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Research Article

Assessment of phenolic composition and antioxidant activity of fermented Andean blackberry beverage enriched with medicinal plants of Ecuador

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Abstract

In recent years there has been a great interest in functional beverages with antioxidant capacity that impart various health implications upon consumption due to the presence of various bioactive components, like phenols. Fruit juices are excellent matrices for the delivery of active components for these functional beverages. This research was focused on developing and testing the biological activity of a new fermented blackberry (*Rubus glaucus* Benth) beverage enriched with different plants as a new product on the functional food market. Several Ecuadorian species (spices, aromatic, and medicinal) have been tested for the highest antioxidant activity and phenolic content. Methods like Folin Ciocalteu, α-diphenyl-α-picrylhydrazyl free-radical-scavenging method (DPPH), free-radical-scavenging activity (ABTS), and Ferric reducing antioxidant power (FRAP) were used. Among the tested plants, arrayán (*Myrcianthes hallii*), plantain (*Plantago major*), ishpingo (*Ocotea quixos*), and ataco (*Amaranthus quitensis*) were selected. The study demonstrates the ability of medicinal plants to improve the total phenolic content and antioxidant capacity, but also the sensory properties of the functional drink compared to the blackberry fermented drink. The highest values of the analyzed characteristics (total phenolic content, antioxidant capacity, sensory evaluation), were obtained in the case of the treatment consisting of fermented blackberry beverage enriched with 4 g of medicinal plants.

Keywords: antioxidants; Arrayán; DPPH; Ecuador; medicinal plants; *Rubus glaucus*

Introduction

Functional beverages, a subsector of the functional food industry and the fastest-growing sector of the functional food market have become increasingly popular among conscientious consumers due to their perceived health benefits (Jahidul and Kabir, 2019). Functional beverages represent one of the essential

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components in our life contributing to nutritional well-being. Current efforts to understand and develop functional beverages to promote health and wellness have been increased (Yilmaz-Akyuz *et al*., 2019).

Compared with current drug treatments, functional foods showed lower or no adverse effects; from this point of view being good candidates for this purpose (Yilmaz-Akyuz *et al*., 2019). Fruits, a rich source of bioactive components represent a significant part of functional beverages. The berries fruits, rich in nutrients and phytochemicals, are known for their beneficial health effects; extracts from berries showed increasing interest, being implicated in functional food combined with other herbal extracts (Cerrato *et al*., 2022).

Rubus glaucus Benth (known as mora de Castilla, Andean raspberry/blackberry) is found in high tropical zones of Latin America (Colombia, Ecuador, Panamá, Guatemala, Honduras, México, and Salvador) (Idárraga-Piedrahita *et al*., 2011). The Andean blackberry is a perennial semi-erect climbing shrub, which produces fruits (red-blue drupes) after the first year (Idárraga-Piedrahita *et al*., 2011). These fruits are important in the human diet due to their high content of antioxidants (predominant group of phenolic acids and flavonoids), nutrients, and proteins (Rodarte Castrejón *et al*., 2008). Due to its pleasant flavor and taste, the beverage with blackberry fruits is highly esteemed by consumers (Meret *et al*., 2011).

It is proven that long-term exposure to high levels of psychological stressors may cause diverse neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases (Kumar *et al*., 2006). Plants and fruits with antioxidative properties have been investigated for preventing oxidative stress-related diseases.

In the last time, plants are used to enrich the beverage due to their chemical composition, sensory and organoleptic properties, and possible health-promoting effects (Manousi *et al*., 2019). The advantages of using natural products to prevent oxidative stress consist of low toxicity, a mixture of multiple antioxidants in the product, the ability to react to most or all types of ROS, and easy accessibility. The protective effect of plants against oxidative stress may be due to the presence of polyphenols (Wojdyło *et al*., 2007).

In our study, we chose ten species (spices and medicinal plants) and investigated their antioxidant characteristics. All of these species have long traditions of use in treating different illnesses. In the case of some of them, this was the first attempt. Only four species from different families were chosen. *Myrcianthes hallii* (O. Berg) McVaugh (arrayán) (Myrtaceae family) is in long-standing use in folk medicine due to the presence of many known antibacterial polyphenolic compounds (Carvajal *et al.,* 2016). *Plantago major* L. (plantain) (Plantaginaceae family) has very long traditions over the world in the treatment of numerous diseases (Samuelsen, 2000). *Ocotea quixos* Lam. (ishpingo) (Lauraceae family) represents a tree native and cultivated in the Amazonian region, characterized by numerous biological activities (antimicrobial, antioxidant, antiplatelet, anti-inflammatory, larvicidal, etc). Traditionally is used as a food aroma, being similar to cinnamon (*Cinnamomum verum* J. Presl) (Gilardoni *et al*., 2021). *Amaranthus quitensis* Kunth (ataco) (Amaranthaceae family) showed great social, cultural, and food significance for the native population. Ataco is a semi-cultivated species, distributed only in South America, on a small scale in the Ecuadorian highlands (DENAREF, 2016).

Therefore, in the present study, we screened for high antioxidant activity and phenolic content of plants used in Ecuador as medicine and spices. The plants with higher characteristics have been used to develop a functional beverage based on a fermented blackberry drink.

Materials and Methods

Plant collection

The medicinal plants taken into account, due to their medicinal properties attributed, were represented by *Myrcianthes hallii* (O. Berg) McVaugh (arrayán), *Plantago major* L. (plantain), *Ocotea quixos* Lam. (ishpingo) and *Amaranthus quitensis* Kunth (ataco). The species were chosen based on their total phenolic content and antioxidant capacity after screening more than ten species. Samples were collected in July 2018 from Montalvo, Pastaza Ecuador, and Quito, Pichincha Ecuador, collection sites.

Extract preparation

The collected plants were shade dried to obtain approximately 50 g dry sample which was powdered in the Ultra Centrifugal Mill ZM 200 (Thomas Scientific, USA) to 60-mesh size and used for solvent extraction. For sample preparation, 1 g of dry sample was extracted with 10 mL of 95% methanol (Sigma Aldrich, USA) at 23 °C. After 24 h, the extracts were centrifuged at 6.000 rpm for 15 min to collect and filter the supernatants through a 0.45 µm pore size filter (Sigma Aldrich, USA).

Quantification of total phenolic content

The total phenolic content (TPC) was determined by the spectrophotometric method described by Machu *et al.* (2015). Briefly, an aliquot of 0.4 mL of each extract diluted in 5 mL of Milli-Q water was added to the 1.5 mL of Folin-Ciocalteu's phenol reagent (Sigma Aldrich, USA). After 5 min, 0.4 mL of 20% Na_2CO_3 and 0.8 mL of distilled water were added and incubated at 23 °C for 1 h in the dark. The absorbance was measured at a wavelength of 765 nm, using a gallic acid (Sigma Aldrich, USA) calibration curve to extrapolate TPC concentrations. The calibration curve was obtained by preparing standard solutions of different concentrations of gallic acid in 95% methanol. The assay was carried out in triplets for each sample. The TPC was expressed as mg of gallic acid equivalents (GAE) per g of dried sample (mg GAE $\cdot$ g^{-1} d.w.).

Antioxidant assays

Each extract with an initial concentration of 0.1 g $\cdot$ dm^{-3} was diluted with 95% methanol to prepare serial concentrations for antioxidant assays. Technique-specific reference chemicals were used for comparison in all assays. The estimation of the antioxidant capacity through DPPH, ABTS, and reducing power was carried out in triplicate.

DPPH radical scavenging activity assay

The free radical scavenging activity of the extracts was measured by 2,2′- diphenyl-1-picrylhydrazyl (DPPH) assay according to a modified protocol from Shikamura *et al.* (2014). A 0.15 mM DPPH (Sigma Aldrich, USA) stock solution was prepared using 95% methanol. Different extract concentrations were tested for each sample. Aliquots of 100 µL of the extracts were added in 2.9 mL of 0.15 mM DPPH solution and incubated in the dark for 15 min at 23 °C. 95% Methanol was used as blank. Scavenging activity was estimated based on the percentage of DPPH radical removed measured at a wavelength of 517 nm using the following equation:

$$\text{Scavenging effect (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{ x } 100$$

Trolox (Sigma Aldrich, USA) standard solutions ranging from 0.1 to 1.5 mM were prepared to obtain a calibration curve. The calibration curve was used to determine the scavenging effect, the results being expressed in TEAC - Trolox equivalent antioxidant capacity.

ABTS radical scavenging activity assay

The 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid - ABTS) cation scavenging activity was performed according to a modified protocol from Nithiyanantham *et al.* (2012). The ABTS cation stock solution was prepared by combining a 7mM ABTS (Sigma Aldrich, USA) solution and a 2.45 mM potassium persulfate $K_2S_2O_8$ (Sigma Aldrich, USA) solution. The stock solution was incubated in the dark, at 23 °C, for

15 h. A daily fresh working solution was prepared by diluting the stock solution and 95% methanol to an initial absorbance of approximately 0.70 ± 0.02 at 734 nm. 20 µL of extract concentrations were added to 2 mL working solution and incubated in the dark for 7 min at 23 °C where scavenging activity occurs between 20 - 80% (Nithiyanantham *et al*., 2012). Scavenging activity was estimated based on the percentage of ABTS removed measured at a wavelength of 734 nm. A standard curve was obtained using Trolox standard solutions in the 0.1 to 1 mM range. The scavenging activity of samples was expressed in Trolox equivalent antioxidant capacity (TEAC).

Reducing power assay

The reducing power was determined by the ferric reducing antioxidant power assay (FRAP) as described by Benzie and Strain, (1996) with modifications. Briefly, FRAP reagent was prepared in acetate buffer (0.3 M, pH 3.6), 10 mM solution of 2,3,5-Triphenyltetrazolium chloride (TPTZ; Sigma Aldrich, USA) in 40 mM HCl and $FeCl_3 \cdot 6\ H_2O$ (Sigma Aldrich, USA) at 10:1:1 respectively. Then, 100 µL of samples were added to 300 µL of deionized water and 3 mL of working solution. The samples were incubated in the dark, at 23 °C, for 4 min. A 593 nm wavelength was used to measure the absorbance. The calibration curve was constructed using $FeSO_4 \bullet 7H_2O$ (Sigma Aldrich, USA) methanolic standard solutions in different concentrations. Each sample was tested with three replicates. Reducing power was expressed as micromoles of Fe^{2+} equivalents per g of dried sample.

Fermentation of Andean blackberries with medicinal plants

The medicinal plants with the highest antioxidant profile were selected to incorporate into the fermentation process of the Andean blackberries with the following combinations: without medicinal plants (control), with 4 g of each dry plant · dm^{-3} of beverage (Treatment 1), and with 8 g of each dry plant · dm^{-3} of beverage (Treatment 2) (Rohan *et al*., 2014). After washing and weighing the Andean blackberries, water was added to make a 70% blackberry-content beverage. The raw Andean blackberry materials thus prepared were treated using a blender. The beverage was filtered using a mesh, and its pH was adjusted to 5.0. The fermentation process was carried out using adapted 4 L glass tanks at room temperature. These containers were filled with the beverage in a 1: 4 ratio (20% Andean blackberry and 80% water) (Ocaña, 2012) and a dry plant quantity specific to each treatment described above, leaving a headspace of 10 cm. Sterilization of the beverage in the different treatments was performed by adding potassium metabisulfite at a final concentration of 100 ppm and resting for 24 h at room temperature. Then, the sugar concentration was adjusted to 21° Brix by adding ordinary cane sugar, and it was immediately inoculated with Lalvin EC - 1118 (Lallemand Brewing, Canada), using 0.3 g yeast · dm^{-3} of beverage. The containers were corked, leaving a hole that allowed the CO_2 to escape through a valve, and another hole to take a sample to determine the fermentative activity by daily control of the density using a portable densimeter (Mettler Toledo). Once the fermentation process stabilized, the fermented was transferred to clean jars. In these, 100 ppm potassium metabisulfite and 200 ppm potassium sorbate were applied to prevent the proliferation of lactic and acetic acid bacteria and prevent oxidation of the beverage. The Brix degrees were also adjusted until reaching 11° by adding ordinary cane sugar, to finally add egg white albumin at a concentration of 0.15 g · dm^{-3} and let it settle. After eight days, the clarified drink was bottled in 750 mL pasteurized bottles and these were stored at room temperature (Ocaña, 2012).

Antioxidant capacity assessment of fermented beverages

The antioxidant capacity tests described above were performed using 1: 10 dilutions of the fermented drinks obtained from the different treatments with 95% methanol.

Sensory and organoleptic evaluation of the fermented drink

The organoleptic properties and sensory evaluation were conducted through a preference test. The panel consisted of ten trained participants selected from students from the Tourism Engineering of the Universidad de las Fuerzas Armadas ESPE, with knowledge in enology. Before evaluating the organoleptic properties, the samples were placed for 10 min at room temperature (22 ± 1 °C). The participants were asked to assess the sensory parameters divided into three categories: visual, olfactory, and gustatory. The organoleptic properties and sensory evaluation tests were realized based on the method of Cuenca *et al*. (2013) and Almanza-Merchán *et al*. (2015) with few modifications (Table 1). The score was settled within 0 (the lowest), and 7 (the maximum score). A one-way ANOVA was carried out on the results to determine significant differences between treatments.

Table 1. Sensory attributes and acceptability parameters and scores

Visual Attributes	**Scale**
Fluidity Limpidity Color Tone Color Intensity	0 = not perceived at all 7 = strongly perceived
Olfactory Attributes	**Scale**
Aroma Intensity Fructal/Floral Aroma	0 = not perceived at all 7 = strongly perceived
Gustative Attributes	**Scale**
Body Acidity Astringency (tannins) Sweetness Texture	0 = not perceived at all 7 = strongly perceived

Statistical analysis

The statistical analysis for the phytochemical and antioxidant activity of the plant extracts was carried out by using three replicates. Results were expressed by means ± SD (Standard Deviation). The statistical hypotheses were evaluated in a one-way analysis of variances (ANOVA) and a Tukey post hoc was performed for the comparison of differences between treatments. The statistics software XLSTAT pro (2013) was used to perform the analysis (Hammer *et al*., 2001). The Pearson correlation test used to analyze the relationship among metabolites and antioxidant activity of fermented beverages was conducted as described by Khiya *et al*. (2021) with modifications using the R Studio package for statistical analysis.

Results

To identify the most suitable Ecuadorian plant species to use in the new Andean blackberry fermented beverage, a number of ten known spices and medicinal species were analyzed. Through the quantification of total phenols and antioxidant capacity, it was possible to evidence significant differences in the different values of each plant sample. From the total analyzed plants, four plant species (arrayán, ishpingo, ataco, and plantain) were chosen based on their maximum values in all the methods used.

Quantification of total phenolic content and antioxidant activity of plants

The mean values of medicinal plant samples are determined in association with standard deviations (SD) and presented in Table 2.

A one-way ANOVA showed a significant difference ($p < 0.05$) for each plant sample. The total phenolic content varied between plant species, the highest value being registered in the case of *Amaranthus quitensis* (79.551 ± 0.222 mg TEAC · g^{-1} d.w.) and the lowest in the case of *Plantago major* (17.583 ± 0.187 mg TEAC · g^{-1} d.w.). The same trend was observed in the case of antioxidant activity identified through all three methods. Through DPPH and ABTS, no significant differences were observed between *Ocotea quixos* and *Plantago major* (Table 2).

A Pearson correlation was performed to identify the correlation between total phenolic content and antioxidant activity. The highest correlation (0.970) was observed between TPC and FRAP, and the lowest between DPPH and TPC (0.864) (Table 3).

Table 2. The total phenol content and antioxidant capacity of the medicinal plant samples used in the beverage

Medicinal plant species	TPC (mg GAE * g^{-1} d.w.)	Antioxidant activity		
		DPPH (μmol Trolox/g^{-1} d.w.)	ABTS (μmol Trolox/g^{-1} d.w.)	FRAP (Fe^{2+} mM /100g $^{-1}$ d.w.)
Myrcianthes hallii (Arrayán leaf)	58.649 ± 0.295 b	28.208 ± 0.166 b	30.015 ± 0.187 b	643.383 ± 0.126 b
Amaranthus quitensis (Ataco leaf)	79.551 ± 0.222 a	54.39 ± 0.314 a	43.306 ± 0.39 a	701.763 ± 0.717 a
Ocotea quixos (Ishpingo)	36.216 ± 0.226 c	24.753 ± 0.355 c	15.14 ± 0.104 c	360.896 ± 0.902 c
Plantago major (Plantain leaf)	17.583 ± 0.187 g	24.126 ± 0.151 c	15.677 ± 0.183 c	246.724 ± 0.126 d
Annona muricata (Guanábana leaf)	15.855 ± 0.160 e	2.360 ± 1.019 g	14.597 ± 0.329 c	145.187 ± 0.282 e
Phyllanthus niruri (Chancapiedra leaf)	20.661 ± 0.318 d	2.409 ± 0.15 g	15.296 ± 0.416 c	184.051 ± 0.158 e
Moringa oleifera (Moringa leaf)	7.267 ± 0.109 g	14.418 ± 0.385 d	8.887 ± 0.205 d	107.178 ± 0.732 f
Mansoa alliacea (Ajo de monte leaf)	7.176 ± 0.198 g	0.379 ± 0.038 h	1.66 ± 0.084 f	54.310 ± 0.287 g
Cymbopogon citratus (Hierba Luisa leaf)	11.579 ± 0.206 f	3.368 ± 0.118 f	1.454 ± 0.115 f	23.581 ± 0.594 h
Solanum nigrum (Hierba Mora leaf)	12.155 ±0.222 f	5.375 ± 0.136 e	3.845 ± 0.03 e	33.170 ± 0.196 g

Legend: TPC - total phenolic content, DPPH - DPPH radical scavenging activity assay, ABTS – ABTS radical scavenging activity assay, FRAP - ferric reducing antioxidant power assay. The values are represented as mean value ± SD (standard deviation). Different letters denote significant differences (Tukey post hoc $p < 0.05$).

Table 3. The Pearson correlation coefficients between the techniques used to determine the total phenol content and antioxidant capacity

	DPPH	FRAP	TPC	ABTS
DPPH	1	0.868**	0.864**	0.960**
FRAP		1	0.970**	0.954**
TPC			1	0.945**
ABTS				1

Legend: TPC - total phenolic content, DPPH - DPPH radical scavenging activity assay, ABTS – ABTS radical scavenging activity assay, FRAP - ferric reducing antioxidant power assay. ** The correlation is significant at the 0.01 level.

Antioxidant capacity assessment of fermented beverages

Concerning the total phenolic content and antioxidant capacity of the mixing blackberry and the tested plant species, it can be observed an improvement in both treatments compared with the control represented by the Andean blackberry must (Table 4). The statistical analysis showed significant differences ($p < 0.05$) between treatments. The best results were in the case of treatment 2 (blackberry must with 8 g of each dry plant $\cdot dm^{-3}$ of beverage.

Table 4. Total phenolic content and antioxidant capacity of the beverage treatments

Fermented beverages	TPC (mg GAE * g^{-1} d.w.)	Antioxidant activity		
		DPPH (µmol Trolox/g^{-1} d.w.)	ABTS (µmol Trolox/g^{-1} d.w.	FRAP (Fe^{2+} mM /100g^{-1} d.w.)
Control (Andean blackberry must)	0.409 ± 0.040 a	0.692 ± 0.009 a	0.635 ± 0.013 a	3.741 ± 0.136 a
Treatment 1	0.595 ± 0.016 a	1.027 ± 0.101 a	0.595 ± 0.016 a	4.305 ± 0.305 a
Treatment 2	0.849 ± 0.046 b	2.34 ± 0.104 b	0.849 ± 0.046 b	7.828 ± 0.137 b

Legend: TPC - total phenolic content, DPPH - DPPH radical scavenging activity assay, ABTS – ABTS radical scavenging activity assay, FRAP - ferric reducing antioxidant power assay. Treatment 1 – Andean blackberries with 4 g of each dry plant/L of beverage; Treatment 2 – Andean blackberries with 8 g of each dry plant/L of beverage. The values followed by different letters are statistically significant (Tukey post hoc $p < 0.05$).

The established correlation between phenolic compounds and antioxidant activity was evaluated for each treatment for the fermented beverages (Figure 1).

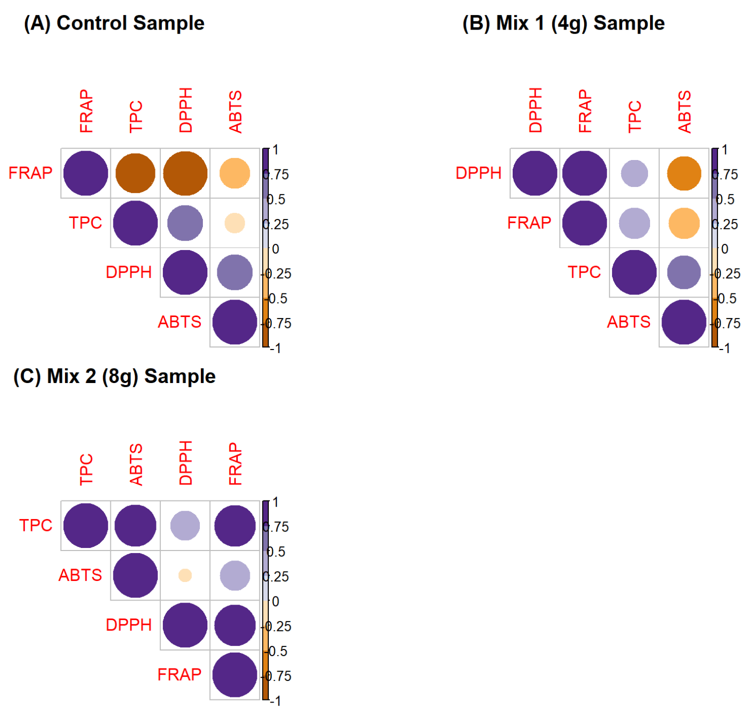

Figure 1. Correlogram representation of the correlation test for total phenolic content and antioxidant activity for every fermented beverage. (A) Correlation test for the control, (B) Correlation test for Treatment 1, (C) Correlation test for Treatment 2

Legend: TPC - total phenolic content, DPPH - DPPH radical scavenging activity assay, ABTS – ABTS radical scavenging activity assay, FRAP - ferric reducing antioxidant power assay. Treatment 1 - beverage mixed with 4 g of each plant, Treatment 2 - beverage mixed with 8 g of each plant.

A strong correlation is observed for treatment 2, which had shown higher phenolic contents than treatment 1 or control beverages. The most important correlation was found in treatment 2 between total phenolic content and ABTS (R = 0.86), followed closely by TPC and DPPH (R = 0.84). For treatment 1, the most relevant correlation was found between TPC and ABTS (R = 0.55), and for the control beverage, the highest positive correlation was described between TPC and DPPH (R = 0.63).

Sensory and organoleptic evaluation of the fermented drink

Statistical analyses were performed to identify the sensory and organoleptic evaluation of different mixes of beverages. To determine the significant differences between treatments, a one-way ANOVA test was performed. Regarding the sensory parameters, for the visual evaluation, it was found that T2 treatment showed the highest score for the four evaluated attributes, followed by T1 treatment and control. In the case of fluidity, there were differences between treatments and control (Figure 2).

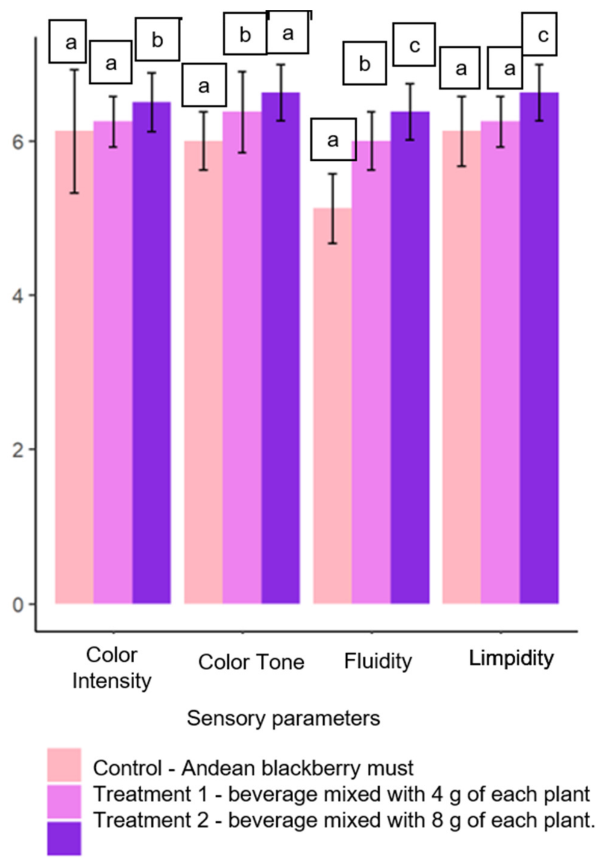

Figure 2. Visual evaluation of the Andean blackberry beverages
Control: Andean blackberry must, Treatment 1 - beverage mixed with 4 g of each plant, Treatment 2 - beverage mixed with 8 g of each plant. The values are represented as mean value ± SD (standard deviation). Different letters denote significant differences between treatments (Tukey post hoc p < 0.05).

The olfactory evaluation consisted of the intensity of the aroma the and floral/fructal aroma evaluation showed that in the case of the Intensity attribute, treatment 2 presented a higher score, with slight differences between treatment 1 and control. Instead, for the type of aroma there were slight differences between treatments, with treatment 1 presenting the highest score for a floral/fructal aroma, and the control sample having the lowest score (Figure 3).

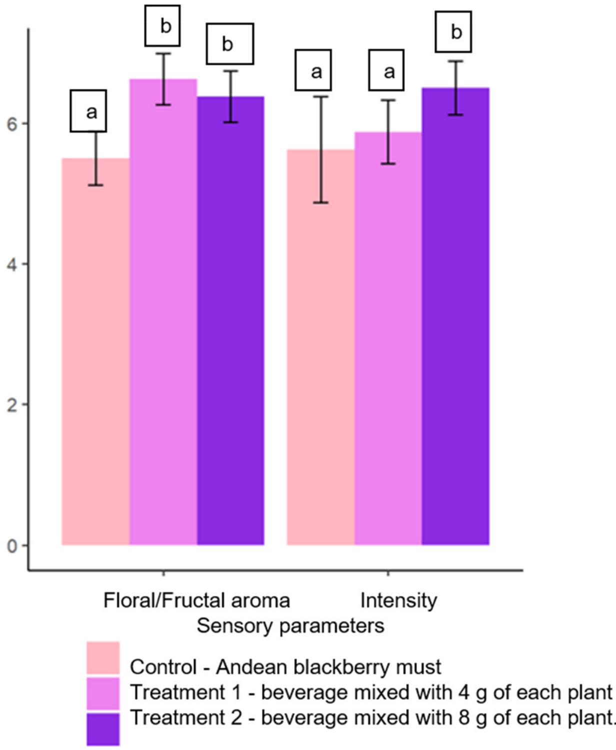

Figure 3. Olfactory evaluation of the Andean blackberry beverages
Control: Andean blackberry must, Treatment 1 - beverage mixed with 4 g of each plant, Treatment 2 - beverage mixed with 8 g of each plant. The values are represented as mean value ± SD (standard deviation). Different letters denote significant differences between treatments (Tukey post hoc $p < 0.05$).

Attributes like acidity, astringency, wine body, sweetness, and texture were tested for the gustative evaluation. For acidity and astringency attributes, T2 treatment showed the highest score, followed by T1 treatment and control. For wine body, sweetness, and texture, the T1 treatment has a higher score. The control sample presented a higher sweetness score compared with the T2 treatment, but for the other attributes, the score was the lowest (Figure 4).

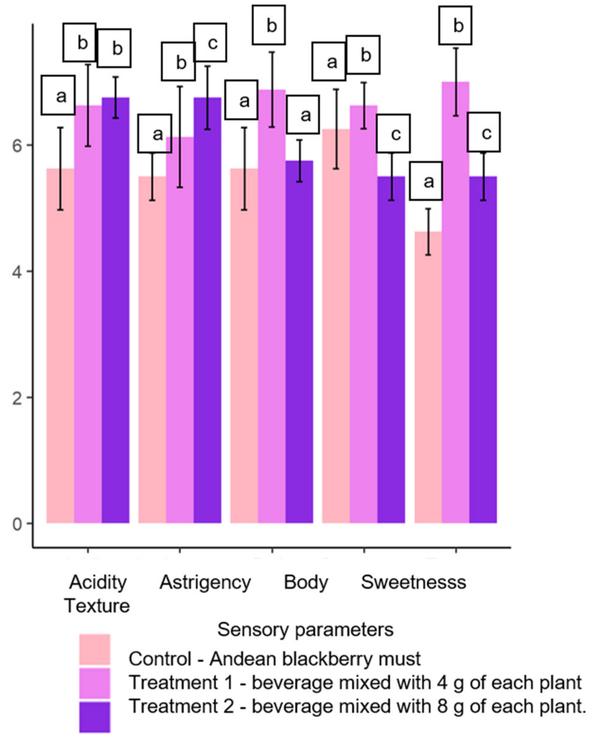

Figure 4. Gustative evaluation of the Andean blackberry beverages
Control: Andean blackberry must, Treatment 1 - beverage mixed with 4 g of each plant, Treatment 2 - beverage mixed with 8 g of each plant. The values are represented as mean value ± SD (standard deviation). Different letters denote significant differences between treatments (Tukey post hoc $p < 0.05$).

The overall results from the sensory evaluation showed no significant differences ($p < 0.05$) between Aroma and Appearance, with the T2 treatment (blackberry beverage mixed with 8 g of each plant) having higher values, while the T1 treatment (blackberry beverage mixed with 4 g of each plant), was characterized by a higher score for Taste (Figure 5).

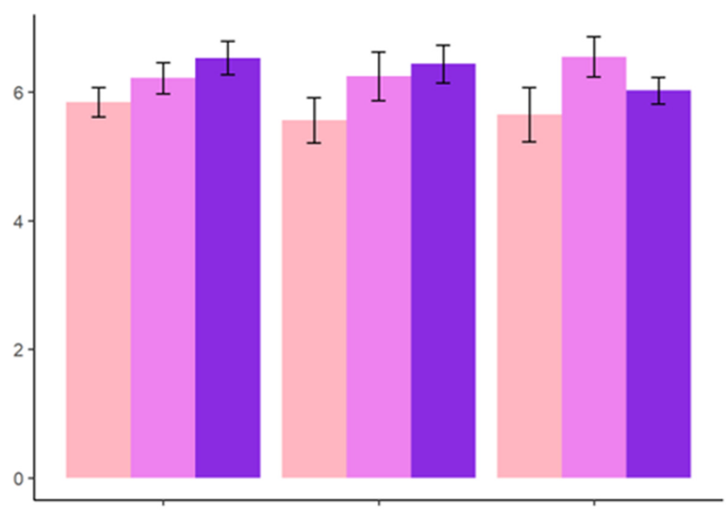

Figure 5. Overall evaluation of the appearance, aroma, and taste of the Andean blackberry beverages
Control: Andean blackberry must, Treatment 1 - beverage mixed with 4 g of each plant, Treatment 2 - beverage mixed with 8 g of each plant. The values are represented as mean value ± SD (standard deviation). Different letters denote significant differences between treatments (Tukey post hoc $p < 0.05$).

Discussion

Functional beverages play an essential role in our modern life and contribute to our well-being by improving the general physical conditions of the human body or decreasing the risk of disease progression. Functional beverages have the ability not only to reduce cancer risk, improve physical and mental conditions, and boost the immune system, but also to be an antistress, and antiaging (Tolun *et al*., 2019). The blackberry represents a good candidate for a nutraceutical beverage due to its bioactive compounds that render their beneficial health effects. Blackberries are used in various forms (ice cream, jelly, jam, tea, juice, etc.) in food and also in pharmaceutical industries due to their bioactive compounds - phenolic compounds (Kaume *et al*., 2012; Veberic *et al*., 2014). The phenolic compounds from blackberries have numerous health benefits (anti-carcinogenic, anti-inflammatory, anti-mutagenic, antiviral, antimicrobial, anti-atherosclerotic, anti-proliferative, antitumorigenic, and anti-neurodegenerative activities) (Turkben *et al*., 2010; Nile *et al*., 2014). Ecuadorian cultivars of Andean blackberry are relevant natural sources of high antioxidant compounds, as multiple studies have reported the composition of phenolic acids, such as gallic and p-coumaric acid derivates as well as proanthocyanidins and quercetin and kaempferol derivates (Alarcón-Barrera *et al*., 2018). As seen in multiple Andean plants, the Andean blackberry adapts to different heights above sea level in a range within 1.200 to 3.500 meters, with a temperature of 16 to 18 °C, ambient humidity of 70 to 80%, and rainfall of 1.200 to 1.700 millimeters per year (Ministerio de Agricultura y Desarrollo Rural, 2013). The Andean blackberry is the most consumed variety in Ecuador since this species is more resistant to common pests and diseases that affect this crop, and it also has higher productivity than the other varieties (Cevallos, 2020).

A fermented Andean blackberry beverage can become a functional drink due to the potency of the fermentation process which involves a series of reactions that modify the chemical components of the substrate and encourage the breakdown of plant cell walls, which helps to liberate or produce various antioxidant compounds. This biotechnological process could be considered a tool to increase the bioactive compounds and functional properties of fruit materials (Zhao *et al*., 2021).

Today's increased awareness of functional beverages has resulted in attempts to modify available drinks to have higher health benefits. Plants such as aromatic, spices, and medicinal ones that have high phenolic content and other bioactive compounds, can be used for the production of functional beverages mainly because of the antioxidant capacity of their phytochemicals (Jiménez-López *et al*., 2018).

The fermentation process that produces also ethanol, can represent a polar solvent for the extraction of bioactive compounds from the plants and helps improve the ratio of nutritive to antinutritive components in plants, which encourages the production of new functional foods or beverages.

The election of plant candidates for improving the biological characteristics of the functional beverage has been inspired by folk medicine and taking into account that plants even not native to Ecuador but grown in this country characterized by a high UV intensity, can have increased antioxidant activity due to the synthesis of bioactive compounds necessary to protect itself from the abiotic stress.

Quantification of total phenolic content and antioxidant activity

Starting from ancient times humans earned to use the secondary metabolites from bark, seeds, fruits, and other parts of the plant, due to the need for medicines to fight against the diseases that have always plagued our species. Science has allowed us to elucidate the role of metabolites present in plants that are part of traditions from different regions, giving rise to drugs whose active ingredient is of plant origin (Petrovska, 2012).

Enriching food with medicinal plant species characterized by antioxidant capacity may present some advantages like preserving food quality by protecting the primary and secondary metabolites from oxidation, rancidity, and resulting degradation and also, promoting human health through antioxidants (National Academy of Sciences, 2000).

Natural antioxidants in beverages are getting more and more attention due to their health and functional properties, and the expectation is that the development of natural antioxidants, such as flavonoids, and phenolic compounds inside plants, will enhance safety (Zhao *et al*., 2021).

Screening the literature, no studies concerning the antioxidant capacity of *Amaranthus quitensis* (ataco) and *Myrcianthes hallii* (arrayán) plant species were identified. In the case of ataco, Poveda, (2013) registered a 94.61 mg · g $^{-1}$ GAE of phenols, a comparative value with that obtained in the present investigation. Carvajal et al. (2016) reveal the high phenolic content (> 29 flavonoids) in Arrayán. Noriega and Dacarro (2008) confirm the presence of flavonoids and phenolic compounds in *Ocotea quixos* foliar samples. In *Plantago major* (plantain) samples, Mohammed et al. (2011) and Lukova et al. (2018) detail the total content of phenols (13.05 and 17.18 mg ·g $^{-1}$ GAE respectively), similar values to each other and to that obtained in our study (17.58 mg · g $^{-1}$ d.w. GAE). The antioxidant activity of this species was revealed in several studies using different techniques of evaluation (Mohammed *et al.,* 2011; Lukova *et al.,* 2018; Speisky *et al.,* 2011).

Characterization of the blackberry fermented drink

The fermentation process of beverages is built on the yeast's ability to convert sugar into alcohol, esters, and other volatile and non-volatile compounds (Duarte *et al*., 2010). During fermentation, the phenolic content and antioxidant activity are increased (Johnson *et al*., 2013).

According to Larsson et al. (2000), the phenolic compounds present in plants can inhibit the processes of alcoholic fermentation since they are metabolites that can be involved in the defense mechanisms of the plant against pathogenic agents. In our investigation, no inhibition was observed in the fermentation of plant treatments. Possible causes may be represented by some phenolic compounds that inhibit fermentation and others do not depend on the structure of the compound in contact with the yeast. It is so that it can correlate the structural conformation of the molecule to its toxicity, which this defined by the position of functional groups or unsaturated carbon-carbon bonds that play a fundamental role in its toxicity (Temitope *et al*., 2014). Another possible cause may be represented by that *S. cerevisiae* has developed a certain tolerance to this type of compound and the fact that these have a negative in its development is more related to the type of compound and the concentration of it in the must (Adeboye *et al.*, 2014).

In our research, we chose four medicinal plants to enrich blackberry wine and to investigate their effect on the total phenol content and antioxidant capacity. In the quantification of total phenols and antioxidant capacity of the treatments, a significant increase was observed related to the concentration of plants in contact with the must during the process of fermentation. This means that the polar compounds dissolved in the drink end, which provides this quality to the drink, are not affected by the process of fermentation, nor by clarification. Despite this, Cuellar et al. (2017) show that the phenolic content of the must decreases if the fermentation process is maintained active despite having reached the Brix stabilization point, the cause of this phenomenon may be related to the reduction of compounds that are produced in the stationary phase of yeast growth (Caridi *et al*., 2004; Chen *et al*., 2005). The maximum concentration of total phenols was found in the T2 treatment (0.849 ± 0.046 mg/g GAE), the value is lower than that obtained in wines from grapes between 1.3 mg/g and 3.5 mg/g GAE (Salazar *et al*., 2011). Likewise, the antioxidant capacity value obtained by DPPH assay in the T2 treatment (2.34 ± 0.104 mg Trolox · dm^{-3}) may be compared with that from grape wines but is less than the range obtained by Stratil et al. 2008 (between 2.91 to 7.3 mg Trolox · dm^{-3}).

The reason that the total content of phenols and the antioxidant capacity are less than the values mentioned, is that the drink is carried out at a concentration of 20% (V/V).

Sensory and organoleptic evaluation of the fermented drink

The sensory evaluation demonstrated that there is a significant difference between the control and T2 treatment, with the aroma the organoleptic characteristic that presented a different value evaluated by the tasters. The most appealing aroma for tasters in the T2 treatment is due to the presence of volatile compounds, which do not necessarily have to be related to antioxidant capacity or phenolic compounds, this is the case of the monoterpenes described by Rodríguez et al. (2016).

Previous studies have described health benefits and sensory properties attributed to fermented berry products that are related to high amounts of polyphenolic compounds present in blackberries (Cordeiro Caillot *et al*., 2018), as found in the present study in the case of T2 treatment.

Conclusions

The market for functional beverages represents the largest and fastest-growing segment of the functional foods sector. Functional beverages impart various health implications upon consumption due to the presence of various bioactive components, such as phenols, flavonoids, carotenoids, peptides, vitamins, and minerals. The ingredients that are building blocks of the beverages are especially fruit or vegetable juices. In our case, due to the multitude of bioactive compounds contained, we chose the Andean blackberry. Fermentation is a biotechnological process that can improve the nutritional value and organoleptic characteristics of the beverage. The addition of plants to the fermentation process significantly increased the total phenol content, antioxidant capacity, and sensory evaluation of the Andean blackberry beverage. The total concentration of phenols and antioxidant capacity were different significantly between treatments. As stated above, the addition of 4 g of each herbal selected sample enhanced the antioxidant activity of the functional beverage based on Andean blackberry, being the one with the highest values of the analyzed characteristics and suggesting that these functional beverages might protect against oxidative stress caused by psychological stress. This work provides the influence of plant addition to the fermented Andean blackberry functional beverage on its antioxidant activity, phenolic content, and organoleptic characteristics.

Authors' Contributions

Conceptualization RAM; Formal analysis NJLG; Funding acquisition RAM; Methodology NJLG; Writing - original draft RAM and RDC; Writing - review and editing RAM and RDC. All authors contributed equally to this work. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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