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Nutritional and nutraceutical properties of wild cranberry: cahuiche (*Vaccinium leucanthum* Schltdl.)

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ABSTRACT

Objective: To determine the physical and chemical characteristics, nutritional and nutraceutical compounds of cahuiche fruits (*Vaccinium leucanthum* Schltdl.) at consumption maturity, harvested in five places of the locality of Ciénega Grande, Omitlán, Hidalgo, Mexico.

Design/methodology/approach: Cahuiche fruits were harvested with simple randomized sampling, and a proximal and mineral, morphological and chemical analysis (sugars, total soluble solids and acidity) was conducted. For the evaluation of the content of nutraceutical compounds (anthocyanins, phenols, flavonoids and total tannins) and antioxidant activity, six solvents were used with the aim of identifying the one of highest extraction yield, except for the evaluation of vitamin C (ascorbic acid, AA).

Results: Cahuiche presented high values (expressed in fresh weight) of Ca (43.24 mg 100 g^{-1}), S (28.61 mg 100 g^{-1}), protein (1.95 g 100 g^{-1}), lipids (1.67 g 100 g^{-1}), and raw fiber (6.67 g 100 g^{-1}); in addition to high concentrations of anthocyanins (267.50 mg EC 100 g^{-1}) and phenolic compounds (407.36 mg EAG 100 g^{-1}). The use of methanol-H_2O-HCl (90:10:1) allowed obtaining the best extraction yields of anthocyanins and total soluble phenols.

Limitations on study/implications: Follow-up studies are needed to evaluate the nutritional value and antioxidant compound content of cahuiche products, since their consumption is mostly processed.

Findings/conclusions: Cahuiche is an underutilized wild cranberry with high potential for its use as a crop, since it is a species with high nutritional and nutraceutical value.

Keywords: extraction solvents, antioxidant activity, phenolic compounds, anthocyanins.

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INTRODUCTION

Vaccinium leucanthum Schltdl is a native tree from Mexico whose edible fruit is an underutilized wild cranberry. This species grows in Mexico's temperate forests, such as those in the state of Hidalgo, where it is used in some of its municipalities (Molina-Mendoza *et al.*, 2012; Wilbur and Luteyn, 2008), such as Huasca de Ocampo and Omitlán de Juárez. This fruit is commonly called cahuiche, and it is part of the ancestral diet of the peoples, since it is used for the elaboration of preserves, sweets, jellies, jams and liquors, as well as an ingredient in various dishes such as adobo, mole and salsa, among others.

However, its consumption is local, without commercial exploitation as in other cranberry species, despite its nutraceutical potential; it could be considered a species with functional properties.

Diverse studies point out that the leaves and the fruits of the genus *Vaccinium* provide benefits to health due to their biological activities, such as antioxidant potential, anti-microbial, anti-inflammatory, anti-cancer activity, and glycemic regulation (Fan *et al*., 2020; Meléndez-Jácome *et al*., 2021). In addition, the wild species of *Vaccinium* produce fruits with higher nutraceutical value, due to their high content of antioxidant compounds compared to cultivated species (Meléndez-Jácome *et al*., 2021).

There are few studies about the yields and the extraction capacity of different solvents on the metabolites responsible for the antioxidant activity in cranberry fruits. Some metabolites can interact with the food matrix components, and may be extracted selectively due to greater chemical affinity with a solvent in particular. The recovery of antioxidant compounds of plant materials is generally achieved through different techniques and extraction solvents, considering the chemical composition and the unequal distribution in the plant matrices (Sultana *et al*., 2009). This is important since it allows establishing appropriate means to evaluate and to quantify antioxidant compounds in *Vaccinium* plant materials, in the most efficient and accurate way.

In this context, Sánchez-Franco *et al*. (2018) reported that the fruits of *V. leucanthum* are an important source of minerals, dietary fiber, protein, phenol compounds, vitamin C and anthocyanins; the latter three metabolites confer to them a high nutraceutical potential. In this study, cahuiche fruits were characterized which were harvested in Huasca de Ocampo, Hidalgo, located at an altitude of 2,100 masl; in contrast, this fruit grows at more than 2,400 masl in Omitlán de Juárez, Hidalgo. These characteristics impact the mineral and phytochemical composition of the fruits, in addition to the genotype, type of soil, environmental conditions (temperature, altitude, radiation, precipitation), nutrition of the plant, season of flowering, degree of maturity at the time of harvest, as well as the post-harvest storage (Zorenc *et al*., 2016). The objective was to evaluate the physicochemical, nutritional and nutraceutical characteristics of the fruits of *Vaccinium leucanthum* harvested in Omitlán de Juárez, Hidalgo; also, to determine the most effective solvent for the extraction of antioxidant compounds (phenols, flavonoids, condensed tannins, and total anthocyanins).

MATERIALS AND METHODS

Biological material

Fruits of cahuiche (*V. leucanthum*), free of disease and visible damage, were randomly collected in state of consumption maturity, in five places of the locality of Ciénega Grande, Omitlán, Hidalgo (20° 11' 41" N, 98° 39' 54" W, 2637 m of altitude), during the fructification in November, 2022.

Taxonomic certification

In each collection site of "cahuiche" fruits, samples were collected from vegetative and reproductive structures (leaves, flowers and fruits) from wild "cahuiche" plants (*V.*

leucanthum) for their taxonomic identification in the Herbarium "Jorge Espinosa Salas", from the Department of Agricultural Preparatory of Universidad Autónoma Chapingo: record number 36228.

Soil characterization of the sampling site

Soil samples were taken in the various fruit collection points, considering a compound sample for the analysis of the physical and chemical quality of the soil. The results were interpreted according to the reference values by Castellanos *et al.* (2000): sandy loam (58.8% sand, 27.3% loam, 13.9% clay), pH 5.98 (moderately acid), apparent density 1.05 g cm^{-3}, 0.16 dS m^{-1} (free of salts), 8.41% organic matter (very high), 22.8 mg of N kg^{-1} (medium or adequate), 3.11 mg of P kg^{-1} (very low), 854 mg of K kg^{-1} (high), 2.96 mg of Ca kg^{-1} (medium or adequate), 546 mg of Mg kg^{-1} (moderately high), 59.93 mg of Fe kg^{-1} (very high), 2.13 mg of Cu kg^{-1} (moderately high), 3.48 mg of Zn kg^{-1} (moderately high), 29.44 mg of Mn kg^{-1} (high), and 1.39 mg of B kg^{-1} (medium or adequate).

Physical and chemical characterization

The fruit weight was determined through an electronic scale (Scout Pro SP2001 Ohaus®, USA). The equatorial diameter (ED) and the length (L) of the fruit were determined using a digital Vernier (INOX IP54 Caliper, Grass Valley, USA), and the rate between L/ED was considered to determine the shape index. The total soluble solids (TSS, °Brix) were evaluated through a digital refractometer (PAL-1 ATAGO®, Japan); the titratable acidity (TA) through the technique described by the Association of Official Analytical Chemists (AOAC, 2005), the content of total soluble sugars through the antrona method described by Witham *et al.* (1971). The rate between TSS/TA was considered to determine the sweetness index.

Nutritional characterization

The percentage of moisture, lipids, raw fiber, ash and total carbohydrates was determined using the methods described by the AOAC (2005). The mineral analysis (P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, B and Mo) was carried out by inductively coupled plasm optical emission spectroscopy (ICP-OES, model 725-ES, Agilent®), prior to acid digestion of multiple elements in microwave. The N content was determined through the combustion method by Dumas AOAC (2005).

Obtaining plant extracts

The extracts were prepared separately from six solvent mixtures: water 100%, acetone at 80%, absolute ethanol, methanol at 80%, methanol/H_2O/HCl (90:10:1), and trifluoroacetic acid ($C_2HF_3O_2$ at 1%).

Quantification of nutraceutical compounds and antioxidant activity

For the quantification of the nutraceutical components and the antioxidant activity, plant extracts prepared with the diverse solvents were used, except for the quantification of vitamin C, where the extracts were prepared according to what was reported by the two methods for their determination.

The quantification of nutraceutical compounds and antioxidant activity was carried out according to the following methods: 1) Total anthocyanins (TAN): the differential pH method described was used; 2) Total flavonoids: it was determined by the colorimetric method proposed by Chang *et al.* (2020); 3) Total soluble phenolic compounds: it was carried out through the Folin-Ciocalteu method; 4) Condensed tannins: it was quantified through the use of the H_2SO_4/vanillin reagent according to what was described by Scalbert *et al.* (1989); 5) Vitamin C: the quantification was done through the volumetric method of the AOAC (2005) and the spectrophotometric method (indophenol-xylene) described by Burdurlu *et al.* (2006); 6) Antioxidant activity: it was determined through the DPPH method (free radical 2,2-Diphenyl-1-picrylhydrazyl), proposed by Brand-Williams *et al.* (1995); ABTS (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), according to that described by Miller *et al.* (1993); and by the method of the ferric reducing ability of plasma (FRAP) method, according to what was described by Benzie and Strain (1996).

Type of study, experimental design and statistical analysis

The experimental unit was one kilogram of fruit per sampling site, obtained from 10 "cahuiche" trees, with five repetitions defined by the sampling site. The physicochemical and nutritional characterization was a study of descriptive nature. For the nutraceutical characterization, an experimental study under a completely randomized design with five repetitions was carried out, where each solvent used for the phytochemical extraction was considered as treatment. All the data were reported as the mean ± standard deviation of five repetitions, values expressed in fresh weight. The data were subjected to analysis of variance, Tukey's means comparison ($P \leq 0.05$), and Pearson's correlation analysis through the Statistical Analysis System software (SAS version 9.2).

RESULTS AND DISCUSSION

According to the morphological characterization, cahuiche fruits are very small round globular berries, given their diameter <7 mm and fruit weight <0.18 g (Table 1), characteristic contrary to what is found in fruits of other cranberry species. According to Frías-Ortega *et al.* (2020), for example, the fruits of *V. corymbosum* L., the most commercial blueberry species in the world, can reach medium (≥12 mm - <16 mm) and large (≥16 mm)

Table 1. Physical and chemical characteristics of cahuiche (*Vaccinium leucanthum*) fruits at consumption maturity, harvested in Ciénega Grande, Omitlán, Hidalgo.

Morphological parameters	Values	Chemical parameters	Values
Fruit weight (g)	0.12±0.06	Total soluble solids (TSS, °Bx)	16.25±1.01
Equatorial diameter (mm)	6.01±1.04	Titratable acidity (TA, % citric acid)	1.49±0.14
Pole diameter (mm)	5.62±0.92	Total soluble sugars (% glucose)	13.51±2.04
Shape index	0.93±0.02	TSS / TA ratio	9.09±1.24

Data are expressed as mean ± standard error of five repetitions. Values reported in fresh weight (fw).

sizes. This could be explained because cahuiche does not receive agronomic management, since it is a wild plant; therefore, it can be subjected to prolonged periods of stress, factor that can condition the size of the fruit, since during the fruit's filling and maturation, the water content is essential to achieve a greater weight gain (Bryla and Strik, 2007).

The total soluble solids (TSS) and titratable acidity (TA) were slightly higher than those found by Sánchez-Franco *et al.* (2018) in *V. leucanthum* (TSS=14.67±0.38 °Bx and TA=1.06±0.02%) cultivated in another municipality of the state of Hidalgo, Huasca de Ocampo. This could be associated with differences in the soil between the two municipalities, since Heeb *et al.* (2005) refer that an increase in sugars and organic acids in fruits that are cultivated in soils with higher content of inorganic nitrogen (NO_3^- and NH_4^+) is common; in this sense, it is important to point out that according to the soil analysis performed, the cahuiche that was harvested for this study grew in soils with adequate nutritional availability of inorganic N for the plant.

The results from the nutritional analysis indicated that cahuiche is a fruit with high contents of carbohydrates, raw fiber, lipids, nitrogen, Ca, Mg and S (Table 2). In this regard, Sánchez-Franco *et al.* (2018) reported only lower values of protein (1.07%), lipids (0.40%) and energetic value (78.77 kcal 100 g^{-1}). Compared to other cranberry species, Karlsons *et al.* (2018) found higher concentrations of P (19.3±2.2 and 16.5±3.3 mg 100 g^{-1}) and K (81.6±6.6 and 110.8±8.9 mg 100 g^{-1}) in fresh fruits of *V. corymbosum* and *V. myrtillus*, respectively, compared to cahuiche. The differences observed between the species of *Vaccinium* reported in other studies could be explained by the different edaphoclimatic conditions of the sites where the plants grew, factors that influenced the nutritional quality of the fruits (Perez-Lainez *et al.*, 2019).

The analysis of nutraceutical metabolites showed that cahuiche is an excellent source of total anthocyanins (TA), total flavonoids (TF) and total soluble phenolics (TSP) (Table 3), as well as vitamin C (volumetric volume: 23.69±3.95 mg EAA 100 g^{-1}; spectrophotometric method: 41.33±6.89 mg EAA 100 g^{-1}). The presence of condensed tannins in the cahuiche fruit was not detected. Cahuiche, in comparison to other berries, presented

Table 2. Nutritional compounds of cahuiche (*Vaccinium leucanthum*) fruits at consumption maturity, harvested in Ciénega Grande, Omitlán, Hidalgo.

Variable	Values	Variable	Values
Moisture (%)	66.84±1.92	Ca (mg 100 g^{-1})	43.24±5.09
Protein (%)	1.95±0.15	Mg (mg 100 g^{-1})	16.09±0.86
Lipids (%)	1.67±0.23	S (mg 100 g^{-1})	28.61±1.35
Ash (%)	0.20±0.02	Fe (mg 100 g^{-1})	0.80±0.08
Carbohydrates (%)	22.67±1.46	Cu (mg 100 g^{-1})	0.13±0.01
Crude fiber (%)	6.67±0.75	Zn (mg 100 g^{-1})	0.15±0.04
Energetic value (Kcal 100 g^{-1})	113.49±7.79	Mn (mg 100 g^{-1})	1.80±0.77
N (mg 100 g^{-1})	250.66±29.27	B (mg 100 g^{-1})	0.30±0.04
P (mg 100 g^{-1})	14.73±1.66	Mo (mg 100 g^{-1})	0.12±0.11
K (mg 100 g^{-1})	37.74±10.90	-	-

Data are expressed as mean±standard error of five repetitions. Values reported in fresh weight (f.w).

Table 3. Nutraceutical compounds in cahuiche (*Vaccinium leucanthum*) fruits harvested at consumption maturity, using six solvents for extraction.

Solvent	TA (mg CE 100 g^{-1} f.w.)	TF (mg QE 100 g^{-1} f.w.)	TSF (mg GAE 100 g^{-1} f.w.)
Water	3.37±0.92 e	46.08±3.58 c	126.21±8.28 c
Acetone 80%	162.84±33.45 cd	98.95±9.89 a	356.61±34.60 ab
Ethanol	135.49±5.90 d	116.06±19.91 a	295.12±32.01 b
Methanol 80%	188.70±33.78 bc	94.08±4.42 a	348.82±31.64 ab
Methanol / H_2O / HCl (90:10:1)	267.50±21.24 a	71.14±8.44 b	407.36±13.59 a
$C_2HF_3O_2$ al 1%	220.84±8.53 b	53.49±3.98 bc	317.78±28.18 b
HSD	46.33	22.83	62.75

Data are expressed as mean±standard error of five repetitions. Values reported in fresh weight (f.w.). Different letters in the same column indicate significant statistical differences (Tukey P≤0.05). HSD: honest significant difference, TA: total anthocyanins, TF: total flavonoids, TSF: total soluble phenolics, CE: cyanidin-3-glucoside equivalents, QE: quercetin equivalents, GAE: gallic acid equivalents.

higher concentrations of anthocyanins (red pigments) and flavonoids, although with values closer to other species of phenolic compounds: *V. corymbosum* (29.72 mg EC 100 g^{-1}, 47.53 mg QE 100 g^{-1}, 305.38 mg GAE 100 g^{-1} and 73.21 mg EAA 100 g^{-1}); other berries such as strawberry (16.03 mg EC 100 g^{-1}, 38.17 mg QE 100 g^{-1}, 621.92 mg GAE 100 g^{-1} and 90.13 mg EAA 100 g^{-1}); raspberry (14.69 mg EC 100 g^{-1}, 9.61 mg QE 100 g^{-1}, 357.83 mg GAE 100 g^{-1} and 92.17 mg EAA 100 g^{-1}), and blackberry (58.61 mg EC 100 g^{-1}, 87.03 mg QE 100 g^{-1}, 850.52 mg GAE 100 g^{-1} and 52.41 mg EAA 100 g^{-1}) (De Souza *et al.*, 2014). Sánchez-Franco *et al.* (2018) reported higher values for cahuiche of vitamin C (102.3 mg EAA 100 g^{-1}), total phenolic compounds (1090.3 mg GAE 100 g^{-1}) and flavonoids (112.0 mg QE 100 g^{-1}), than those found in this study.

Regarding the antioxidant activity, capacity of a substance to inhibit the oxidation of biomolecules (lipids, proteins and nucleic acids) and to avoid the alteration of the cellular functions of the organism, Table 4 shows the antioxidant capacity of the cahuiche fruit determined by three methods. The results showed that wild fruits of *V. leucanthum* have a higher antioxidant capacity than blueberry (*V. corymbosum*) (588 μM TE 100 g^{-1} p.f.), cherry (883 μM TE 100 g^{-1} p.f.), raspberry (627 μM TE 100 g^{-1} p.f.), strawberry (787 μM TE 100 g^{-1} p.f.), and blackberry (1323 μM TE 100 g^{-1} p.f.) by the ABTS method (De Souza *et al.*, 2014). Sánchez-Franco *et al.* (2018) reported lower antioxidant activity by the DPPH (1.29 mM TE 100 g^{-1} p.f.) and ABTS (1.03 mM TE 100 g^{-1} p.f.) methods in cahuiche collected in another municipality of Hidalgo.

In general, the differences found in the contents of nutraceutical components and antioxidant activity between the various studies of cahuiche and other species of berries, could be because of the genetic variability between species and genotypes, state of maturity, edaphoclimatic conditions of the place of origin, as well as the techniques for extraction, preparation and analysis of these metabolites (Perez-Lainez *et al.*, 2019).

In most of the extracts, the antioxidant activity observed in cahuiche was higher with the ABTS method (Table 4). According to Floegel *et al.* (2011), the differences found in the antioxidant activity determined by various methods can be due mainly to the polarity of the antioxidants present in the matrix of the fruit and the chemical action

Table 4. Antioxidant capacity of cahuiche (*Vaccinium leucanthum*) fruit pulp harvested at consumption maturity, using six solvents for the extraction.

Solvent	AA by DPPH (mM TE 100 g^{-1})	AA by ABTS (mM TE 100 g^{-1})	AA by FRAP (mM TE 100 g^{-1})
Water	0.63±0.15 e	1.21±0.08 d	0.65±0.15 d
Acetone 80%	2.71±0.32 ab	6.39±1.09 a	3.80±0.63 bc
Ethanol	2.51±0.35 b	4.71±0.84 b	3.35±0.69 c
Methanol 80%	2.82±0.21 a	6.33±0.62 a	4.03±0.47 b
Methanol / H_2O / HCl (90:10:1)	2.04±0.23 c	3.30±0.40 c	5.13±0.16 a
$C_2HF_3O_2$ al 1%	1.77±0.13 d	3.10±0.21 c	3.76±0.38 bc
HSD	0.21	0.72	0.64

Data are expressed as mean±standard error of five repetitions. Values reported in fresh weight (f.w). Different letters in the same column indicate significant statistical differences (Tukey $P \leq 0.05$). HSD: honest significant difference, AA: antioxidant activity, ET: trolox equivalents.

foundation of each of the methods. The ABTS assay is based on the generation of a free radical that allows quantifying this biological activity both in hydrophilic and lipophilic antioxidant systems, so it has been reported that this method in most of the fruits allows detecting higher antioxidant capacity; in contrast, the DPPH assay is the most effective one for hydrophobic systems. On the other hand, the FRAP method acts only by the transference mechanism of an unpaired electron, while ABTS acts by transference of an unpaired electron as well as the transference mechanism of a hydrogen atom (Fonseca-García *et al.*, 2014).

Regarding the solvent for extraction, it has been described that ethanol is the most effective solvent to recover phytochemicals, specifically anthocyanins (Nistor *et al.*, 2021); however, in this study the use of ethanol only allowed extracting 49% less of anthocyanins compared to the use of methanol/H_2O/HCl (90:10:1), mixture that allowed obtaining the best extraction yields of anthocyanins and total soluble phenolics (Table 3), and the highest value of antioxidant capacity by the FRAP method (Table 4), nearly double compared to other methods.

Pearson's correlation analysis (Table 5) indicated that: 1) anthocyanins presented a positive correlation with the high antioxidant activity detected by FRAP in those extracts where CH_3OH/H_2O/HCl was used as a solvent; 2) the highest antioxidant activity detected by the ABTS assay in acetonic extracts was highly correlated to the presence of total soluble phenolic compounds, since this solvent allowed extracting higher amounts of these metabolites (Table 4), in concentrations that are statistically equal to those obtained through extraction with methanol/H_2O/HCl (90:10:1) and methanol at 80% (Table 3); and 3) through the DPPH assay, the highest antioxidant capacity was observed in methanol extracts at 80%, strongly associated to the higher concentration of flavonoids compared to other extracts (Table 4). This brings to light the importance of continuing to study the effect of the solvent in the evaluation of the antioxidant capacity of foods through various methods such as those evaluated here, since each food is a complex and different matrix; also, the polarity of each solvent can affect the transference of electrons and the transference

Table 5. Pearson's correlation coefficients between the phytochemical compounds and antioxidant activity for the various solvents used for the extraction of nutraceutical compounds of cahuiche (*Vaccinium leucanthum*).

Variable	H_2O	Acetone 80%	Ethanol	CH_3OH 80%	CH_3OH / H_2O / HCl	$C_2HF_3O_2$ 1%
DPPH/TA	0.29	0.73	0.03	0.68	−0.13	−0.52
DPPH/TF	0.76	0.57	0.24	0.99**	0.66	−0.99**
DPPH/TSF	0.90*	0.98**	0.47	0.54	0.40	−0.90*
ABTS/TA	0.13	0.74	−0.67	0.44	0.98**	−0.66
ABTS/TF	0.64	0.57	−0.49	0.99**	0.51	0.29
ABTS/TSF	0.81	0.98**	−0.26	0.28	0.75	−0.15
FRAP/TA	0.27	0.48	0.85	0.66	0.99**	0.96**
FRAP/TF	0.74	0.81	0.94*	0.99**	0.60	0.73
FRAP/TSF	0.88*	0.99**	0.99**	0.52	0.81	0.95*

Note: *P≤0.05 (significant); **P≤0.01 (highly significant). TA: total anthocyanins, TF: total flavonoids, TSF: total soluble phenolics. ABTS, DPPH and FRAP: methods for determining the antioxidant activity.

of hydrogen atoms, key aspects in the measurements of the antioxidant capacity in foods (Pérez-Jiménez & Saura-Calixto, 2006).

CONCLUSIONS

Cahuiche is an underused wild cranberry with high potential for its exploitation as a crop, since it is a species with high nutritional and nutraceutical potential whose consumption could provide benefits to the health of the inhabitants where this plant is located, due to its high contents of fiber, protein, Ca, S, lipids, anthocyanins and other phenolic compounds; however, it is recommended to conduct more studies for its use in the food industry and the effect of its transformation on its nutraceutical potential. The solvent used for extraction is determinant to achieve better yields for the quantification and analysis of phenolic compounds, anthocyanins, flavonoids and the measurement of antioxidant activity. The values of antioxidant capacity in *Vaccinium* species should only be compared when the measurements have been carried out by the same solvent method. In cahuiche, the best extraction solvents were methanol-H_2O-HCl (90:10:1), methanol at 80%, and acetone at 80%.

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