Andean Blueberry

Subjects: Biology

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Andean blueberry (Vaccinium floribundum Kunth), also known as mortiño, is a promising wild berry of the family Ericaceae that grows spontaneously in the Andean regions of Ecuador. The demand for these small (~8 mm diameter), black, and round fruits has been increasing due to their antioxidant characteristic, similar to other Vaccinium species, such as cranberry, blueberry, or bilberry, mostly related to the high content of (poly) phenolic compounds.

Keywords: mortiño ; Vaccinium floribundum ; HPLC–MS/MS ; anthocyanins ; antioxidant ; antimicrobial ; toxicity ; zebrafish

1. Introduction

The consumption of berries has been associated with health-promoting effects, such as reductions in the incidence of degenerative and chronic diseases (cardiovascular diseases, type 2 diabetes, and certain types of cancer, among others), mainly due to the presence of bioactive compounds (phenolic compounds, vitamins, and carotenoids), associated with radical scavenging capacity and epigenetic mechanisms [1]. Clinical intervention studies have also shown that phenolic compounds from berries, particularly anthocyanins, are able to improve the profile of inflammatory markers and the total antioxidant status, these effects being more evident with chronic dietary interventions [2].

Andean blueberry (*Vaccinium floribundum* Kunth), also known as **mortiño**, is a promising wild berry of the family Ericaceae that grows spontaneously in the Andean regions of Ecuador. The demand for these small (~8 mm diameter), black, and round fruits has been increasing due to their antioxidant characteristic, similar to other *Vaccinium* species, such as cranberry, blueberry, or bilberry, mostly related to the high content of (poly) phenolic compounds.

The phytochemical evaluation of these fruits is essential to assess their potential health-promoting effects before an intervention study, establishing their characteristics for use in the food, nutraceutical, and pharmaceutical industries. Unlike many other Ibero-American fruits and vegetables, the carotenoid profile of Andean blueberry is basically unknown [3]. The study of carotenoids is very important as they are very versatile compounds with many applications in agro-food and nutricosmetics [4][5]. As far as we know, only few works have published the profile and content of phenolic compounds in *V. floribundum* Kunth evaluated by HPLC–MS/MS [6][7]. These results are varied and influenced by many factors, including differences among varieties, maturity of the fruit, environmental parameters, and pre-/postharvest handling [8]. Aside from phytochemical evaluation, in vitro antioxidant capacities [7][9] and antimicrobial activities [10] have been reported in Andean blueberry. However, further experiments are required before taking a step ahead through in vivo assays and clinical trials. In this sense, the aim of this study was to evaluate the phytochemical profile of *V. floribundum* Kunth from the local market in Machachi (Ecuador) by HPLC–DAD–MS/MS and assess its antioxidant capacity in vitro by $ABTS^{\cdot}$ (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), $DPPH^{-}$ (2,2-Diphenyl-1-picrylhydrazyl) and ORAC (oxygen radical absorbance capacity) methods and its antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, identifying substantial differences with previous reports. In addition, the in vivo toxicity effect by the zebrafish embryogenesis test and the in vivo antioxidant capacity using the zebrafish animal model (thiobarbituric acid reactive substances (TBARS) test) were evaluated for the first time, simulating physiological conditions through an aqueous extract. Additionally, the presence of lectins in Andean blueberry as antinutritional factors were newly investigated. Finally, the bioaccessibility of phenolic compounds was studied after an in vitro gastrointestinal digestion, evaluating also the antioxidant activity in the different phases of digestion, which may ultimate the physiological effect and role of Andean blueberry within the organism. These results make advances in the knowledge about the health benefits linked to Andean blueberry consumption related to bioactivity, bioaccessibility, and safety, being essential before carrying out further in vivo assays and clinical trials.

2. Physicochemical Characterization

Andean blueberry fruits (*Vaccinium floribundum* Kunth) had high water content (~89%) and appropriate size (weight, length, and diameter) within the quality standards for blueberries (Table 1); nevertheless, these parameters are very varied among species and varieties [11][12]. Sugar concentration and pH are important parameters for evaluating blueberry quality. This fruit had low pH (2.6), titratable acidity (TTA) of 1.6%, and high amount of soluble sugars (11.2 °Brix), according to the expected range of pH 2.7–3.8, TTA values between 0.3% and 1.3%, and >11 °Brix reported for other blueberry cultivars, these values also being influenced by environmental and growing conditions [13][14][15].

Table 1. Physicochemical characterization of Andean blueberry (*Vaccinium floribundum* Kunth).

Parameters	Content
Weight (g unit $^{-1}$)	3.5 ± 0.05[1]
Length (cm unit^{-1})	1.75 ± 0.04
Diameter (mm unit^{-1})	8.5 ± 0.75
pH	2.61 ± 0.05
Moisture (%)	88.69 ± 0.08
°Brix	11.17 ± 0.03
Titratable acidity (% citric acid)	1.62 ± 0.00

[1] Mean values of three determinations ± standard deviation (SD). Fresh weight basis.

In this sense, Andean blueberry is a sweet fruit with a pleasant acid flavor that could be consumed not only fresh but also as derived products, such as juice, jam, jelly, or wine, or could be used as food ingredient with potential technological applications, such as antioxidant and dying properties [13][16].

3. Identification and Quantification of Bioactive Compounds

In Andean blueberry fruits, mainly phenolic compounds were detected and one carotenoid was found. The characterization of phenolic compounds of these fruits was performed by the identification of individual compounds by HPLC-DAD-ESI/MS^n (Table 2) and the subsequent quantification using HPLC-DAD (Table 3), revealing a wide range of different (poly) phenols. A total of 16 phenolic compounds were identified following their main ion $[M-H]^-$ (*m*/*z*) and MS^n fragmentation ions.

Table 2. Phenolic compounds detected and characterized tentatively in Andean blueberry samples (*n* = 3) by HPLC-DAD-ESI/MS^n. Compounds were numbered by their elution order.

Peak Number	Rt (min)	DAD λ (nm)	$[M-H]^-$	Fragment Ions (MS^n)	Phenolic Compounds [1]
1	6.0	330	707 (2[M−H]$^-$) 353	191, 179	3-*O*-Caffeoylquinic acid *
2	10.8	330	353	191	5-*O*-Caffeoylquinic acid
3	16.7	280, 520	465	303	Delphinidin-3-*O*-hexoside I
4	18.5	280, 520	465	303	Delphinidin-3-*O*-hexoside II
5	19.6	280, 520	449	287	Cyanidin-3-*O*-hexoside I
6	20.8	280, 520	435	303	Delphinidin-3-*O*-pentoside
7	21.8	280, 520	449	287	Cyanidin-3-*O*-hexoside II
8	23.9	280, 520	419	287	Cyanidin-3-*O*-pentoside
9	26.6	320	335	179, 161, 131	Caffeoylshikimic acid
10	28.7	360	433	323, 179, 161	Caffeic acid derivative
11	33.6	360	463	301	Quercetin-3-*O*-hexoside I

Peak Number	Rt (min)	DAD λ (nm)	$[M-H]^-$	Fragment Ions (MS^n)	Phenolic Compounds [1]
12	35.2	360	463	301	Quercetin-3-*O*-hexoside II
13	37.5	360	433	301	Quercetin-3-*O*-pentoside I
14	39.6	360	433	301	Quercetin-3-*O*-pentoside II
15	41.2	360	433	301	Quercetin-3-*O*-pentoside III
16	42.8	360	447	301	Quercetin-3-*O*-rhamnoside

[1] Identification of phenolic compounds based on the ion $[M - H]^-$ (m/z) in the negative mode, fragment ion (MS^n) data, and retention time compared with standards and reference sources. * Dimeric adduct. Rt: retention time.

Table 3. Phenolic compounds and carotenoids quantified in Andean blueberry (*n* = 3).

Phenolic Compounds	Concentration (µg/g DW)	
Hydroxycinnamic acids		
3-*O*-Caffeoylquinic acid	236.1	± 37.7 [1]
5-*O*-Caffeoylquinic acid	845.5	± 1.25
Caffeoylshikimic acid	35.8	± 1.58
Caffeic acid derivative	273.0	± 40.0
Total	1390.3	± 78.9
Anthocyanins		
Delphinidin-3-*O*-hexoside I	395.7	± 58.5
Delphinidin-3-*O*-hexoside II	274.0	± 50.0
Cyanidin-3-*O*-hexoside I	1963.9	± 140
Delphinidin-3-*O*-pentoside	392.1	± 29.5
Cyanidin-3-*O*-hexoside II	71.1	± 22.3
Cyanidin-3-*O*-pentoside	2289.8	± 327
Total	5386.4	± 567
Flavonols		
Quercetin-3-*O*-hexoside I	849.7	± 25.9
Quercetin-3-*O*-hexoside II	70.0	± 13.9
Quercetin-3-*O*-pentoside I	186.0	± 23.1
Quercetin-3-*O*-pentoside II	45.4	± 2.47
Quercetin-3-*O*-pentoside III	683.5	± 23.5
Quercetin-3-*O*-rhamnoside	219.0	± 25.9
Total	2095.5	± 184
Total phenolic compounds	8875.3	± 787
Carotenoids		
Lutein	5.94	± 1.34

[1] Mean values ± standard deviation (*n* = 3). Hydroxycinnamic acids were quantified as 5-*O*-caffeoylquinic acid equivalents, anthocyanins as cyanidin-3-*O*-glucoside equivalents, and flavonols as rutin equivalents.

Four hydroxycinnamic acids were found, all of them being caffeoyl acid derivatives. Compound **1** was found as an adduct of 3-*O*-caffeoylquinic acid; this dimer is usually formed as an artefact of the mass spectrometry analysis, having a $[2M-H]^-$ adduct ion at *m*/*z* 707 and $[M-H]^-$ ion at *m*/*z* 353, which produced MS^2 ions at *m*/*z* 191 and 179, which evidenced its tentative identification [17]. The 5-*O*-caffeoylquinic acid (**2**) also showed $[M-H]^-$ ion at *m*/*z* 353, and the daughter ion at *m*/*z* 191. Compound **9**, caffeoylshikimic acid, gave its characteristic $[M-H]^-$ ion at *m*/*z* 335 with MS^2 fragmentation peaks at *m*/*z* 179, 161, and 131 [6][7][18]. Finally, compound **10** exhibited $[M-H]^-$ ion at *m*/*z* 433 and gave MS^2 fragmentation peaks at *m*/*z* 323, 179, and 161, being characteristic of caffeoylquinic acid derivatives [19]. This information, along with its characteristic spectrum with absorption at 320 nm, led us to the tentative identification of this compound as caffeic acid derivative, according to previous works analyzing *V. floribundum* [6] and *Vaccinium meridionale* [20].

Compounds **3**–**8** were detected as glycosylated anthocyanin derivatives of delphinidin and cyanidin, with the typical molecular ion at *m*/*z* 303 and 287, respectively, bound to a glucose or pentose, with a loss of 162 or 132 mass units, respectively. This anthocyanin profile agrees with previous works analyzing Andean blueberry [6][7]. Compounds **11**–**16** belonged to the flavonoid family, all of them being derivatives of quercetin, with the typical MS^2 fragment of *m*/*z* 301 and a loss of 162 mass units in case of glucose, 132 due to pentose, and 146 because of the deoxyhexoside rhamnose. Other authors also found quercetin-3-glycosides as the predominant flavonols in this fruit [3]. Additionally, small amounts of two different myricetin derivatives were identified in mortiño berries [4].

The quantification of phenolic compounds (Table 3) showed anthocyanins as the main group present in the samples (~60% of the total phenolic compounds). Among them, cyanidin-3-*O*-pentoside and cyanidin-3-*O*-hexoside I were the predominant anthocyanins (~80% of the total), followed by delphinidin hexosides, accounting for 19%. These results agree with the distribution of anthocyanins described in *V. floribundum* before, showing anthocyanin contents in the range 3–10 mg/g DW, mainly constituted by cyanidin glycosides [6][7][21]. This accumulation of delphinidin and cyanidin-type anthocyanins has been related to the deep purple-black color of berries, these contents being affected by differences in the growth conditions or ripening stage of the fruits [22].

Regarding flavonols, these compounds accounted for 24% of the total phenolic compounds, all of them being quercetin glycosides. The contents of quercetin-3-*O*-hexoside I and quercetin-3-*O*-pentoside III were significantly high, corresponding to 70% of the total flavonols, as reported by You et al. [23].

Finally, hydroxycinnamic acids constituted 15.7% of the total, mainly represented by caffeoylquinic acids, the isomer of the chlorogenic acid 5-*O*-caffeoylquinic acid being the most representative compound (Table 3), according to previous studies showing chlorogenic acid derivatives as the main phenolic acids in *V. floribundum* [6][20].

Diverse contents of phenolic acids (1–3 mg g^{-1} DW) and flavonols (2–4 mg g^{-1} DW) were described before by HPLC in Andean blueberry [6][7][20][23], as several factors may affect the concentration of total phenolic compounds in blueberries, such as agronomic factors, cultivars and varieties, geographic region, storage conditions, ripeness, climate, and others, which are reported in the literature with varied contents of total phenolic compounds in *Vaccinium* sp. (0.5–7 mg g^{-1} FW; ~5–40 mg g^{-1} DW) [6][20][23].

On the other hand, the carotenoid content was studied using a rapid resolution liquid chromatography (RRLC) by comparing the chromatographic UV–VIS spectroscopic characteristics with the standards. Results showed lutein (5.94 µg g^{-1} DW = 0.67 µg g^{-1} FW) as the only carotenoid found in Andean blueberry (Table 3). Recently, other authors showed lutein as the main carotenoid in higher concentrations (8.7 µg g^{-1} FW), but also β-carotene in lower amounts (0.7 µg g^{-1} FW) [9]. On the other hand, only β-carotene (0.4 µg g^{-1} FW) was found in Andean blueberry by Vasco et al. [7]. These differences among Andean blueberry fruits affirm that similar varieties may contain diverse individual and total bioactive compounds depending on factors of different nature, including stage of maturity, variety, harvesting season or production, postharvest processing, and storage conditions, among others [24].

4. Determination of Antinutritional Lectins

The most known plant components with agglutination properties are the varied lectin proteins, which are able to reversibly bind sugar structures in the blood cells [25]. These proteins are few of the well-known antinutrients in plants and can be found in legumes, seed extracts, fungi, and some fruits; however, their presence in berries has been little studied. Lectins may exert different responses in the human body, from allergies and gastrointestinal problems to bioactive effects related to their selectivity to bind carbohydrate residues of glycoproteins as markers in cancer research [26]. Thus, with this assay, the hemagglutination effect of Andean blueberry extract due to the presence of lectins and other compounds was evaluated using five different concentrations of the extract (2.5, 1.25, 0.625, 0.312, and 0.156 mg mL^{-1}). Results showed

no agglutination effect of the extracts (data not shown), revealing the possible lack of the lectin, and therefore the absence of an antinutritional effect, in Andean blueberry fruit.

5. Antimicrobial Activity

Vaccinium spp., such as cranberry, blueberry, and bilberry, have shown bactericidal activity against *S. aureus* and *E. coli*, especially in the prevention of urinary tract infections [26]. This activity was associated with the presence of (poly) phenols, mainly flavonol glycosides, anthocyanins, proanthocyanidins, and flavan-3-ols. In this work, only the highest concentration of Andean blueberry aqueous extract tested in this experiment (10 mg mL^{-1}) exhibited significant antimicrobial effects toward *S. aureus* and *E. coli*, the percentages of bacterial growth inhibition being 30% and 43%, respectively (data not shown). These results agree with those of previous works showing antibacterial activities in the range 25–100 mg mL^{-1} of blueberry, the inhibitory effect being higher for Gram-positive bacteria than for Gram-negative bacteria [27]. The concentration of 10 mg mL^{-1} of Andean blueberry extract used in the experiment suggests a weak antibacterial effect of this fruit.

6. In Vitro Gastrointestinal Digestion

The assessment of total phenolic compound content (TPC) and antioxidant capacity during in vitro gastrointestinal digestion of Andean blueberry allowed us to determine how the digestion process affected the stability and, therefore, bioaccessibility, of the dietary (poly) phenols present in this fruit. This experiment resembles the antioxidant role of these fruits in our gastrointestinal tract, where they may exert important beneficial effects against different pro-oxidants (such as diet components) that have been observed to increase oxidative stress before they are absorbed. Our results showed the presence of phenolic contents in all phases of the in vitro digestion (Table 5), representing the availability of these compounds for absorption in the intestinal epithelium and metabolism. No significant changes in TPC were found during the oral and gastric phases, obtaining a bioaccessibility around 85%–90%. Afterward, TPC was recovered in lower contents (51%–56% bioaccessibility), maybe due to degradation processes of these compounds with the intestinal juice treatment, as they are converted to aglycones and glucuronides in the colon. These results agree with those of other authors who found similar losses of (poly) phenols in the intestinal steps [28][29]. Among phenolic compounds, anthocyanins have been found to experience higher losses during gastrointestinal digestion than flavonols and caffeic acid derivatives, all of them being affected by enzymes, pH levels, and secretions in the digestive tract in real physiological conditions [30]. Once the release of phenolic compounds from their matrix into the intestinal lumen has been studied, the further step would be the study of their transport through the epithelium into the body and their availability to be metabolized and absorbed after reaching the colon.

Table 5. Total phenolic compounds (TPC), bioaccessibility, and antioxidant capacity determined in the initial, oral, gastric, intestinal, and final phases during in vitro digestion.

Gastrointestinal (GI) Phase	Total Phenolic Content (mg GAE g^{-1})	% Loss	% Bioaccessibility	Antioxidant Capacity (µmol Trolox g^{-1})
Initial	**11.27 ± 0.20 [1] a**			**40.53 ± 2.40 b**
Oral	**10.10 ± 0.94 a**	**10**	**90**	**41.67 ± 1.73 b**
Gastric	**9.54 ± 1.09 a**	**15**	**85**	**25.94 ± 2.14 c**
Intestinal	**6.36 ± 0.36 b**	**43**	**56**	**68.67 ± 2.98 a**
Final	**5.74 ± 0.62 b**	**49**	**51**	**63.97 ± 4.79 a**

[1] Mean values (n = 3). Different lowercase letters indicate statistically significant differences among gastrointestinal phases.

Regarding the antioxidant capacity evaluated by the ABTS method, it showed a high decrease in the gastric phase (Table 5), maybe due to the lower chemical reaction of bioactive compounds with acid pH. Afterward, the antioxidant capacity found during the intestinal and final steps of the gastrointestinal digestion (64–69 µmol TE g^{-1}) was significantly higher compared with that found during the initial and oral steps (41–42 µmol TE g^{-1}). This fact could be explained by changes in the structural form of (poly) phenols in the intestine, affected by neutral pH and enzymatic activities, which promote multiple forms of metabolites in the intestinal lumen, such as phenolic acids, resulting in a higher ability to scavenge free radicals [30][31]. Finding phenolic compounds after intestinal digestion showed their availability to be metabolized and absorbed after reaching the colon. Apart from showing antioxidant capacity, (poly) phenols may act as digestive enzyme

inhibitors, affecting the activity of α-glucosidase, α-amylase, and lipase, which may contribute to the control of diabetes type II and obesity, delivering other health benefits attributed to the ingestion of berries as part of the diet [32][33].

7. Conclusions

Andean blueberry is a relevant source of phenolic compounds, mainly anthocyanins, which may be responsible for its high antioxidant capacity. In addition, the freeze-dried extract of Andean blueberry did not show toxicity and could be included in the safe category as a natural ingredient. These characteristics make Andean blueberry suitable to be used as a functional ingredient with potential technological applications in the food industry, such as natural antioxidant or dye, or in the pharmaceutical industry for the development of nutraceuticals. Due to the substantial differences in phytochemical profile among *Vaccinium* spp. and varieties reported in the literature, the identification and quantification of bioactive compounds of Andean blueberry performed in this work is part of the study of this berry as an interesting candidate for the further evaluation of its health benefits through in vivo assays and clinical trials. In this work, the in vitro simulated digestion showed a gradual release of phenolic compounds but a sustained antioxidant activity, increasing the reliability of antioxidant data described for berries. It should be note that further in vivo and clinical studies with Andean blueberry should highlight the real effect of these bioactive compounds in the body, as the absorption and bioavailability could be affected by different interindividual factors.

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