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ORIGINAL ARTICLE

Antioxidant and antimicrobial activity of mortiño (Vaccinium floribundum Kunth) against multi-resistant pathogenic bacteria

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ABSTRACT

Introduction: bacterial resistance to antibiotics represents a critical global public health problem, prompting the search for new natural antimicrobial sources to combat multidrug-resistant bacteria.
Objective: to evaluate the antioxidant and antimicrobial activity of *Vaccinium floribundum* Kunth from Ecuador against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*.
Methods: ethanol extracts were obtained by maceration (leaves) and Soxhlet extraction (fruit), yielding 11,18 % and 41,16 %, respectively. Qualitative phytochemical screening identified secondary metabolites. Antioxidant capacity was assessed using the DPPH assay. Antimicrobial activity was determined by agar diffusion method against *L. monocytogenes*, *S. aureus*, *B. cereus*, and *E. coli*.
Results: leaves contained flavonoids and tannins; fruits contained flavonoids, tannins, phenols, and diterpenes. Antioxidant capacity was 86,42 % (leaf) and 27,93 % (fruit). Leaf extract inhibition zones were: *L. monocytogenes* (20,67 mm), *S. aureus* (17,83 mm), *B. cereus* (18,50 mm), and *E. coli* (13,67 mm). Fruit extract showed lower activity: *L. monocytogenes* (14,00 mm), *S. aureus* (11,67 mm), *B. cereus* (11,50 mm), and *E. coli* (9,83 mm). The highest inhibition percentage compared to vancomycin was for *S. aureus*: 89 % (leaf) and 38,4 % (fruit).
Conclusions: the leaf extract demonstrated superior antioxidant and antimicrobial activity against both Gram-positive and Gram-negative bacteria, representing a potential natural source for the development of therapeutic agents against multidrug-resistant bacteria.

Keywords: Plant Extracts; Phytochemicals; Products With Antimicrobial Action; Vaccinium.

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INTRODUCTION

Bacterial resistance to antibiotics poses a growing threat to global public health, generating considerable levels of morbidity and mortality and high healthcare costs.[1,2] This phenomenon is mainly attributed to the excessive and inappropriate use of antibiotics, leading to therapeutic ineffectiveness and hindering the management of common infections such as pneumonia, urinary tract infections, tuberculosis, gonorrhea, and sepsis. It is estimated that approximately 50% of antibiotic prescriptions are inappropriate and at least 30% are administered unnecessarily, a situation that favors the spread of multidrug-resistant bacteria and the emergence of serious diseases.[3,4,5]

Globally, in 2019 approximately 4,95 million deaths were associated with multidrug-resistant bacterial infections, of which 1.27 million were directly attributable to antimicrobial resistance.[6] In Latin America and the Caribbean, this problem caused 338,000 deaths related to antibiotic resistance and 84,300 directly attributed deaths, transmitted through direct contact, contaminated food, fecal-oral and respiratory routes.[7] In Ecuador, the Ministry of Public Health reported 8,924 cases and 93 cases of diseases transmitted by contaminated food and water in 2020 and 2021 respectively.[8,9]

Particularly worrying is the high level of resistance identified in rural areas of Ecuador, where Escherichia coli shows 79,8 % resistance to ampicillin and Staphylococcus aureus shows 55,4 % resistance to oxacillin.[10,11,12] Faced with this problem, the World Health Organization established a list of priority bacteria that have shown resistance to different third-generation antibiotics, promoting research and innovation of potential products with high antimicrobial activity capable of counteracting these multidrug-resistant bacteria.

In this context, medicinal plants represent an extraordinary source for the development of new drugs against infectious diseases. Historically, plants have been the primary therapeutic alternative for 80 % of the world's population, and particularly in rural areas of Ecuador, to address various health problems.[13,14]

The medicinal value of plants depends primarily on their secondary metabolites, which exert antimicrobial, anticancer, anti-inflammatory, antidiabetic, antioxidant, and antidiuretic activities, among others. This therapeutic versatility has generated considerable scientific interest due to the efficiency of these new compounds and concerns about the side effects of modern medicine. Several studies have documented antibacterial mechanisms of medicinal plants against specific pathogens, including Eucalyptus globulus, Vaccinium macrocarpon, and Cinnamomum verum.[15,16]

In research conducted in Ecuador on the use of medicinal plants as potential antimicrobials, the methanolic extract of dandelion and guaviduca showed a high bactericidal effect against E. coli.[17] On the other hand, in the study by Bayas et al.,[18] it was demonstrated that the ethanolic extract of chilca leaf and flower was more effective against Listeria and Salmonella, while the stem extract was more effective at inhibiting E. coli. Likewise, the genus Vaccinium spp. is important due to its antioxidant capacity and its potential health benefits, as it contains phenolic acids, flavonoids, proanthocyanidins, coumarins, hydrolyzable tannins, carotenoids, and anthocyanins.[19]

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Rev Ciencias Médicas. 2025; 29(S1): e6950

According to Garzón et al.,[20] the anthocyanins and other antioxidants in this berry exhibit anti-inflammatory, anticancer, and antineurodegenerative effects, and also possess antimicrobial activity against Gram-positive and Gram-negative microorganisms. Thus, the antioxidant and antimicrobial capacity of plant sources represents a potential therapeutic alternative for the pharmaceutical industry in the treatment of infectious diseases and could also be a nutraceutical with high potential. For this reason, the present research was conducted, with the objective of evaluating the antioxidant and antimicrobial activity of the Ecuadorian species Vaccinium floribundum Kunth against Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Listeria monocytogenes.

METHODS

A non-observational, quasi-experimental, prospective longitudinal study was developed, using basic microbiological laboratory equipment, certified analytical reagents and reference bacterial strains: Staphylococcus aureus ATCC 12600, Listeria monocytogenes ATCC 19115, Bacillus cereus ATCC 10876 and Escherichia coli ATCC 11775. The plant material of Vaccinium floribundum Kunth (leaves and fruits) was collected in the Santa Rosa de Totoras community, belonging to the San Miguel canton, Bolívar province (Ecuador).

The fresh material underwent a disinfection process by immersing it for five minutes in a solution of 20 mL of 5 % sodium hypochlorite diluted in 100 mL of distilled water; subsequently, it was rinsed thoroughly with water to remove any disinfectant residue. The samples were oven-dried at 50 °C for four days, pulverized using a laboratory mill, and stored in airtight Ziploc-type bags until analysis.

Vegetable extracts

For the leaf sample, a hydroalcoholic extraction was implemented in which 100 g of the powdered sample was mixed with 500 ml of 96 % ethanol for two days with periods of agitation and stored at room temperature in the dark. The liquid extract was collected, and the process was repeated for one day before filtration. For the fruit sample, the Soxhlet extraction method was used. 500 ml of 96 % ethanol was added to the flask, and a filter paper cartridge containing 25 g of the sample was placed inside the extraction tube. The mixture was heated until all the solvent was recirculated through the sample. To separate the solvent from the leaf and fruit extracts, they were evaporated using a rotary evaporator and stored at 4°C until further analysis.[21,22] The yield of the extracts was calculated using the following equation:

$$\%Rendimiento = \frac{X_{\text{Peso del extracto después de la evaporación del solvente}}}{X_{\text{Peso seco del polvo vegetal antes de la extracción}}} * 100$$

Phytochemical screening

The qualitative analysis of secondary metabolites from the leaf and fruit of the mortiño was carried out using seven detailed tests by Abubakar[23] and Pujol,[24] with certain modifications.

- Dragendorff's essay: s1 ml of the extract and 1 ml of Dragendorff's reagent were placed in a test tube and homogenized; the presence of an orange-red precipitate is positive for alkaloids.
- Foam Test: s1 ml of the extract was diluted with distilled water 5 times and shaken vigorously for five minutes; the formation of 2 mm of foam in the test tube indicates the presence of saponins.

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(S1): e6950

- Shinoda's essay: s1 ml of the extract, 1 ml of concentrated hydrochloric acid and pieces of metallic magnesium ribbon were mixed, left to stand for five minutes and 1 ml of amyl alcohol was added; the change of color to deep red indicates a positive test for flavonoids.
- Ferric Chloride Test: sThree drops of 5 % ferric trichloride were added to 1 ml of the extract and shaken; the bluish-black coloration indicates the presence of phenols.
- Gelatin Test: s1 ml of the extract was mixed with a 1 % gelatin solution and shaken; the test is positive when a white precipitate forms.
- Borntrager's essay: sThe extract was dissolved with 1 ml of chloroform, 1 ml of 5 % sodium hydroxide and shaken, left to stand until the separation of the phases and the change of color of the aqueous alkaline phase to red or pink, the test is positive for quinones.
- Copper acetate test: sThe extract was dissolved in distilled water and three drops of copper acetate were added; the solution is positive for diterpenes by taking on an emerald green color.

Antioxidant capacity

Antioxidant activity was determined by DPPH free radical scavenging according to Ginting et al.,[25] with modifications. 0,0059 g of DPPH was dissolved in 100 mL of methanol-water (80:20) to form a DPPH stock solution at a concentration of 150 µM. This solution was covered with aluminum foil and stirred for 30 min. A 500 µM Trolox stock solution was used as a standard, prepared by dissolving 0.0125 g of Trolox in 100 mL of methanol-water (50:50) and stirring. From this solution, dilutions of 50, 100, 200, 300, 400, and 500 µM were prepared. The blank (180 µl of DPPH diluent + 20 µl of distilled water), control (180 µl of DPPH solution + 20 µl of distilled water), and extract (180 µl of DPPH solution + 20 µl of diluted extract) were added to a 96-well microplate. The microplate was incubated at room temperature in a dark place, covered with aluminum foil, for 40 minutes. Finally, the absorbance was measured at 515 nm using a spectrophotometer. The experiment was performed in quadruplicate, and the antioxidant activity was calculated using the following equation:

$$\%\ Inhibición\ DPPH = \left[1 - \left(\frac{A_{\text{absorbancia de la muestra}} - A_{\text{absorbancia del blanco}}}{A_{\text{absorbancia del control}} - A_{\text{absorbancia del blanco}}}\right)\right] \times 100$$

Antimicrobial activity by diffusion on agar

The method used was... Carrillo,[26] with some modifications. First, the bacteria were activated with Mueller-Hinton broth (5 ml) and incubated at 37°C with shaking at 200 rpm for 24 hours. To achieve a turbidity of 0,5 McFarland in the bacterial culture (BaCl2 1 % and H2SO4 1 %), the culture was diluted with broth. The absorbances of the bacterial cultures were read at 540 nm to verify that they were within the McFarland range. Subsequently, the standardized culture was inoculated with sterile swabs across the entire surface of a Petri dish containing Mueller-Hinton agar. Using the tip of a sterile blue tip, four 7 mm diameter wells were made. In each well, 100 µl of the leaf and fruit extract at different concentrations of 1, 10, 25, 50, 100 mg/ml, and 500 µg/ml was added, along with a positive control (gentamicin and vancomycin) and a negative control (sterile distilled water). Six replicates were performed for each concentration. The samples were incubated at 37°C for 24 hours, and the inhibition zones were measured in millimeters. The percentage of inhibition was calculated using the following equation:

$$\%\ Inhibición\ = \left(\frac{\emptyset\ HE_{\text{Diámetro del halo extracto}} - \emptyset\ HB_{\text{Diámetro del halo blanco}}}{\emptyset\ HC_{\text{Diámetro del halo control positivo}} - \emptyset\ HB_{\text{Diámetro del halo blanco}}}\right) \times 100$$

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(S1): e6950

Statistical analysis

The antimicrobial activity of the obtained extracts was evaluated against the four bacterial strains using established methods for microbial inhibition assays. For the statistical analysis of the inhibition zones, an analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparison test with a 95 % confidence level, using STATGRAPHICS software.

RESULTS

The yield of the fruit extract (41,163 %) was significantly higher than that of the leaf extract (11,189 %), which is attributed to the extraction method used and the content of soluble compounds in each plant matrix.

The secondary metabolites flavonoids and tannins were present in greater intensity in both extracts (Table 1). Saponins, phenols, and diterpenes were also identified in the leaf at low concentrations, while alkaloids and quinones were absent. Phenols and diterpenes were additionally detected in the fruit at moderate intensity, with only quinones absent.

Table 1. Secondary metabolites identified in extracts of V. floribundum Kunth.

Metabolites	Rehearsal	Sheet	Fruit
Alkaloids	Dragendorff	-	+
Saponins	Foam	+	+
Flavonoids	Shinoda	++	++
Tannins	Gelatin	++	++
Quinones	Borntrager	-	-
Phenols	Ferric Chloride	+	++
Diterpenes	Copper acetate	+	++

Grades: The plus sign (+) indicates presence and the minus sign (-) indicates absence. Metabolite intensity: (++) Moderate and (+) Low.

The antioxidant capacity of the leaf extract (86,422 % DPPH inhibition, 488,344 µmol Trolox Equivalent/L) was significantly higher than that of the fruit extract (27,930 %, 152,367 µmol Trolox Equivalent/L), demonstrating high antioxidant potential attributable to the content of polyphenolic compounds, especially flavonoids and tannins (Table 2).

Table 2. Antioxidant capacity of leaves and fruits of V. floribundum Kunth.

Plant matrix	% Inhibition	µmol Trolox Equivalent/L
Fruit	27,930 ± 1,016	152,367 ± 5,780
Sheet	86,422 ± 0,275	488,344 ± 6,163

The concentration of 100 mg/ml exhibited maximum antimicrobial activity. The leaf extract showed sensitivity in S. aureus (17,83 mm), L. monocytogenes (20,67 mm), and B. cereus (18,50 mm), with E. coli in the intermediate range (13,67 mm). The fruit extract showed moderate antimicrobial activity, with L. monocytogenes (14,00 mm) as the only microorganism

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(S1): e6950

in the susceptible range, while S. aureus, B. cereus, and E. coli showed resistance. The best percentage of inhibition compared to positive controls was S. aureus (89 % leaf, 38,4 % fruit) with respect to vancomycin, and L. monocytogenes (68,3 % leaf, 35 % fruit) with respect to gentamicin. Statistical analysis confirmed significant differences (P ≤ 0,05) between concentrations and controls (Table 3).

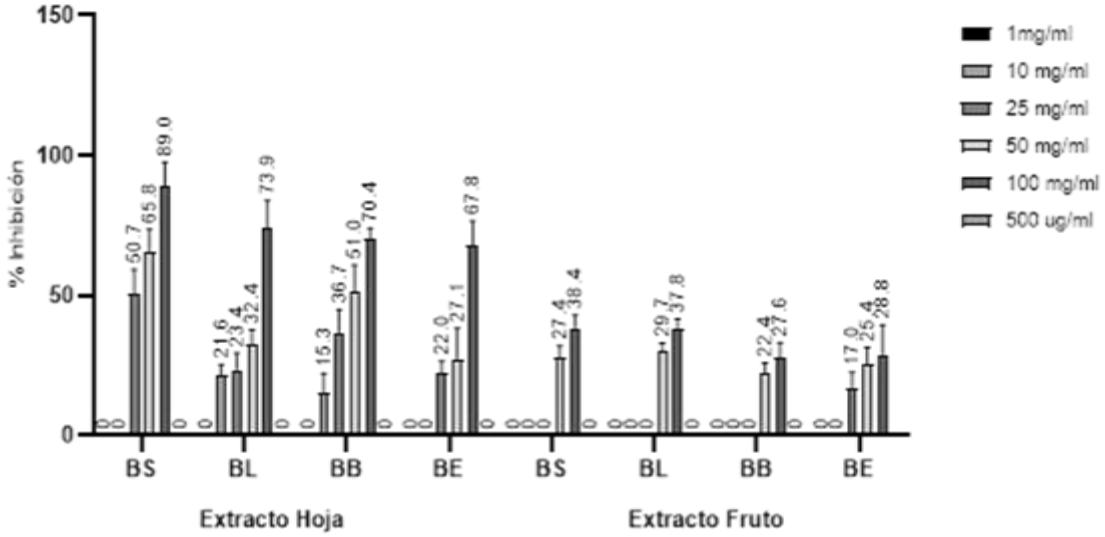
Table 3. Inhibition halos (mm) of V. floribundum Kunth extracts against pathogenic bacteria.

Extract	Concentration	S. aureus Average (OF)	L. monocytogenes Average (OF)	B. cereus Average (OF)	E. coli Average (OF)
Sheet	1 mg/ml	0	0	0	0
Sheet	10 mg/ml	0	11,000 (0,632)	9,500 (1,049)	0
Sheet	25 mg/ml	13,167 (0,983)	11,333 (1,033)	13,000 (1,265)	9,167 (0,408)
Sheet	50 mg/ml	15,000 (0,894)	13,000 (0,894)	15,333 (1,506)	9,667 (1,033)
Sheet	100 mg/ml	17,833 (0,983)	20,667 (1,751)	18,500 (0,548)	13,667 (0,816)
Sheet	500 µg/ml	0	0	0	0
Sheet	Vancomycin	19,167 (0,753)	25,500 (1,049)	23,333 (0,816)	16,833 (1,169)
Sheet	Gentamicin	24,667 (1,033)	27,000 (1,549)	28,167 (0,983)	22,333 (1,033)
Fruit	1 mg/ml	0	0	0	0
Fruit	10 mg/ml	0	0	0	0
Fruit	25 mg/ml	0	0	0	8,667 (0,516)
Fruit	50 mg/ml	10,333 (0,516	12,500 (0,548)	10,667 (0,516)	9,500 (0,548)
Fruit	100 mg/ml	11,667 (0,516)	14,000 (0,632)	11,500 (0,837)	9,833 (0,983)
Fruit	500 µg/ml	0	0	0	0
Fruit	Vancomycin	19,167 (0,753)	25,500 (1,049)	23,333 (0,816)	16,833 (1,169)
Fruit	Gentamicin	24,667 (1,033)	27,000 (1,549)	28,167 (0,983)	22,333 (1,033)

Grades: SD (standard deviation). Statistical analysis ANOVA with Tukey test (P ≤ 0,05)

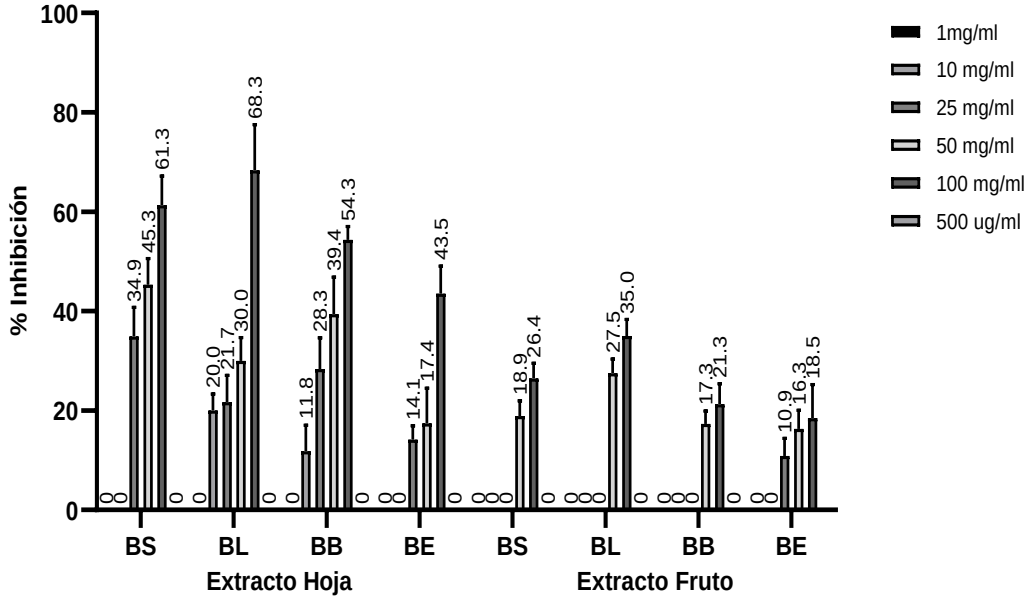
Comparative analysis of antimicrobial efficacy against reference antibiotics revealed varying percentages depending on the bacteria tested and the control used (Figs. 1 and 2). Compared to vancomycin, the leaf extract achieved remarkable inhibition percentages: S. aureus (93,0 %), E. coli (81,2 %), L. monocytogenes (81,0 %), and B. cereus (79,3 %), demonstrating high antimicrobial activity approaching that of the reference antibiotic. The fruit extract showed lower percentages against vancomycin: S. aureus (60,9 %), E. coli (58,4 %), L. monocytogenes (54,9 %), and B. cereus (49,3 %), maintaining moderate activity but consistently lower than that of the leaf extract. Compared to gentamicin, the leaf extract showed the following inhibition percentages: L. monocytogenes (76,5 %), S. aureus (72,3 %), B. cereus (65,7 %), and E. coli (61,2 %), while the fruit extract showed the following inhibition percentages: L. monocytogenes (51,9 %), S. aureus (47,3 %), E. coli (44,0 %), and B. cereus (40,8 %). These results confirm that the leaf extract has significantly higher antimicrobial activity than the fruit extract, achieving between 61,2 % and 93,0 % of the efficacy of conventional antibiotics depending on the bacteria and control used.

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(S1): e6950



Grades: The extracts were evaluated at a concentration of 100 mg/ml against four pathogenic bacteria.

Fig. 1 Percentage of antimicrobial inhibition of V. floribundum Kunth extracts compared to Vancomycin.



Grades: The extracts were evaluated at a concentration of 100 mg/ml against four pathogenic bacteria.

Fig. 2 Percentage of antimicrobial inhibition of V. floribundum Kunth extracts compared to Gentamicin.

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(S1): e6950

DISCUSSION

As a result of a soxhlet extraction, a high percentage yield of 41,163 % was obtained from the fruit extract, while 11,189 % was obtained from the leaf extract by maceration, yields that show differences with the results obtained by Gomes,[27] from the extract of the Vaccinium myrtillus berry, which was 12,84 %.and the 41 % reported by Koshovyi,[28] in the leaves of Vaccinium corymbosum L.

According to Frías and Rosales,[29] factors such as temperature, contact time with the solvent, solvent/mass ratio, solvent concentration, particle size, solvent polarity, chemical composition of the components to be extracted and molecular size influence the performance of an extraction.

In the leaf of the mortiño V. floribundum Kunth there was a greater intensity of flavonoids and tannins, showing similarities in the study of the phytochemical profile of the leaf of V. vitis-idaea L. where there was a higher content of flavonoids.[30] Another study of the leaf of V. myrtillus shows that the polyphenols that stand out are hydroxycinnamic acids, flavanols, flavonols and flavanolignans and anthocyanins, in addition to small amounts of triterpenes.[31] According to Llivisaca et al.,[32] the content of polyphenols and other bioactive compounds can vary significantly by growth conditions such as altitude and temperature, geographical location of the mortiño, radiation and by the development and maturation of the fruit and leaf.

Furthermore, flavonoids, tannins, phenols, and diterpenes were significantly present in the fruit extract compared to the leaf, metabolites that are similar to those found in research on the Andean blueberry (V. floribundum Kunth) from the Ecuadorian highlands, in a study conducted by Alarcón et al.,[33] which found high content of flavonoids, tannins, phenolics, anthocyanins, and lutein. Likewise, the fresh and fermented extract of V. floribundum Kunth fruit from Peru showed anthocyanins, flavonoids, phenolic acids, and proanthocyanidins, with the presence of quinones in the fermented extract.[34]

In the analysis of antioxidant activity, a higher percentage of DPPH inhibition was recorded in the leaf extract (86,422 % and 488,344 µmol Trolox Equivalent/L) compared to the fruit extract (27,930 % and 152,367 µmol Trolox Equivalent/L). In another study by Ștefănescu,[35] high percentages of DPPH inhibition were recorded in the leaves of three Romanian blueberry varieties: V. corymbosum L. (Toro, Elliot, and Nelson) at 70,41 %, 68,42 %, and 58,69 %, respectively; V. myrtillus L. at 61 %; and V. vitis-idaea L. at 63 % and 490.85 µM TE/g.[36,37]

Even so, the antioxidant capacity of the V. floribundum Kunth leaf extract under study remains high; this could be attributed to the variable content of polyphenols, anthocyanins, and flavonoids. On the other hand, the DPPH inhibition percentage of the fruit extract is approximately similar to that reported by Bunea et al.,[38] for V. chlorosibyum Duke (29,96 %), Bluecrop (46,64 %), and Elliot (43,48 %).

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Rev Ciencias Médicas. 2025; 29(S1): e6950

There is greater inhibition in the leaf and fruit extract at a concentration of 100 mg/ml. Forming inhibition halos (mm) in L. monocytogenes of20,667 ± 1,751, B. cereus with18,500 ± 0,548, S. aureus with17,833 ± 0,983and E. coli with13,667 ± 0,816 in the leaf extract. Furthermore,*S. aureus*(89 %)and*L. monocytogenes*(68,3 %)They showed a higher percentage of antimicrobial activity compared to the positive controls. According to the study reported by Llivisaca et al.,[32] *S. aureus*It exhibits an inhibition zone of 12 mm (resistant), and E. coli of 29 mm (sensitive) to the extract from the sheet of*V. Floribundum* Ecuadorian Kunth. Another study mentions the inhibitory potential of the leaf of*V. Oxycoccos* facing *E. coli* (23,17 mm) and S. aureus (25,48 mm).[39]

Whereas, in the fruit extract, the inhibition halos were in*L. monocytogenes*of 14,000 ± 0,632,*S. aureus*with11,667 ± 0,516,*B. cereus*with11,500 ± 0,837 and E. coli with9.833 ± 0,983, and similarly, the antimicrobial percentages with the two positive controls were low against all pathogens compared to the leaf. These data are similar to those presented by Brzezowska et al.,[40] who indicated that the fruit powder from three variations of V. corymbosum L. showed no antimicrobial activity against E. coli, S. enterica, S. aureus, and L. monocytogenes (data not shown). Likewise, low inhibition zones were reported in V. myrtillus L. against S. aureus (7 mm), E. coli (9,1 mm), B. subtilis (7 mm), and B. cereus (10,26 mm).[41]

CONCLUSIONS

Vaccinium floribundum Kunth (mortiño) from Ecuador is notable for its richness in bioactive compounds, with flavonoids and tannins as predominant metabolites that support its antioxidant and antimicrobial potential. The leaf extract showed significantly higher antioxidant activity than the fruit extract, achieving 86,42 % DPPH radical inhibition and 488,34 µmol TE/L, attributed to its high concentration of polyphenols capable of neutralizing free radicals. Regarding antimicrobial activity, the leaf extract was effective against Gram-positive and Gram-negative pathogenic bacteria, generating high inhibition zones against L. monocytogenes, B. cereus, and S. aureus, with sensitivities comparable to conventional antibiotics. The fruit extract showed reduced efficacy, with sensitivity only to L. monocytogenes. These findings position mortiño, especially its leaves, as a promising source of compounds with therapeutic, food, and nutraceutical applications, recommending further studies to isolate and characterize its active components.

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This study did not receive external funding. The research was conducted using the researchers' own resources and those of the participating institutions.

Conflict of Interest
The authors declare that they have no conflicts of interest in relation to the research, authorship and publication of this scientific article.

Authorship Contribution
EJGP: Conceptualization, Research, Methodology, Formal Analysis, Writing - original draft, Writing - revision and editing.
GLVA: Supervision, Validation, Resources, Writing - review and editing, Project management.
EEVA: Methodology, Formal Analysis, Validation, Writing - revision and editing.
IRTU: Resources, Data curation, Visualization, Writing - review and editing.
All authors have read and approved the final version of the manuscript.

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