flavonoids (mg catechin equivalents)	Total monomeric anthocyanins (mg cyanidin 3-glucoside equivalents)
Aqueous	922.26 ± 91.12[a]	65.12 ± 7.31[a]	148.00 ± 32.44[a]	182.57 ± 21.47[a]
Ethanolic	922.30 ± 54.92[a]	146.95 ± 6.46[b]	341.95 ± 14.45[b]	374.05 ± 24.08[b]
Methanolic	1 187.88 ± 87.48[b]	78.59 ± 8.38[a]	372.17 ± 35.23[b]	578.89 ± 38.50[c]

[a,b,c]different letters in the same column indicate a statistically significant difference ($P < 0.05$)

efit to humans (Sánchez-Franco et al. 2019) and can be positive for the processing and extraction of phenolic compounds key to the food industry and the production of functional foods.

Content of total flavonoids. Table 1 shows that the ethanolic extract presented the highest concentration of quercetin (146.95 ± 6.46 mg QE, $P < 0.05$). These results are higher than those reported by Sánchez-Franco et al. (2019) and García-Mateos et al. (2024), who found quercetin concentrations of 112.0 ± 3.6 mg QE·(100 g)$^{-1}$ and 116.06 ± 19.91 mg QE·(100 g)$^{-1}$ fruit containing flavonoids in the same variety respectively. No significant differences ($P < 0.05$) were found in catechin contents in ethanolic and methanolic extracts. De Souza et al. (2014) reported total flavonoids of 47.53 ± 2.40 mg CE per 100 g dry fruit in blueberry from the *Vaccinium corymbosum* species whose fruits were harvested when they were physiologically mature. The results obtained in the present work are higher than those reported by the authors above. The concentration of bioactive compounds may vary according to factors, such as pH and acidity of the soil, solar radiation, post-harvest handling, and storage, even when comparing fruits of the same genus and species (De Souza et al. 2014). Flavonoids are beneficial bioactive molecules found in human foods.

Content of total monomeric anthocyanins. Methanolic extract exhibited the highest concentrations of anthocyanins (Table 1) when compared against ethanolic and aqueous extracts ($P < 0.05$). Sánchez-Franco et al. (2019) reported 267.1 ± 7.1 mg cyanidin-3-glucoside (C3GE) per 100 g dry fruit regarding anthocyanin content. The present work found a higher content in cahuiche berries: 578.89 ± 38.5 mg C3GE·(100 g)$^{-1}$ dry sample of the fruit. It must be considered that, although the same genus was studied, there are factors that define changes in the concentration of compounds. According to the literature review by Abreu-Guirado et al. (2008) on the phytochemistry of *Vaccinium* spp., flavonoids are the most widely studied and described family of compounds in the largest numbers of species and cultivars. Among them, the most reports have been conducted on anthocyanin-type pigments: cyanidin, delphinidin, malvidin, petunidin, and peonidin; and hyperoside, epi-catechin, and proanthocyanidins, mainly in the fruit. The results obtained from bioactive compounds and the concentration sequence were total phenols > anthocyanins > flavonoids, and they are similar to those in the report by De Souza et al. (2014) in species like raspberry, Abeywickrama et al. (2016) in cranberry, and Sánchez-Franco et al. (2019), Bernal-Gallardo et al. (2024) and García-Mateos et al. (2024) in *Vaccinium leucanthum* Schltdl. Anthocyanins are polyphenolic compounds in *Vaccinium*, which neutralise free radicals. The latter damage DNA, accelerate aging, and promote the onset of diseases caused by stress, malnutrition and other unhealthy habits. Studies suggest that the consumption of anthocyanins is beneficial to human health; for example, it prevents diabetes and is an adjuvant treatment in islet transplantation; it improves gut health, and prevents cancer, among others (Verediano et al. 2021). Therefore, according to the results obtained and the high concentration of anthocyanins, cahuiche is a potential candidate for use in human diet thanks to its beneficial effects in health.

Antioxidant capacity. According to Table 2, all the extracts showed high antioxidant activity expressed as mg Trolox equivalents by ABTS assay; the aqueous and methanolic extracts presented no significant differences ($P < 0.05$). Inhibition was > 90% in all extracts using the same method, which evidences the high potential of cahuiche as a berry with antioxidant activity. When the activity was measured using DPPH, methanolic extract exhibited the most activity ($P < 0.05$) expressed in mg ascorbic acid, and inhibition reached 44.06% in the same extract. According to Hassimotto et al. (2005), the antioxidant values were classified in high (> 70% inhibition), medium (40–70% inhibition), and low (< 40% inhibition). Per this clas-

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Table 2. Antioxidant activity of (*Vaccinium leucanthum* Schltdl.) per 100 h extract (dry base)

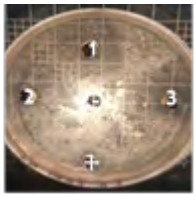
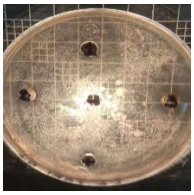
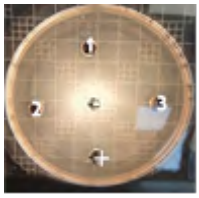
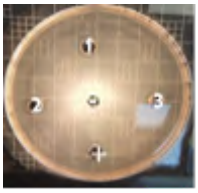
Type of extract	ABTS antioxidant activity (mg Trolox equivalents)	ABTS antioxidant activity [inhibition (%)]	DPPH antioxidant activity (mg ascorbic acid equivalents)	DPPH antioxidant activity [inhibition (%)]
Aqueous	1 398.57 ± 4.12^{a}	98.00 ± 0.23^{a}	556.57 ± 15.78^{b}	29.06 ± 0.85^{a}
Ethanolic	1 316.82 ± 8.36^{b}	93.42 ± 0.46^{b}	264.47 ± 7.89^{a}	13.24 ± 0.42^{b}
Methanolic	1 403.33 ± 0.00^{a}	98.26 ± 0.00^{a}	833.77 ± 72.99^{c}	44.06 ± 3.95^{c}

^{a,b,c}different letters in the same column indicate a statistically significant difference ($P < 0.05$); data expressed as mean ± SD; ABTS – 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid; DPPH – 2,2-diphenyl-1-picrylhydrazyl

sification, methanolic extract shows high antioxidant activity. Similar results were reported by Bernal-Gallardo et al. (2024) and García-Mateos et al. (2024) showing greater antioxidant activity in *Vaccinium leucanthum* Schltdl. as measured by the ABTS and DPPH methods. Then, following the results obtained, cahuiche berries present a marked functional activity, which renders these berries as an affordable alternative to foods with high antioxidant potential.

Antibacterial activity. Table 3 shows the results of inhibition zones (mm) present in cahuiche (*V. leucanthum* Schltdl.*)* extracts against food bacteria *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), *Escherichia coli* (O157:H7 E09), and (ATCC 14028). According to the results obtained from Gram-positive bacteria, inhibition zones were obtained at > 10 mm with the methanolic extract similar to positive control (EDTA), $P < 0.05$); however, there was no antibacterial activity against the Gram-negative bacteria evaluated. Recently Bernal-Gallardo et al. (2024) reported antibacterial effect against the enterobacteria *Salmonella choleraesuis* ATCC12022, *Escherichia coli* ATCC 12792 and *Shigella flexneri* ATCC 10708 with methanolic extracts of *V. leucanthum* Schltdl. Still, there are reports on the antimicrobial activity of *Vaccinium* fruits, which potentially act directly and suppress some microorganisms through several action mechanisms that prevent the development of infectious diseases; for instance, type A (PAC) proanthocyanidins inhibit the growth of some pathogen agents; affect the cytoplasmic membrane and cell permeability; and interfere with microbial metabolism. In addition, they chelate iron, which points at a direct damage of the regular functions in bacteria, leading

Table 3. Inhibition zones (mm) of antibacterial activity in *Vaccinium leucanthum* Schltdl. extracts against pathogen bacteria

Type of extract	Bacteria			
	Staphylococcus aureus (ATCC 25923)	*Listeria monocytogenes* (ATCC 19115)	*Escherichia coli* (O157:H7 E09)	*Salmonella typhymurium* (ATCC 14028)
Aqueous (1)	8.27 ± 0.36^{b}	8.44 ± 2.02^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ethanolic (2)	10.45 ± 1.93^{ab}	9.20 ± 1.16^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Methanolic (3)	13.49 ± 0.96^{a}	11.73 ± 0.33^{ab}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
EDTA(control +)	13.99 ± 3.10^{a}	13.975 ± 4.49^{b}	18.320 ± 5.10^{b}	19.513 ± 2.84^{b}
Water (control –)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Inhibition zones				

^{a,b,c}different letters in the same column indicate statistically significant differences ($P < 0.05$); data expressed as mean ± SD; EDTA – ethylenediaminetetraacetic acid

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to failed adhesion and the formation of biofilms (Jagannathan and Viswanathan 2018). Antimicrobial activity is given by PAC type A and flavan-3-ol polymers in the species *V. macrocarpon*, which affect adhesion and damage motility in bacteria like *Pseudomona aeruginosa* and *Helicobacter pylori* (Zepon et al. 2019). They also alter electrostatic charges (zeta potential) that, in turn, affects the superficial hydrophobicity of microorganisms, preventing bacterial adhesion (Rodríguez-Pérez et al. 2016).

Antihypertensive activity of methanolic cahuiche extract. The methanolic extract of the cahuiche (*V. leucanthum* Schltdl.) sample showed ACE inhibition of 80.20 ± 2.46%, which is likely related to the presence of phenolic compounds previously described. Recently Bernal-Gallardo et al. (2024) reported 42.28 ± 2.4 of ACE inhibition for *V. leucanthum* L. These authors mentioned that the quercetin and chlorogenic acid have been reported to be ACE inhibitors. On the other hand, high blood pressure or hypertension plays a key role in human homeostasis. Therefore, there is a wide variety of drugs that regulate blood pressure, among which are ACE inhibitors. As first-line drugs, they are highly efficient, and their action mechanism is based on the conversion of angiotensin I into angiotensin II, an essential vasoconstrictor in the renin-angiotensin-aldosterone system (García-Donaire 2013). Unfortunately, side effects have been reported due to the use of antihypertensive drugs, such as nausea, loss of appetite, dizziness, and diarrhoea, among others. Then, recent works have focused on plant-source antihypertensive compounds that allow for ACE inhibition (Esquivel-Gutiérrez et al. 2012). In addition to the relevant ACE inhibition found in cahuiche samples, several studies as that by Martínez-Ramírez et al. (2024), have used vegetable samples with inhibitory profile. They evaluated aqueous extracts of *Hibiscus sabdariffa* and hibiscus acid and found inhibition of up to 90%. Some other studies, as the one by Aguayo-Rojas et al. (2024) found that the ACE inhibition in methanolic quince (*Cydonia oblonga* Miller) extracts was 45%, related to the phenolic compound profile evaluated. This places cahuiche fruit as a promising sample with biological activity for future studies on extraction and isolation of antihypertensive bioactive compounds.

CONCLUSION

Cahuiche (*Vaccinium leucanthum* Schltdl.) berries show a great potential for uses in the food and pharmaceutical industries and as functional fruits due to the biological activities found *in vitro*. They include antioxidant, antihypertensive, and antibacterial activity because of large concentrations of bioactive compounds.

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