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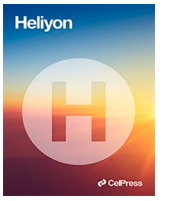

Research article

Chemical composition and antioxidant properties of native Ecuadorian fruits: *Rubus glabratus* Kunth, *Vaccinium floribundum* Kunth, and *Opuntia soederstromiana*

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ABSTRACT

The native berries of South America present promising marketing opportunities owing to their high antioxidant content, notably rich in anthocyanin and phenolic compounds. However, Ecuador's endemic fruits, primarily found in the wild, lack comprehensive data regarding their phytochemical composition and antioxidant capacity, underscoring the need for research in this area. Accordingly, this study evaluated the total phenolic, anthocyanin, flavonoid, resveratrol, ascorbic acid, citric acid, sugars, and antioxidant content of three native Ecuadorian fruits: mora de monte (*Rubus glabratus* Kunth), mortiño (*Vaccinium floribundum* Kunth), and tuna de monte (*Opuntia soederstromiana*). Determination of resveratrol, ascorbic acid, citric acid, and sugars was determined by HPLC analysis, and UPLC analysis was used to determine tentative metabolites with nutraceutical properties. Antioxidant capacity was assessed using cyclic voltammetry and the DPPH method; differential pulse voltammetry was used to evaluate antioxidant power. Analysis of results through UPLC-QTOF mass spectrometry indicated that *R. glabratus* Kunth and *V. floribundum* Kunth are important sources of various compounds with potential health-promoting functions in the body. The DPPH results showed the following antioxidant capacities for the three fruits: *R. glabratus* Kunth > *O. soederstromiana* > *V. floribundum* Kunth; this trend was consistent with the antioxidant capacity results determined using the electrochemical methods.

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1. Introduction

Ecuadorian vegetables and fruits are known for their pleasing taste and unique aromas; they are also excellent sources of water, fiber, minerals, and vitamins, as well as other substances with health-benefitting properties [1–4]. Ecuador's rich variety of fruits is highly sought after in international markets; however, despite their potential, only a small number of them have been recently incorporated into the food industry, with minimal exploitation primarily limited to local artisanal markets. In Ecuador, the export of non-traditional crops increased from USD 3.0 million in 2010 to more than USD 4.3 million in 2020 [5]. The most significant fruit non-traditional crops (primarily mangos, passion fruit, lemons, pineapples, Inca berries and melons) yielded USD 280 million in exports, 6.5 % of total exports [6].

Previous studies have found a direct correlation between health and a fruit-rich diet [7–9]. Certain fruits may be associated with an observable decrease in the risk of diseases potentially caused by oxidative stress, as found with the use of Andean blueberry or "*mortiño*" [10]. The frequent consumption of antioxidant-rich fruit helps prevent cardiovascular and chronic diseases, including cancer, which causes more than six million deaths per year worldwide. Oxidative stress plays an important role in certain types of cancer [11–13], and antioxidants can reduce the induced carcinogenesis through specific antioxidative mechanisms. Further, underutilized fruits and vegetables may serve as potential novel pigment sources, according to the *Handbook on Natural Pigments in Food and Beverages* (pp. 391–418).

South America has a wide variety of native berries with high marketing potential due to their antioxidant power, derived from their high levels of anthocyanin and phenolic compounds. Ecuadorian edible fruits are often only available *in situ* in wild populations, which is the case for those evaluated in this study: *Rubus glabratus* Kunth (*mora de monte* o *mora andina*) and *Vaccinium floribundum* Kunth (*mortiño*), which are considered wild berries, and *Opuntia soederstromiana* (*tuna de monte*), a type of prickly pear. Furthermore, these fruits have special cultural value, and their flavors are essential in many different native recipes.

The Ecuadorian highlands, where the selected fruits grow naturally, encompass extensive areas of land abundant in biodiverse ecologic resources, including unique native fruits and vegetation. In addition, the area's specific attributes, such as the higher levels of solar radiation, have influenced the adaptation of species to combat metabolic stress through the production of secondary metabolites (antioxidants) in greater quantities, which are beneficial for consumers [14].

V. floribundum Kunth, a fruit endemic to the Andes region from Venezuela to Bolivia, can almost exclusively be found either in artisanal markets or wild in its natural habitat [15]. Its purple coloration makes it desirable for the preparation of wines, desserts, and other traditional foods. *R. glabratus* Kunth is currently increasing in commercial value; however, despite its growing popularity and being a rich source of antioxidants like anthocyanin, phenols, and polyphenols, relevant available information is still scarce. Plants from the genus *Opuntia*, including *O. soederstromiana*, thrive throughout the Americas from north to south and are cultivated in diverse climates. *O. soederstromiana* is classified as an endemic fruit of Ecuador [16] and is found specifically in the arid climate of the Ecuadorian highlands, as it has adapted to high levels of hydric stress. Further, *O. soederstromiana* is a non-climacteric fruit and must be harvested in the proper stage of ripening for both research and commercialization purposes [16].

Collecting comprehensive data regarding the phytochemical composition and antioxidant capacity of Ecuador's endemic fruits is crucial, given the scarcity of relevant literature. Accordingly, the current study aimed to evaluate antioxidant activity and total phenolic, anthocyanin, flavonoid, resveratrol, ascorbic acid, citric acid, and sugar content, as well as identify potential metabolites with nutraceutical properties, in three endemic Ecuadorian fruits: *R. glabratus* Kunth, *V. floribundum* Kunth, and *O. soederstromiana.*

2. Materials and methods

2.1. Reagents

The following reagents were used in this study: Folin–Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH, 97 %), acetonitrile high-performance liquid chromatography [HPLC] grade, methanol (HPLC grade, ≥99.9 %), and glacial acetic acid (99.9 %); these were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate, *meta*-phosphoric acid, gallic acid, and potassium hydroxide (analytical grade) were purchased from Merck (Darmstadt, Germany). Tetrabutylammonium hexafluorophosphate was purchased from Acros Organics, (Geel, Belgium). Methanol (analytical grade) and formic acid (HPLC grade) were purchased from Fisher Scientific (Loughborough, UK). Chemical standards for citric acid, glucose, fructose, cellobiose, ascorbic acid, and resveratrol (purity ≥99 %, Sigma-Aldrich, St. Louis, MO, USA) were used to construct the calibration plot.

2.2. Equipment

HPLC with UV–Vis detection (Hitachi LaChrom Elite L-2420 UV–Vis detector) was performed using the Agilent 1260 LC and a Hach DR6000 spectrophotometer; additionally, UPLC with a QTOF-mass spectrometer (Waters, Model Xevo G2 QTOF, USA) was performed with an ACQUITY UPLC BEH C18 column model (2.1 mm × 100 mm, particle size 1.7 μm). An ultrasonic bath (40 kHz, 100 W, Danbury, CT, USA), vortex mixer (Heathrow Scientific Vortex HS), hot plate magnetic stirrer (magnetic stirrer with heater), potentiostat (CH Instruments, Model 70), reference electrode (Ag/AgCl, 3 M KCl) (CH Instruments), freeze dryer (Labogene, CoolSafe Touch 55-4-115v 60 Hz model), analytical balance (Mettler Toledo MS204-S/Z), moisture analyzer (Mettler Toledo HB43–S), ultra-low freezer (Infrico Medcare, LTFU40S), and water purifier (Rephile Genie 5, 110V dispenser) were also used.

2.3. Collection and treatment of fruit samples

Fruits were obtained in February and March 2022, which correspond to the growing season when fruit is traditionally harvested and consumed in Ecuador. *V. floribundum* Kunth *and R. glabratus* Kunth were purchased at their optimal ripeness stage from a popular market in La Esperanza (0.2070587, latitude-78.0919395, longitude), a rural town in Ibarra canton, Imbabura province. For both species, the fruit was an intense purple color (Figs. S1A and C), which indicates the proper state of maturity; fruits weighed 0.12 ± 0.02 g/fruit and 0.17 ± 0.03 g/fruit, respectively. *O. soederstromiana* (Fig. S1B) was purchased from a popular market in Ambuquí (0.4246116, latitude −77.9633976, longitude). The *O. soederstromiana* fruits were light red in color (indicating a proper state of maturity) and weighed 57 ± 0.25 g/fruit. All samples were collected on three separate occasions (minimum of 500 g) at one-week intervals. For *O. soederstromiana*, five fruits were considered one replicate, 60 for *R. glabratus* Kunth, and 30 for *V. floribundum* Kunth.

The collected samples of each fruit were thoroughly washed in sterile distilled water and a commercial germicide containing acetic acid (5 % wt/wt) and hydrogen peroxide (3 % w/wt). Fruit samples were frozen immediately after purchase for 24 h at −22 °C and then stored at −80 °C until lyophilisation. Fruit samples were freeze-dried for 48 h at −22 °C and 0.150 hPa. Freeze-dried samples were stored at −22 °C until analyzed within three weeks. The lyophilized samples were manually crushed using a ceramic mortar and pestle into a fine powder to perform the required analysis.

2.4. Preparation of ethanolic extracts

Ethanolic extracts according to the procedure established by Stafussa et al. [17], were obtained. A total of 2 g of each sample was added to a 20 mL ethanol/water solution (1:5 v/v) and magnetically stirred for 120 min at room temperature (21°C) in darkness. The supernatant was separated by centrifugation at 5000 rpm, for 10 min and then kept under refrigeration (4 °C) in brown containers.

2.5. Antioxidant properties

2.5.1. Determination of antioxidant capacity by DPPH assay

The antioxidant capacity of each ethanolic fruit extract was determined by DPPH assay, as described by Stafussa et al. [17]. The reaction mixtures were prepared in 5 mL brown glass vials containing 2.75 mL of ethanol +1.75 mL of DPPH (5 % v/v ultrapure water) + 0.5 mL of ethanolic extract. The vials were kept in darkness for 30 min to facilitate the reaction. Readings were taken at an absorbance of 517 nm. To construct the calibration plots, AA (ascorbic acid) standard solutions in concentrations from 0.88 to 28.11 μM were used. The results were recorded in AA equivalent μmol per 100 g (AAE 100 g^{-1}). A higher value of AAE 100 g^{-1} indicates greater antioxidant capacity.

2.5.2. Superoxide anion assay by cyclic voltammetry

For the cyclic voltammetry (CV) analysis, a potentiostat was used with an electrochemical cell (10 mL) consisting of three electrodes: a glassy carbon-working electrode (3 mm in diameter), a graphite counter electrode, and a reference electrode (Ag/AgCl, 3 M KCl).

Analysis was performed according to Pérez-Cruz et al. [18]; cyclic voltammograms were done using a scan rate of 100 mV s^{-1} and a potential window from 0 to −1.2 V (*vs.* Ag/AgCl). The superoxide radical anion ($O_2^{\bullet-}$) was generated *in situ* in the electrolytic media of air-saturated dimethylsulfoxide (C_2H_6OS) using tetrabutylammonium hexafluorophosphate ($C_{16}H_{36}F_6NP$) as the supporting electrolyte. Before each measurement, the working electrode was polished using alumina in decreasing granulometry (0.3, 0.1, and 0.05 μm, respectively) and then washed and rinsed in distilled water under ultrasound conditions for 1 min [19–21].

According to Pérez-Cruz et al. [18], the relative decrease of the anodic peak in the respective voltammograms is expressed using the dimensionless parameter $[(Ip_a^0\text{-}Ip_a^s)/Ip_a^0]$, where Ip_a^0 is the peak current in the oxidative scan without the substrate in the medium (ethanolic fruit extract), and Ip_a^s is the peak current in the oxidative scan with the fruit extract. Using this procedure, the electrochemical index (AI_{50}), defined as the concentration necessary to reduce the anodic current peak by 50 %, was calculated. This value was determined by its interpolation over the center of the resulting line of a plot of the dimensionless parameter $[(Ip_a^0\text{-}Ip_a^s)/\ Ip_a^0]$ *vs.* the concentration of each fruit extract (ppm). A higher AI_{50} value means less antioxidant capacity.

2.5.3. Determination of electrochemical index by differential pulse voltammetry

Differential pulse voltammetry (DPV) was conducted using the same electrochemical assembly as in the previous determination. The analysis was carried out in a 0.1 M buffer acetate electrolytic solution (pH = 5), using a pulse width of 0.005 s, potential window of −1.2 to 1.6 V *vs.* Ag/AgCl, pulse amplitude of 0.5 s, and a scan rate of 17 mV s^{-1}.

Using the described procedure, the electrochemical index (EI) was calculated with Equation (1). According to previous studies, EI is directly proportional to antioxidant power [22].

$$EI = Ip_{a1}\ E_{pa1}^{-1} + Ip_{a2}\ Ep_{a2}^{-1} + \cdots + I_{pan}\ E_{pan}^{-1}, \quad (1)$$

where I_{pan} is the respective current of the anodic peak in the voltammogram, and E_{pan} is the respective potential of the anodic peak in the voltammogram.

2.6. Phytochemical analysis

2.6.1. Determination of total phenolic content

Quantification of total phenolic content (TPC) in each fruit extract was performed using the Folin–Ciocalteu method, according to the methodology established by Stafussa et al. [17]. The reaction mixture for the analysis was carried out into 10 mL volumetric flasks using 7 mL of ultrapure water +0.5 mL of Folin–Ciocalteu (FC) reagent **(**5 % v/v**)** + 0.1 mL of fruit extract (prepared in: 2.3). After 3 min, 1.5 mL of sodium carbonate was added, and the volume of the mixture was adjusted to 10 mL with ultrapure water. The absorbance was measured at 765 nm, and a gallic acid equivalent (GAE) calibration plot of 1.54–5.63 μg mL^{-1} was used for quantification. The results were recorded in mg GAE 100 g^{-1} of fruit.

2.6.2. Determination of content of anthocyanin and flavonoid

The extractions were performed by adding 2 g of the lyophilized fruit to a 1.5 N HCl solution in 85 % ethanol, maintaining a ratio of 1:25 (w/v). The extracts were left for 24 h in refrigeration (4 °C) in darkness. The extracts were subsequently filtered using a 45 μm filter, and the anthocyanin and flavonoid content were analyzed using UV–Vis spectroscopy at wavelengths of 535 nm and 374 nm, respectively.

Anthocyanin and flavonoid content were calculated using Equation (2) and the respective absorption coefficients of 982 and 766 $(g/100\ mL)^{-1}\ cm^{-1}$:

$$Anthocyanin\ content\left(\frac{mg}{100g}\right)=\frac{(ABSxdilution\ factors)\ \text{x}\ 1000}{\left(sample\ dried\ weight\ \text{x}\ \varepsilon^{1\%}_{1cm,535}\right)}, \tag{2}$$

where ABS is sample absorbance detected at 535 nm, and $\varepsilon^{1\%}_{1cm,535}$ is the absorption coefficient for anthocyanin. Flavonoid content was calculated using the same equation using a wavelength of 374 nm and its respective absorption coefficient.

2.6.3. HPLC analysis of ascorbic acid and resveratrol

For ascorbic acid (AA) quantification, the procedure by Chebrolu et al. [23] was followed. Freeze-dried fruit samples (0.25 g) in 5 mL brown glass vials were mixed with *meta*-phosphoric acid (MPA) at 3 % (w/v) using a constant solution volume. Reversed-phase HPLC with isocratic elution was carried out using an Agilent TC C-18 column (150 mm × 4.6 mm, 5 μm) and methanol/water (15:85 v: v; with a pH value of 2.5 adjusted with MPA) as the mobile phase. The flow rate was maintained at 0.9 mL min^{-1}, and the sample injection volume was 10 μL; a UV–Vis detector at a wavelength of 245 nm was used with a run time of 10 min per sample at room temperature (~21 °C). The calibration plot was constructed using AA standard solutions (from 0.5 to 200 μg mL^{-1}) dissolved in the extracting solution, Fig. S2.

Resveratrol (RSV) quantification was performed according to Sun et al. [24], with slight modifications. A total of 50 mg of the lyophilized samples was weighed in 4 mL brown glass vials and dissolved in 2 mL of extracting solution (ethanol: water, 70:30, v/v) by stirring for 35 min. The extraction process was conducted in an ultrasonic bath; the resulting supernatant fractions were filtered using a 0.45 μm disposable syringe filter (Fisher Scientific). Reversed-phase HPLC with isocratic elution was conducted using an Agilent TC C-18 column (150 mm × 4.6 mm, 5 μm). A water/acetonitrile/acetic acid solution (70:29.9:0.1, v/v/v) was used as the mobile phase, with a 1 mL min^{-1} flow rate, 10 μL injection volume, 306 nm UV–Vis detector wavelength, 30 min run time per sample, and at 30 °C. RSV standard solutions (0.12–8.2 μg mL^{-1}) were dissolved in the mobile phase to construct the calibration plot (Fig. S3).

2.7. HPLC analysis of sugar and citric acid

Citric acid and carbohydrate content (cellobiose, fructose, and glucose) were quantified for each fruit sample using the procedure by Ávila Núñez et al. [25] with reversed-phase HPLC. Measurements were performed using a Rezex ROA-Organic Acid (8 %) column (300 mm × 7.8 mm) at 10 μm and an isocratic elution of 0.005 N H_2SO_4 as mobile phase with a flow rate of 0.44 mL min^{-1}; refractive index measurement at 30 °C was used as a detection mechanism.

Lyophilized samples of the fruits (250 mg) were suspended in 4 mL of deionized water in 4 mL brown glass vials; ultrasound-assisted extraction was performed for 30 min. The final supernatant fractions were filtered through a 0.45 μm membrane (Fisher Scientific) before being injected into the chromatographer.

Calibration plots were constructed from solutions containing different concentrations of the analytes (citric acid, cellobiose, glucose, and fructose) after diluting the 20 mg mL^{-1} standard solutions, ranging from 0.1 to 4 mg mL^{-1} for all analytes (Fig. S4).

2.8. Evaluation of the profile of metabolites of ethanol extracts of Rubus glabratus Kunth and Vaccinium floribundum Kunth by UPLC-QTOF mass spectrometry

Phenolic profiles of the ethanol extracts of *R. glabratus* Kunth and *V. floribundum* Kunth were determined using UPLC with a QTOF-mass spectrometer (Waters, Model Xevo G2 QTOF, USA). An ACQUITY UPLC BEH C18 (2.1 mm × 100 mm, particle size 1.7 μm) column model was used. The extracts were the same as those used to determine RSV. The mobile phase comprised a binary system composed of A: 99.99 % MilliQ water/0.01 % formic acid (HPLC grade) and B: 99.99 % acetonitrile (HPLC grade)/0.01 % formic acid (HPLC grade). The linear gradient began with 1 % A for 1 min, followed by 1–20 % A for 10 min, 20–25 % A for 10 min, 25–35 % A for

5 min, 35–50 % for 5 min, and finally 4 min at initial conditions.

Mass spectrometer (Figs. S5 and S6) anlayses were performed with an electrospray ionization source in the negative mode with a capillary voltage of 0.5 kV, cone gas flow rate of 20 L h^{-1}, desolvation gas flow rate of 900 L h^{-1}, source temperature at 140 °C, and desolvation temperature at 450 °C, with sampling cone and source offset at 40 and 80, respectively. The mass spectrometry experiments were carried out in conjunction with energy ramp collision (EC): low EC of 6 eV and high EC from 20 to 30 eV. The data obtained were analyzed with the equipment library.

Data acquisition was processed with UNIFI™ v1.8.0 (Waters), which was used for filtering, screening, and matching the chemical compounds. The data processing parameters were set as follows: the selection of adducts of +H, +Na, +K, $+NH_4$, -e, –H, +COOH, and +Cl, +e; retention time range 0.1–45.0 min; retention time tolerance was 0.2 min; peak intensities count mass accuracy tolerance set at ±5 ppm, mass range 100–1200 Da.

2.9. Data analysis

Samples were analyzed in triplicate. Statistical analysis was performed using Excel 2016. Results are expressed as (mean ± standard deviation). Each value is the mean ± SD of three determinations performed in triplicate on three replicates of fruits. Differences between means were first analyzed by a one-way ANOVA followed by a post hoc multiple comparison Tukey test ($p < 0.05$). Pearson correlation coefficients were calculated to determine the correlation between the methods used to ascertain antioxidant capacity.

3. Results and discussion

3.1. Antioxidant properties

The use of simple methods to determine antioxidant properties with various forms of free radical generation has become increasingly common. However, inconsistent results have made it difficult to determine the usefulness of one method over another and have also led to complications regarding accurately typifying, characterizing, and comparing different samples. In the present study, we have chosen electrochemical methods, CV for antioxidant capacity and DPV for antioxidant power, whose inherent selectivity and versatility, according to the experimental conditions, allow the redox behavior and specific classes of antioxidant species to be determined in the analyzed samples. Results have been compared to those from the most widely used traditional method to determine antioxidant capacity—the DPPH assay [4].

Antioxidant capacity results from the DPPH method are shown in Table 1. As the magnitudes of antioxidant capacity are expressed as μmol AAE 100 g^{-1}, the results show that the fruits exhibit antioxidant capacity as follows: *R. glabratus* Kunth > *O. soederstromiana* > *V. floribundum* Kunth.

The superoxide anion scavenging ability of each ethanolic extract was evaluated via CV (Fig. 1). Additions of ethanolic extract to an electrolytic medium led to a decrease of the $O_2^{\bullet-}$ anodic peak current (Ipa), Fig. 1A–C, regardless of the fruit. Depending on the nature of the phenolic compounds present in the extract, this decrease could be concomitant with an increase in the cathodic current peak and/or with the appearance of a prepeak [26]. An irreversible cathodic prepeak, approximately 4.5 V, was observed in the cyclic voltammograms for the extracts of *R. glabratus* Kunth (Fig. 1A) and *O. soederstromiana* (Fig. 1B). Cathodic prepeak could correspond to product reduction formed after reaction with $O_2^{\bullet-}$, concerning polyphenols like flavonoids [26]. In addition, after the analysis, R^2 values of 0.994, 0.999, and 0.998 for *R. glabratus* Kunth, *O. soederstromiana, V. floribundum* Kunth, respectively, were found when comparing the dimensionless parameter $[(Ip_a^0\text{-}Ip_a^s)/Ip_a^0]$ to the extract concentration in solution (Fig. 2); this indicates that the experimental data fit a linear model. The AI_{50} values (concentration required to reduce the anodic current peak by 50 %) are reported in Table 1. Extracts with the lowest AI_{50} value correspond to the highest antioxidant capacity against the $O_2^{\bullet-}$ generated *in situ* in the electrolytic media [18]. The extract with the highest antioxidant capacity was *R. glabratus* Kunth, followed by *O. soederstromiana* and *V. floribundum* Kunth. This trend is consistent with that found for antioxidant capacity evaluated using the DPPH method.

Fig. 3 shows the DPV voltammograms obtained for each fruit extract from which the EI values reported in Table 1 were determined. The EI is defined as the total content of polyphenols in a sample that can be obtained electrochemically through the non-selective oxidation of all polyphenols. However, the antioxidant species identity and antioxidant power can be estimated using the oxidation

Table 1
Antioxidant capacity evaluation.

Fruit	μmol AA 100 g^{-1} using DPPH	AI_{50} (ppm)	EI (μA V^{-1})
***R. glabratus* Kunth**	1501.86 ± 0.37	0.499 ± 0.0014	$2.26 \times 10^{-5} \pm 2.85 \times 10^{-7}$
***V. floribundum* Kunth**	1144.52 ± 0.23	0.653 ± 0.007	$1.16 \times 10^{-5} \pm 2.48 \times 10^{-8}$
O. soederstromiana	1012.09 ± 0.36	0.569 ± 0.011	$1.26 \times 10^{-5} \pm 2.46 \times 10^{-8}$

Note: Values are expressed as means ± standard deviation (SD of triplicate assays). Mean values within a column were evaluated using one-way ANOVA and were found to not be statistically significant ($p > 0.05$).
- Ascorbic acid (AA).
- Electrochemical index (EI).
-Electrochemical index (AI_{50}).

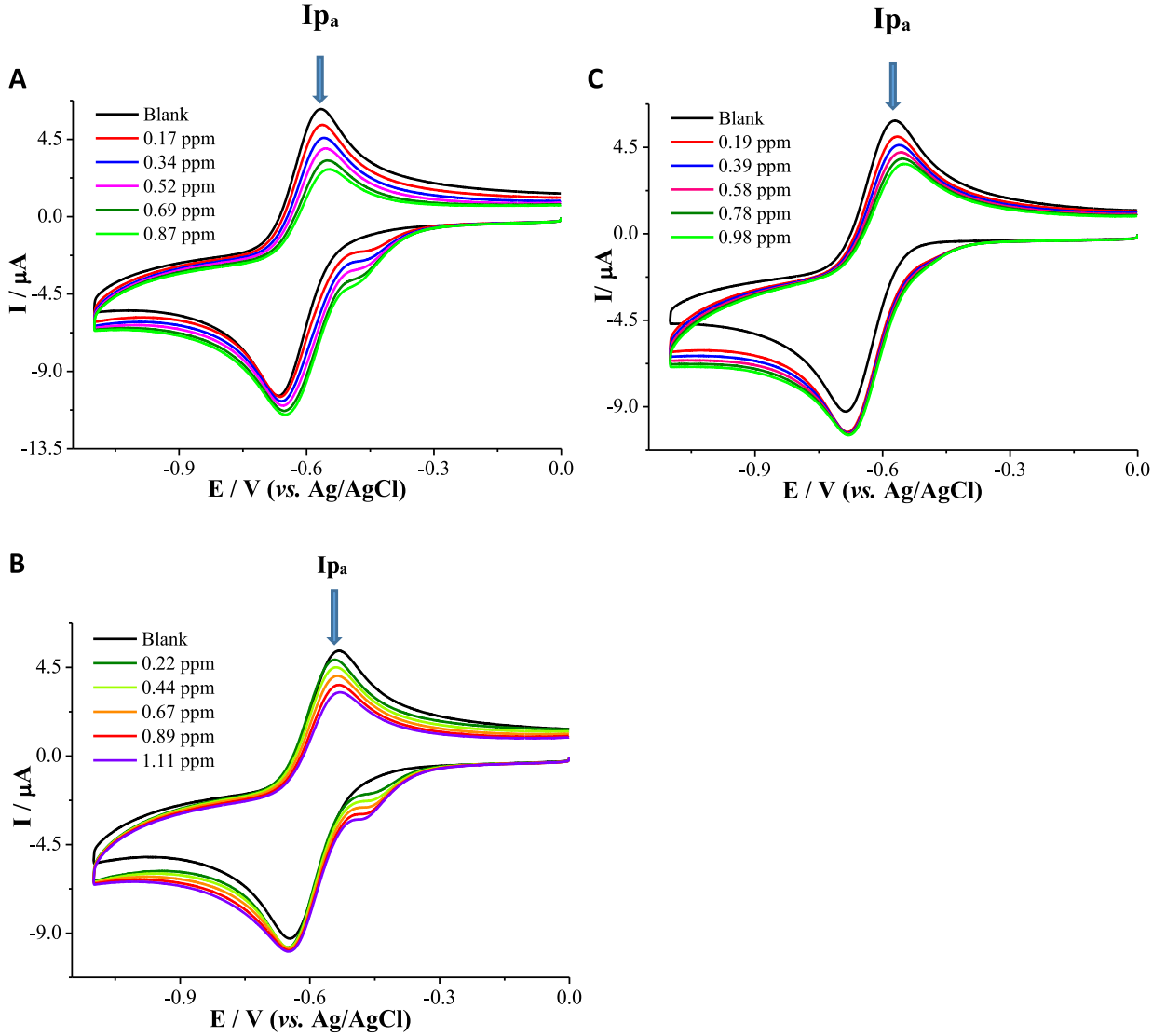

Fig. 1. Cyclic voltammogram for the redox coupling of $O_2^{\bullet-}$ with increasing concentration of extracts (indicated in the insert) in C_2H_6OS + tetrabutylammonium hexafluorophosphate, scan rate 17 mV s^{-1}. (A) *R. glabratus* Kunth, (B) *V. floribundum* Kunth and (C) *O. soederstromiana*.

potential of the different fractions that comprise the conglomerate of antioxidant species present in the sample [27]. According to this definition, the number of signals obtained is proportional to the number of electroactive antioxidant species present in the extract; the signals at lower oxidation potentials in the voltammograms are associated with chemical species with greater electron donation capacity, while the higher current signals indicate a higher concentration of such species dissolved in the solutions. Thus, the voltammograms in Fig. 3 suggest the presence of three types of species with antioxidant capacity in the extract of *R. glabratus* Kunth (Fig. 3A), five in the extract of *V. floribundum* Kunth (Fig. 3B), and four in the extract of *O. soederstromiana* (Fig. 3C). The antioxidant species present in the *O. soederstromiana* ethanolic extract have lower antioxidant capacity than those in the *R. glabratus* Kunth and *V. floribundum* Kunth extracts, as the oxidation potentials for the species in the *O. soederstromiana* extract are greater than those for the species of the other two fruit extracts. On the other hand, the comparison of the current intensities for each signal in the voltammogram of Fig. 3 suggests a higher content of antioxidant species in the *R. glabratus* Kunth extract than in the *O. soederstromiana* and *V. floribundum* Kunth extracts.

The EI value, according to Equation (1), is directly proportional to the antioxidant power of the chemical species and is usually correlated with the content of phenolic compounds (TPC) in the analyzed sample. The highest EI value corresponded to the *R. glabratus* Kunth ethanolic extract, followed by the *O. soederstromiana* and *V. floribundum* Kunth extracts (Table 1). This result is consistent with those obtained by the TPC (Table 2), DPPH, and CV methods (Table 1), which also showed that *R. glabratus* Kunth had the highest antioxidant capacity and TPC of the three fruits; *O. soederstromiana* had an intermediate-level antioxidant capacity and *V. floribundum*

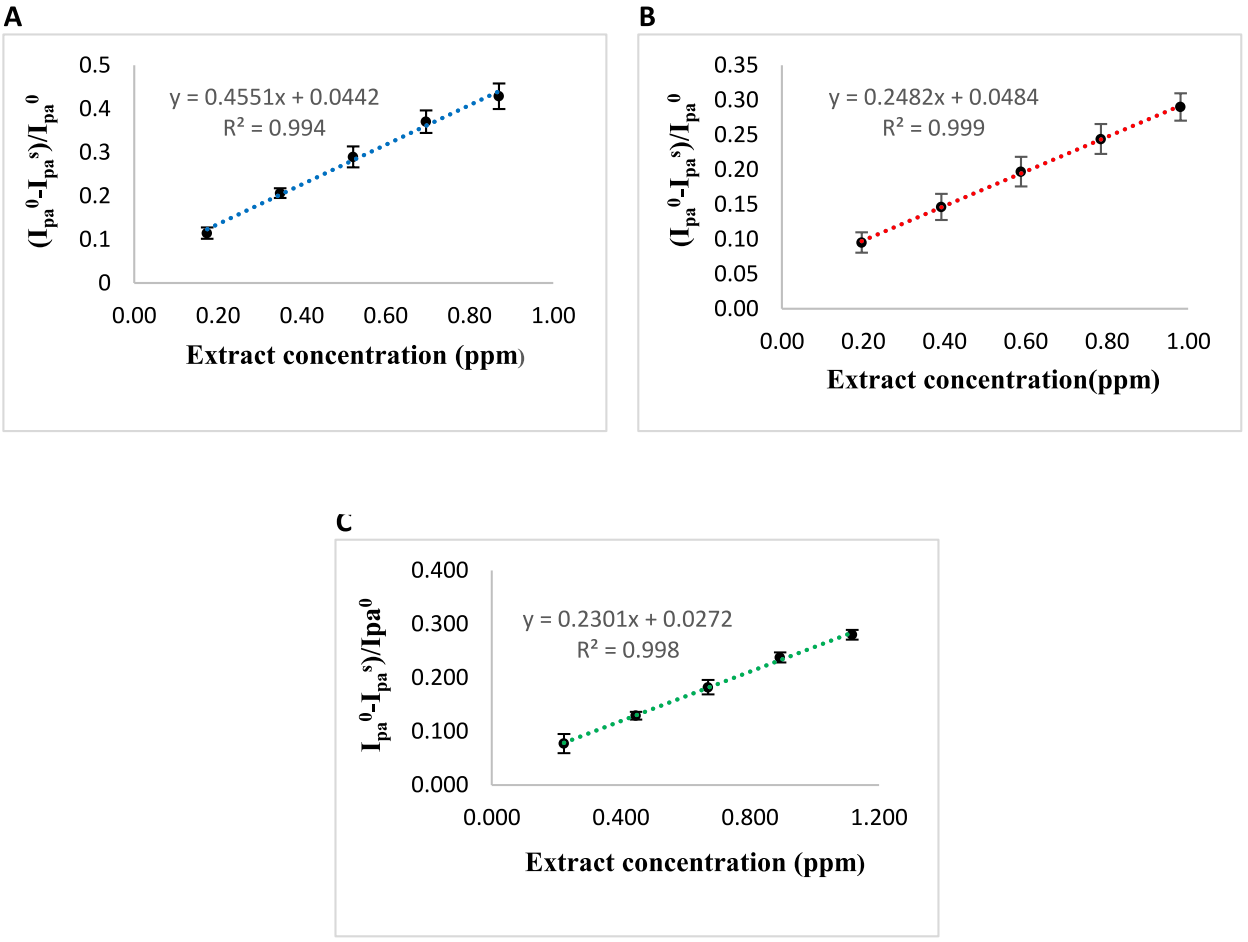

Fig. 2. Dimensionless parameter $[(Ip_a^0\text{-}Ip_a^s)/Ip_a^0]$ *vs* concentration of fruit extract. (A) *R. glabratus* Kunth, (B) *V. floribundum* Kunth and (C) *O. soederstromiana*.

Kunth the lowest antioxidant capacity. Consistency between results was statistically evaluated using the Pearson correlation coefficient. A higher DPPH analysis result corresponds to greater antioxidant capacity. Likewise, a higher EI value corresponds to the greater content of antioxidant species and their reducing power in the sample; thus, these values are directly proportional. The Pearson correlation coefficient was 0.98 in our study for these values. Conversely, a lower AI_{50} value corresponds to a higher antioxidant capacity of the substrate on the electrochemically generated superoxide anion; thus, this value is inversely proportional to the DPPH and EI values. We found a correlation of −0.95 with the DPPH value and −0.88 with the EI. These high correlation rates show that the spectrophotometric and electrochemical analyses used in this investigation are in agreement.

3.2. Phytochemical analysis

3.2.1. Total phenolic content

Polyphenols are the most abundant reducing agents in the human diet and interact with other compounds, such as vitamin C and carotenoids, to protect the body from oxidative stress. These reducing agents have been the focus of both scientific and industrial interest because of increased attention on the consumption of functional foods. The results of TPC for each ethanolic extract of the selected fruits are shown in Table 2.

Total phenolic content for *R. glabratus* Kunth was 22.69 ± 0.66 mg GAE g^{-1}, which is consistent with the value reported for the same fruit (i.e., 23.40 ± 6.3 mg GAE g^{-1}) by Ref. [28]. Likewise, it is similar to that reported by Ref. [29] of 21.67 ± 8.35 mg GAE g^{-1}. Both previous studies were conducted in Ecuador. However, our results contrast with those of A. Gawron-Gzella et al.[30], who examined *Rubus genus* blackberries in Poland; they found TPC ranged from 70.50 to 136.04 mg GAE 100 g^{-1}.

O. soederstromiana had a phenolic content of 20.98 ± 0.17 mg GAE g^{-1}. However, results from similar studies on fruit from the genus *Opuntia* have varied widely. For *Opuntia ficus-indica*, mainly found in Mexico by Refs. [31,32], a range between 5.5 and 7.6 and 12.79 ± 1.07 mg GAE g^{-1} was reported, respectively. Our study's result is considerably higher, which may be associated with differences between the geographical areas where the fruits were collected, as it has been shown that geographic location can greatly

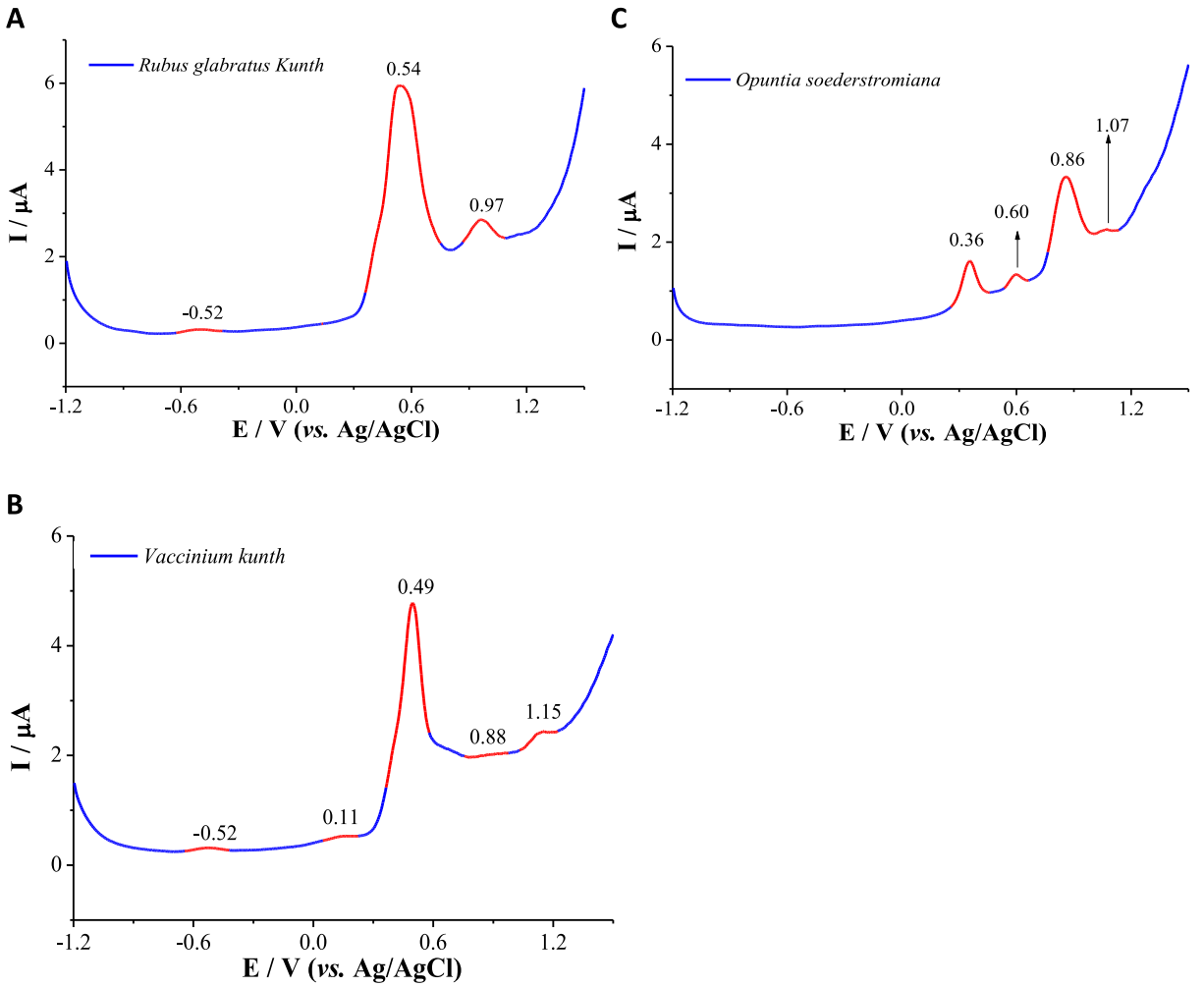

Fig. 3. Differential pulse voltammogram for the ethanolic extract of each fruit in acetate buffer, pH 4.5, scan rate of 17 mV s^{-1} (A) *R. glabratus* Kunth, (B) *V. floribundum* Kunth, and (C) *O. soederstromiana*.

Table 2
Results of phytochemical analysis.

Fruit	Total phenolic content (mg GAE 100 g^{-1})	Total anthocyanin (mg 100 g^{-1})	Total flavonoids (mg 100 g^{-1})	Fructose (g 100 g^{-1})	Cellobiose (g 100 g^{-1})	Citric acid (g 100 g^{-1})	Glucose (g 100 g^{-1})	RSV µg g^{-1}	AA (mg 100 g^{-1})
***R. glabratus* Kunth**	22.69 ± 0.66	205.24 ± 0.55	6.29 ± 0.05	12.2 ± 0.02	0.091 ± 0.067	0.597 ± 0.02	3.821 ± 0.015	0.14 ± 0.03	3.36 ± 0.07
***V.floribundum* Kunth**	20.31 ± 0.03	572.15 ± 0.55	53.32 ± 0.06	18.01 ± 0.6	0.074 ± 0.003	4.613 ± 0.012	14.837 ± 0.048	ND	6.58 ± 0.26
O. soederstromiana	20.98 ± 0.17	19.03 ± 0.22	4.07 ± 0.05	4.96 ± 0.61	0.010 ± 0.017	3.358 ± 0.045	8.21 ± 0.97	0.66 ± 0.04	9.44 ± 0.09

Note: Values are expressed as means ± standard deviation (SD of triplicate assays). Mean values within a column were evaluated using one-way ANOVA and were found to not be statistically significant ($p > 0.05$).
- Gallic acid equivalent (GAE).
- Ascorbic acid (AA).
- Resveratrol (RSV).
-ND: not detected.

affect the number of analytes found in similar fruits [33]. Another factor that can influence the results is the type of species studied. Ecuadorian fruit has high levels of bioactive compounds relative to that of other countries, even neighbouring countries such as Peru and Colombia, which may be due to climatic conditions and land characteristics [34].

Total phenolic content for *V. floribundum* Kunth corresponded to 20.31 ± 0.03 mg GAE g^{-1}, a considerably higher value than that found by Ref. [35]—11.27 ± 0.20 mg GAE g^{-1}—and [29]—1.26 ± 0.04 0 mg GAE g^{-1}—while [36] reported 53.3 ± 5.8 mg g^{-1} of catechin equivalents. Berries vary widely in terms of phenolic composition; therefore, variations among studies are expected since they include fruits from different regions, soils, and time zones and at different ripeness levels. The phenolic content results are also in line with those reported in Table 1. *R. glabratus* Kunth had the highest phenolic content, followed by *O. soederstromiana and V. floribundum* Kunth. High phenolic content corresponds to high antioxidant power. According to the Pearson correlation results between antioxidant capacity and TPC (Table S1), we found positive correlations for the DPPH assay and DPV method and a negative correlation for the CV method. The high correlation between antioxidant capacity and TPC for DPPH and CV is consistent with previous studies [18].

Anthocyanin are natural pigments easily detected by sight, thanks to their particular coloration. The anthocyanin results are summarized in Table 2. *V. floribundum* Kunth had the highest anthocyanin content, 572.15 ± 0.55 mg 100 g^{-1}, which falls within the range obtained by Ref. [37], 328.73–1383 mg 100 g^{-1}. The second-highest content was found in the *R. glabratus* Kunth, 205.24 ± 0.55 mg 100 g^{-1}; this value is lower than that found by Ref. [38], who reported results ranging from 569 to 1192 mg 100 g^{-1}, which may be due to the different geographical location—Central America—of the fruit. Nevertheless, the present study's value coincides with that of Moyer et al. [39] in Oregon: 242 mg 100 g^{-1}. Finally, *O. soederstromiana* had the lowest anthocyanin content, 19.03 ± 0.22 mg 100 g^{-1}; this was expected since its reddish coloration does not visibly indicate high anthocyanin content. To the best of our knowledge, no previous studies have examined total anthocyanin content, so no comparisons of our results can be made.

V. floribundum Kunth presented the highest content of flavonoids, 53.32 ± 0.06 mg 100 g^{-1}, which is consistent with previous studies [40] that have established a range of 54–88 mg 100 g^{-1} for fruits from the genus *Vaccinum.* Next was *R. glabratus* Kunth (6.29 ± 0.05 mg 100 g^{-1}), which is lower than the results from Ref. [41]. However, this variation can be considered normal, as this previous study was carried out in Brazil, and flavonoids vary according to the fruit's geographical origin [33]. Finally, *O. soederstromiana*, with 4.07 ± 0.05 mg 100 g^{-1}, had the lowest concentration of flavonoids, a result that cannot be compared owing to the lack of previous relevant studies.

3.2.2. HPLC analysis of carbohydrates and citric acid

Many fruits are high in carbohydrates, which gives them their characteristic taste. These carbohydrates can vary considerably from one fruit to another. The analysis showed high variability regarding the different types of sugars examined (cellobiose, glucose, and fructose) in the selected fruits, Fig. S4. In addition, citric acid, considered one of the most important organic acids in fruits, was also determined. The results are summarized in Table 2.

Glucose content in *R. glabratus* Kunth, 3.821 ± 0.015 g 100 g^{-1}, was consistent with the results by Hassimotto et al. [41] (2.44 g 100 g^{-1}); however, the fructose content was considerably higher in our study 12.2 ± 0.02 g than that in Hassimotto et al. [41], 1.91 g 100 g^{-1}. Citric acid content (0.597 ± 0.02 g 100 g^{-1}) was in line with results from Ref. [42], who determined 5 mg g^{-1} for fruit from the *Rubus* genus. Regarding *V. floribundum* Kunth, glucose and fructose content, 14.837 ± 0.04 and 18. 012 ± 0.06 g 100 g^{-1}, respectively, were considerably higher than the respective 5.5 and 5.3 g 100 g^{-1} reported previously [43]. With respect to citric acid, 4.613 ± 0.01 g 100 g^{-1} was obtained for *V. floribundum* Kunth, which was higher than previous studies (1.33 ± 0.06 g 100 g^{-1}) [42]. For *O. soederstromiana*, both, glucose and fructose content, 8.21 ± 0.97 and 4.96 ± 0.61 g 100 g^{-1}, respectively, were higher than that found by Gurrieri et al. [44], 3.16 ± 0.03 and 2.85 ± 0.02 g 100 g^{-1}, respectively. Regarding citric acid, this study's result of 3.358 ± 0.05 g 100 g^{-1} was higher than that of Teles et al. [45] (1.78 ± 0.12 g 100 g^{-1}) for fruits of the same genus. Although the degree of maturity of the fruit is indeed a determinant of sugar and organic acid content in this specific case, it was difficult to collect all fruits at the same stage of maturation, as they are native fruits harvested using artisanal methods. Therefore, significant differences in these fruit components are normal and acceptable.

3.2.3. HPLC analysis of ascorbic acid and resveratrol

Ascorbic acid is one of the most studied and utilized antioxidants in the food industry; therefore, finding new biological sources rich in AA is a priority in food research. However, studying this metabolite is complicated by its instability, as it is very sensitive to environmental factors such as light and oxygen, as well as to its method of extraction. The AA content (Table 2 and Fig. S2) of *V. floribundum* Kunth was 6.58 ± 0.26 mg 100 g^{-1} in a freeze-dried sample, which coincides with results from Kalt et al. [43] and Arvinte et al. [46], who reported 5.8–6.357 mg AA 100 g^{-1} in berries from the *Vaccinium* genus, but it was lower than the 9 mg 100 g^{-1} reported by Piluzza et al. [29]. For *R. glabratus* Kunth, AA content has been generally found to vary little; the results were within the range established by Ref. [47] of 1.3–16.4 mg 100 g^{-1}. We obtained 3.36 ± 0.07 mg AA 100 g^{-1} of the lyophilized sample, which was lower than that reported by Refs. [36,48], who found 10.1 mg AA 100 g^{-1}. These variations could be due to the fact that the previous studies were carried out using different *Rubus* fruits, as indicated by Ref. [29]; likewise, functional compounds can vary significantly depending on ripeness. In our research, fully ripe fruits were distinguished from 100 % blue fruit (Fig. S1A), while in Ref. [49], they were carried out in different states of maturity of the fruit, which may not coincide with that of our analyzed fruit. Regarding *O. soederstromiana*, 9.44 ± 0.09 mg AA 100 g^{-1} was found in the freeze-dried sample, which is high compared to the 3.5–6.5 mg AA 100 g^{-1} found by Ref. [50] in their study on another *Opuntia* fruit (*Opuntia ficus-indica*) but lower than the results by Ref. [51]. In general, AA content in fruits is especially affected by the light received by the plant [14]; a greater amount of light will result in a greater amount of AA in the fruit. This could explain the high values of AA found in the analyzed fruits in comparison with the same fruits from other studies, as sunlight is more intense in the equatorial zone. On the other hand, some studies have attributed differences

in AA content in fruits to their ripeness [52].

Resveratrol is a phytoalexin found in certain fruits. It has antioxidant, anti-inflammatory, and anti-mutagens properties [53], and it is the main stilbene in withered berries and grapes. Because of its beneficial properties, the current study used HPLC to determine the RSV content of the fruit extracts. The results are shown in Table 2 and Fig. S3.

No positive RSV results were obtained for *V. floribundum* Kunth despite a recovery of 89.1 % for the validation method. However, this does not indicate a total absence of the chemical species, since the concentration value found in the samples may have been below the quantification limit of the technique used. However, our results are consistent with Ref. [54], who did not detect RSV in several of the fruits of the *Vaccinum* genus using a detection limit of 9 fmol, a value well below the detection limit established in the current study.

Resveratrol was identified in *R. glabratus* Kunth in a concentration of 0.14 ± 0.03 μg of RSV per g of lyophilized sample. This functional compound was not detected by Ref. [55] in *Rubus idaeus* L.

For *O. soederstromiana*, 0.66 ± 0.04 μg of RSV per g of lyophilized sample was identified, which is inconsistent with the results from Ref. [56], who found 1.5–2.6 μg of RSV per g of lyophilized sample. These variations may be caused by the sampling method of the previous study, wherein the analysis was performed using exclusively fruit pulp, while that of the present study used the entire fruit. However, in another study on *Opuntia* fruit by Ref. [57], the absence of RSV was reported in two genera of this species.

Variations in RSV determinations could be due to external factors such as fruit maturity and geographic origin or the sensitivity of the bio-functional compound [46]. It should be noted that RSV analysis is difficult due to its sensitivity to both light and oxygen, which causes it to decompose very shortly after extraction.

Multiple comparison Tukey tests (data not shown) using the results of phytochemical analysis (DPPH, AI50, EI, TPC, and anthocyanin, flavonoid, ascorbic acid, sugar, and citric acid content) showed the greatest significant difference in terms of antioxidant capacity between *R. glabratus* Kunth and *V. floribundum* Kunth. The analysis suggests *R. glabratus* Kunth as the fruit with the greatest contribution in terms of antioxidant capacity.

Table 3
Metabolite profile of extracts of *Rubus glabratus* Kunth and *Vaccinium floribundum* Kunth.

Fruit	Identified compounds	Chemical Formula	Observed *m/z*	Observed Rt (min)	Adducts	Biological activities	References
***R. glabratus* Kunth**	Indole-3-acetonitrile-6-*O*-Beta-D-glucopyranoside[a]	$C_{17}H_{19}N_2O_8$	379.1146	0.64	+HCOO	Anti-influenza A Inhibitory effects on nitric oxide production	[58,59]
	Feroxin B	$C_{35}H_{35}O_{12}$	647.2151	2.40	-H	Nutria-pharmaceutical applications Psoriasis-Like Skin Inflammation	[60,61]
	3,6-Bis-*O*-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranose	$C_{20}H_{19}O_{14}$	483.0779	6.37	-H	–	[62]
	Caffeoylxanthiazonoside	$C_{27}H_{30}O_{13}NS$	608.1447	10.03	+HCOO	Antiallergic rhinitis effect Protective effects	[63,64]
	Periplocoside L[b]	$C_{29}H_{47}O_9$	539.3234	23.19	+HCOO	Cytotoxicity on HepG2 and LO2 cells	[65]
	alpha-D-Glucopyranose-1-hexadecanoate[c]	$C_{23}H_{43}O_9$	463.2905	31.22	+HCOO	–	[66]
***V. floribundum* Kunth**	Flavenochromane C	$C_{21}H_{19}O_6$	367.1183	0.56	-H	Neuroprotective effect Cytotoxicity on Human Tumor Cell Lines	[67,68]
	Xanthotoxin (Methoxsalen)	$C_{12}H_7O_4$	215.0345	0.56	-H	Anticonvulsant effect Treatment of vitiligo	[69,70]
	Polygalaxanthone III	$C_{26}H_{29}O_{17}$	613.1394	0.67	+HCOO	Antioxidant and antidiabetic effects Anti-inflammatory effect	[71,72]
	Interiorin D	$C_{29}H_{27}O_8$	503.1695	0.69	-H	–	[73]
	Smiglaside D	$C_{47}H_{49}O_{22}$	965.2763	0.82	-H	Cytotoxic Activity	[74]
	Pseudobrucine[a]	$C_{23}H_{25}O_5N_2$	409.1778	5.50	-H	Anti-arthritis activity	[75]
	Epigallocatechin 3-*O*-gallate	$C_{16}H_{15}O_9$	503.0848	6.05	+HCOO	Antioxidant and antitumor activity	[76]
	Strycnine[a]	$C_{22}H_{23}O_4N_2$	379.1659	6.31	+HCOO	Anti-proliferative and cytotoxic effects	[77]
	Vomicine[a]	$C_{22}H_{24}O_4N_2$	425.1723	6.32	+HCOO	Antimalarial and cytotoxic activities	[78]

Note.
[a] Alkaloid compound.
[b] Steroidal compound and.
[c] Monosacharide derivative.

3.2.4. Phenolic profiles of Robus glabratus Kunth and Vaccinium floribundum Kunth by LC -QTOF mass spectrometry

The use of traditional plants with pharmaceutical applications and their impact on human health has been of great importance throughout the centuries, considering that this knowledge was developed long before drugs were formally introduced in the market. Unfortunately, valuable ethnobotanical information about the traditional use of endemic fruits from Ecuador is scarce in the literature. Particularly, *R. glabratus* Kunth and *V. floribundum* Kunth are known as excellent sources of water, fiber, minerals, and vitamins, among other beneficial health-related substances. Table 3 and Figs. S5 and S6 summarize the metabolites found for these two fruits with their corresponding biological activity.

The identity of possible compounds was determined with a phenolic profile using the UPLC-QTOF method. A total of three phenolic constituents, an alkaloid, a steroidal compound, and a monosaccharide, were tentatively identified for *R. glabratus* Kunth. In the same way, a total of six phenolic compounds and three alkaloids were tentatively identified for *V. floribundum* Kunth. Feroxin B, found in *R. glabratus* Kunth, has been recognized for its nutraceutical potential. Likewise, epigallocatechin has been extensively studied as an antioxidant; it can increase the effect of anticancer systemic drugs and mitigate their oxidative side effects [79]. Therefore, the presence of this metabolite in *V. floribundum* Kunth could be related to the antioxidant capacity reported for this fruit. In addition, *Furanocumarin* xanthotoxin is used to treat vitiligo. Although three alkaloids were tentatively identified for *V. floribundum* Kunth, they are more common for the genus *Strychnos* of the Loganiaceae family [80]. The isolation and characterization of these alkaloids are necessary to prove their presence in *V. floribundum* Kunth.

Opuntia soederstromiana is very scarce during the harvest months and is not harvested every year, so it was only possible to obtain test samples for this fruit for the phenolic profile study. In terms of economic value, it is a very important fruit, which is why we are considering it in further research.

4. Conclusions

The fruits analyzed in this study are rich sources of components with antioxidant capacity. The results indicate the antioxidant capacity of the fruits in the following order: *R. glabratus* Kunth > *O. soederstromiana* > *V. floribundum* Kunth. The present study found an inverse correlation between AI_{50} and EI and a positive correlation between DPPH and TPC, which is consistent with previous studies. The antioxidants and other chemical species found in *R. glabratus* Kunth, *V. floribundum* Kunth, *and O. soederstromiana* indicate that these Ecuadorian species are important sources of metabolites with nutraceutical properties; this finding supports ancestral ethnobotanical knowledge and suggests potential industrial applications that have thus far been underexplored. A better understanding of the factors that influence the composition and antioxidant activity of fruits can allow stakeholders to leverage these insights to promote health, validate traditional knowledge, and drive innovation within the food and pharmaceutical industries. The differences between the results of the current study and those of previous research focusing on fruits from the same genus may be owed to verified variations of bioactive compounds related to geographical area, differences in species, ripeness, and moisture, among many other variables. Therefore, in future research, we will aim to fully characterize the edaphoclimatic conditions, stage of maturity, and ripeness, among other factors, when assessing the composition and antioxidant activity of fruits. Currently, we are initiating docking studies to determine the possible role of each phytochemical present in these extracts of these fruits, so that the binding affinity data and the interaction pattern of all the possible phytochemicals in the extract of these fruits reveal what they can synergistically inhibit to prevent the pathologies reported in the literature.

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Data availability statement

Data will be made available on require.

CRediT authorship contribution statement

Raul D. Monge-Sevilla: Validation, Formal analysis, Data curation. **Lenys Fernandez:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Patricio J. Espinoza-Montero:** Writing – review & editing, Resources, Data curation. **Carlos Mendez-Durazno:** Methodology. **Pablo A. Cisneros-Pérez:** Methodology. **David Romero-Estevez:** Methodology. **Diego Bolaños-Méndez:** Validation, Data curation. **Jocelyne Alvarez-Paguay:** Validation, Data curation. **Mónica Jadán:** Resources.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30593.

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