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BIOTECHNOLOGY

Antimicrobial potential of *Vaccinium floribundum* extract fluid against *Staphylococcus saprophyticus* causing urinary tract infections

Potencial antimicrobiano del extracto fluido de *Vaccinium floribundum* frente a *Staphylococcus saprophyticus* causante de infecciones de vías urinarias

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Abstract

Vaccinium floribundum, belonging to the Ericaceae family, was evaluated for its antimicrobial, antioxidant, anti- inflammatory, and anticancer properties due to its high anthocyanin content. The objective of this study was to determine the content of anthocyanins present in the fluid extract of *V. floribundum*, as well as to evaluate its antimicrobial activity in the prevention of UTIs, establishing the minimum inhibitory concentration (MIC) against *Staphylococcus saprophyticus* ATCC 15305, a causative agent of urinary tract infections. The study was conducted at the Life Sciences Laboratories of the Salesian Polytechnic University, beginning with the preparation of 20 % and 50 % ethanolic extracts from fruits harvested in the Province of Cotopaxi, Sigchos Canton; the assays were performed *in vitro*. The concentration of total phenols was quantified using the Folin-Ciocalteu method, yielding 537.26 mg AG/100 mL for the 20 % extract and 621.04 mg AG/100 mL for the 50 % extract. In the quantification of total anthocyanins, 11.6391 mg/L was obtained for the 20 % extract and 43.63 mg/L for the 50 % extract, respectively. Subsequently, the minimum inhibitory concentration (MIC) and the percentage of inhibition were evaluated for the extracts against *S. saprophyticus*; the MIC was 390.63 μg/mL for both extracts, with 88.42 % inhibition for the 20 % extract and 88.44 % for the 50 % extract. Using thin-layer chromatography (TLC), the retention factors (R_f) were 0.42 (cyanidin 3-*O*-glucoside), 0.62 (malvidin), and 0.86 (cyanidin). In conclusion, the 20 % and 50 % ethanolic extracts of *V. floribundum* exhibit antimicrobial activity against *S. saprophyticus*.

Keywords: Microbial control, Mortiño, *Vaccinium floribundum*, ethanolic extracts, anthocyanins.

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Resumen

Se evaluó *Vaccinium floribundum*, de la familia Ericaceae, por sus propiedades antimicrobianas, antioxidantes, antiinflamatorias y anticancerígenas, debido a su alto contenido de antocianinas. El objetivo de este estudio es determinar la cantidad de antocianinas presentes en el extracto fluido de *V. floribundum*, así como evaluar la actividad antimicrobiana en la prevención de infecciones del tracto urinario (IVU), estableciendo la concentración mínima inhibitoria (MIC) frente a *Staphylococcus saprophyticus* ATCC 15305, causante de infecciones urinarias. El estudio se realizó en los Laboratorios de Ciencias de la Vida de la Universidad Politécnica Salesiana, iniciando con la obtención de extractos etanólicos al 20 y 50 % a partir de frutos recolectados en la provincia de Cotopaxi, cantón Sigchos; los ensayos fueron aplicados a nivel *in vitro*. Se cuantificó la concentración de fenoles totales por el método de Folin-Ciocalteu, obteniendo 537.26 mg AG/100 mL de extracto al 20 % y 621.04 mg AG/100 mL al 50 %. En la cuantificación de antocianinas totales se obtuvieron 11.6391 mg/L de extracto al 20 % y 43.63 mg/L al 50 %, respectivamente. Posteriormente, se evaluó la concentración mínima inhibitoria (MIC) y el porcentaje de inhibición de los extractos frente a *S. saprophyticus*; la MIC fue de 390.63 μg/mL para ambos extractos, con un 88.42 % de inhibición para el extracto al 20 % y un 88.44 % para el extracto al 50 %. Mediante cromatografía en capa fina (TLC), los factores de retención (R_f) fueron: 0.42 (cianidina 3-*O*-glucósido), 0.62 (malvidina) y 0.86 (cianidina). En conclusión, los extractos etanólicos de *V. floribundum* al 20 y 50 % presentan actividad antimicrobiana frente a *S. saprophyticus*.

Palabras clave: Control microbiano, Mortiño, *Vaccinium floribundum*, extractos etanólicos, antocianinas.

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1 Introduction

Vaccinium floribundum, known as mortiño in Ecuador, is a wild shrub belonging to the Ericaceae family and the genus *Vaccinium*. It grows at altitudes ranging from 3400 to 3800 meters above sea level in the Andean region. It is a perennial species whose ecosystem is considered sensitive; therefore, there is no propagation method that ensures plant uniformity. *Vaccinium floribundum* contains a variety of phenolic compounds and high concentrations of anthocyanins in the form of pigments in berries, leaves, flowers, and fruits. These compounds possess a sugar moiety at position 3 and an aglycone (Pérez et al., 2020; Llerena et al., 2004).

Recent studies have shown that the active substances in mortiño fruits—fructose and proanthocyanidins—act by preventing the adhesion of bacteria, such as *Escherichia coli*, to the uroepithelium in women, although not in patients with neurogenic bladder. Therefore, the use of this plant species is recommended as a preventive measure in cases of urological pathologies (De León et al., 2009).

Within Urinary Tract Infections (UTIs), Urinary Tract Infection (UTI) is classified as occurring in 1–3 % of school-aged adolescents and increases with the onset of sexual activity, being very common in women between 20 and 50 years of age. According to statistics from the Ministry of Public Health of Ecuador (MSP), 4 out of 10 inhabitants reported urinary tract infections during 2009 (Ministerio de Salud Pública, 2013). In 2019, the National Institute of Statistics and Census (INEC) reported 9,916 cases of genitourinary infections in females aged 18 to 29, of which 9.0 % occurred in the Highlands and 7.2 % in the Coastal region (Instituto Nacional de Estadística y Censos, 2019; Rengifo, 2013; Palacio Rojas et al., 2018).

Focusing on the genus *Vaccinium* and its bactericidal activity in the prevention of urinary tract infections, *Vaccinium floribundum* is known to be associated with the presence of flavonol glycosides and polyphenols (proanthocyanidins, anthocyanins, and flavan-3-ols) (Baenas et al., 2020). Its bactericidal activity can be assessed through the Minimum Inhibitory Concentration (MIC), which consists of a serial dilution technique in a sterile plate containing defined concentrations of an extract against various microorganisms. The lowest microbial growth allows determination of the corresponding inhibition percentage. To apply this technique, it is important to use chemically stable dyes, among which 2,3,5-triphenyltetrazolium chloride (TTC) at 1 % stands out, forming formazan salts that enable detection of microbial colonies at a wavelength of 450 nm, evidenced by a color change from colorless to reddish (Hemeg et al., 2020; Condalab, 2019).

It is necessary to quantify the total phenolic content of *Vaccinium floribundum* samples. Therefore, fluid extracts of mortiño prepared with 20 % and 50 % ethanol are quantified colorimetrically using the Folin–Ciocalteu (F–C) reagent with gallic acid as a standard in a plate. The F–C reagent is characterized by forming phosphomolybdic–phosphotungstic complexes due to the formation of chromogenic oxides under basic pH conditions, resulting in a blue coloration, at which point a chemical reduction occurs. Measurement performed with a UV–visible spectrophotometer determines the absorbance of the blue compounds (phenols), which is directly proportional to their concentration (Cortez et al., 2018). Subsequently, the concentration of monomeric anthocyanins is determined considering the maximum wavelength of the samples, in this case at 520 and 700 nm using the differential pH method (Zapata et al., 2014).

To separate the chemical fractions of crude mortiño extracts, thin-layer chromatography (TLC) was employed, considering the retention factor (R_f) as a fundamental parameter to identify the type of bioactive compounds separated. In this case, these include glycosylated derivatives of anthocyanins, such as pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Zapata et al., 2014). The TLC technique involves a stationary phase (silica gel plate 10×10 cm, factor 254 nm) and a mobile phase composed of solvents of medium polarity in various proportions. These mortiño extracts were compared with a cyanidin-3-*O*-glucoside standard with an R_f value of 0.44 (Santacruz, 2017).

The fluid extracts were obtained through drying and extraction by maceration, a process consisting of leaving the solvent in contact with the plant material for several days under moisture conditions at

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a temperature suitable for the bioactive compounds to be extracted (Urquizo, 2016). The solvents used for each assay were ethanol at concentrations of 20 % and 50 %.

The main objective of this research project is to determine the concentration of phenolic compounds present in the fluid extract of *Vaccinium floribundum* and to evaluate its antimicrobial activity *in vitro*. Additionally, it aims to identify the secondary metabolites present in *Vaccinium floribundum* with antimicrobial potential relevant to the control of pathogens causing urinary tract infections, and to determine the minimum inhibitory concentration (MIC) of cyanidin from *Vaccinium floribundum* against *Staphylococcus saprophyticus*.

2 Materials and Methods

2.1 Obtaining ethanolic extracts

The fruits of *Vaccinium floribundum* were collected in the Sigchos Canton, Cotopaxi province, at 3023 meters above sea level, with coordinates S 00°4242.6 – W 078°5322.1. The classified and cleaned fruits were placed on aluminum trays and dried at 40 °C in an oven for 6 days. The dried material was ground, and 100 g were placed into a 250 mL Erlenmeyer flask, hydrated with 200 mL of ethanol at 20 % and 50 %, respectively. The mixture was stirred for 24 hours, then filtered and concentrated using a rotary evaporator until a final volume of 100 mL was obtained. Finally, the extracts were stored in amber containers for subsequent analyses (Narváez, 2016).

2.2 Determination of total phenolic content

The Folin–Ciocalteu colorimetric method was employed. Starting from a stock solution of gallic acid at a concentration of 2000 ppm, solutions at six different concentrations (100, 150, 200, 250, 300, 350, and 400 ppm) were prepared. From each solution, 0.5 mL were taken, to which 3.95 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent were added. The mixture was allowed to stand for 2 minutes, after which 0.75 mL of 20 % (w/v) sodium carbonate was added. The absorbance was measured at a wavelength of 765 nm after 2 hours of incubation in complete darkness. Using the obtained data, the calibration curve was constructed.

Subsequently, the samples were prepared by taking 0.5 mL of each ethanolic extract of *Vaccinium floribundum* at 20 % and 50 %, and the same procedure as for the calibration standards was followed. To quantify the concentration of total phenols in the extracts (mg GA/100 mL of extract), the following Equation 1 was used (Varela and Musuña, 2020):

$$\text{Abs} = mx + b \tag{1}$$

Where: x is the value of total polyphenols expressed as gallic acid equivalents (mg/mL); Abs is the absorbance at 765 nm; m is the slope of the line; and b is the intercept with the y-axis (Urbano, 2016).

2.3 Quantification of anthocyanins

The differential pH method (Giusti and Wrolstad, 2001) was applied. pH 1 and pH 4.5 buffers were prepared; their detailed composition is described in Table 1.

A volume of 2.5 mL of each ethanolic extract of mortiño at 20 % and 50 % was measured in triplicate and brought to a final volume of 5 mL, followed by sonication for 30 minutes. The extracts were filtered into Falcon tubes, protected from light with aluminum foil, and the pH was adjusted to 2.3 using 2 M HCl. The samples were then left to stand in darkness for 4 hours, with sonication for 5 minutes every 2 hours. After this period, the tubes were centrifuged at 3500 rpm for 30 minutes.

Table 1. Preparation of the buffers used

Buffer	Composition / Reagents
Buffer pH 1	0.076 g of potassium chloride in 40 mL of distilled water.
Buffer pH 4.5	2.27 g of sodium acetate in 40 mL of distilled water.

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Subsequently, 0.5 mL of the supernatant from each Falcon tube was taken and diluted to 5 mL with each of the prepared buffers (pH 1 and pH 4.5) and left to stand for 45 minutes. Then, 100 μL were added into a 96-well microplate to measure absorbance at 510 and 700 nm. Finally, by applying Equations 2 and 3, the anthocyanin concentration present in each ethanolic extract of *Vaccinium floribundum* was determined (Giusti and Wrolstad, 2001; Kayima, 2012).

$$\Delta A = (A_{\lambda max} - A_{700})_{pH1.0} - (A_{\lambda max} - A_{700})_{pH4.5} \quad (2)$$

$$AT\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\Delta A \times PM \times FD \times 1000}{\varepsilon \times 1} \quad (3)$$

Where: ΔA is the absorbance variation; $A_{\lambda \text{max}}$ is the absorbance at the maximum wavelength; A_T corresponds to total anthocyanins (mg/L); F_D is the dilution factor; PM is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); 1000 is the conversion factor from grams to milligrams; and ε is the molar absorptivity coefficient of cyanidin-3-glucoside (26,900) (Giusti and Wrolstad, 2001).

2.4 Determination of the Minimum Inhibitory Concentration (MIC)

Certified ATCC 15305 Microbiologics strains of *Staphylococcus saprophyticus*, in KWIK-STIK presentation, were reactivated by swabbing and from cryobank beads in both solid and liquid Tryptic Soy media (TSA/TSB). They were incubated for 24 hours at 37 °C at a pH of 7.3 ± 0.2.

An overnight culture was obtained by inoculating in Müller–Hinton broth for 24 hours at 37 °C. After this period, the tubes were centrifuged at 3500 rpm for 30 minutes, the supernatant was discarded, and 5 mL of sterile 0.9 % physiological solution was added, followed by vortexing for 3 minutes. By adding small amounts of physiological solution, the suspension was adjusted to 0.5 on the McFarland scale, and the absorbance was measured at 625 nm within the range of 0.08–0.2 to achieve a concentration of 10^8 CFU/mL. The solutions of the commercial and natural antibiotics to be tested were prepared; additionally, the extracts were diluted 1:10 to a final volume of 10 mL (Guzmán, 2018; Veloz, 2018). Subsequently, with all materials and the working area completely sterile, inoculation in the microplate was carried out (León et al., 2011), as detailed in Table 2.

Table 2. Layout of the contents of each well on the microplate for determining the MIC

Treatment	Location	Contents
Blank	Column A12-H12	200 μL MC
Negative control (C_E)	Column A11-H11	200 μL MC
Positive control (C_C)	Rows H9-H1	150 μL MC + 50 μL inoculum
Ethanolic extract samples	Rows A9-B9-C9	150 μL MC + 50 μL Ext + 50 μL inoculum
	Rows A8-A1/B8-B1/C8-C1	150 μL MC + 50 μL inoculum. Serial dilution
Positive control (antibiotic)	Rows D9-E9	150 μL MC + 50 μL Antb + 50 μL inoculum
	Rows D8-D1/E8-E1	150 μL MC + 50 μL inoculum. Serial dilution
Positive control (natural)	Rows F9-G9	150 μL MC + 50 μL thymol solution + 50 μL inoculum
	Rows F8-F1/G8-G1	150 μL MC + 50 μL inoculum. Serial dilution

Note. MC: culture medium; Ext: extract; Antb: antibiotic. The remaining 50 μL of each dilution is discarded.
Source. (León et al., 2011).

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The microplate was incubated for 24 hours at 35 °C with agitation. After the incubation period, 30 μL of 1 % 2,3,5-triphenyltetrazolium chloride (TTC) was added to each of the 96 wells of the microplate and incubated at 37 °C for 40 minutes. Finally, absorbance was measured at 450 nm using a microplate reader.

To determine the Minimum Inhibitory Concentration (MIC), the following equation was applied (Equation 4):

$$\%\text{Inhibition} = 100 - \left(\frac{A \times 100}{B}\right) \tag{4}$$

Where: A is the average absorbance of the three replicates, and B is the absorbance of the positive control (Rosales, 2018).

2.5 Separation of compounds by Thin-Layer Chromatography (TLC)

Prior to the separation of components using Thin-Layer Chromatography (TLC), the ethanolic extracts of mortiño at 20 % and 50 % were concentrated using a rotary evaporator for 2 hours. Subsequently, using a reflux system, they were acidified with 10 mL of 2 M HCl for one hour and then filtered and transferred into amber flasks.

The mobile phase used was a mixture of ethyl acetate, formic acid, acetic acid, and water in the proportion (10:1.1:1.1:2.6), which was also used to condition the chromatographic chamber for 30 minutes (Poole, 2015).

The cyanidin-3-glucoside chloride standard was prepared by dissolving 2 mg of the standard in 1 mL of HPLC-grade methanol (Narváez, 2016).

On a silica gel HPTLC glass plate (Factor 254, size 10×10 cm), a line was drawn 1 cm from the edge of the plate (Corzo, 2019; Pássaro Carvalho et al., 2016). Using a TLC applicator, 7 μL of the cyanidin-3-glucoside chloride standard and 10 μL of the ethanolic mortiño extracts at 20 % and 50 % were applied. The plate was placed in the previously saturated chromatographic chamber for component elution for 1 hour and 15 minutes. The plate was then removed from the chamber, and the solvent was dried using a cold air dryer. The spots generated during the run were observed using a UV visualizer at 254 and 365 nm. Subsequently, the retention factors (R_f) of the standard and the ethanolic mortiño extracts were calculated using Equation 5 (Pássaro Carvalho et al., 2016):

$$R_f = \frac{\text{dist.run by the compound}}{\text{dist.run by the solvent}} \tag{5}$$

2.6 Statistical analysis

Within the experimental design, two concentrations of ethanolic extract of *Vaccinium floribundum* (20 % and 50 %) were evaluated, with three replicates for each concentration. For both the determination of total phenols and the quantification of anthocyanins, data processing was performed using analysis of variance (ANOVA) to establish significant differences with parametric variables. For the functional analysis of the study factors, Tukey's significance test at 0.05 % and the independent samples t-test were applied using the INFOSTAT software.

3 Results and Discussion

3.1 Quantification of total phenols

The calibration curve was constructed using seven concentrations of gallic acid, with a linear regression coefficient (R^2) of 0.9975 (Figure 1).

Based on the absorbance readings at 765 nm from each replicate of the ethanolic extract samples of *Vaccinium floribundum* at concentrations of 20 and 50 %, and using the linear equation, the total phenolic compound concentrations were calculated, expressed as mg of gallic/100 mL of extract. Values of 537.2624 were obtained for the 20 % ethanolic extract and 621.0393 for the 50 % extract.

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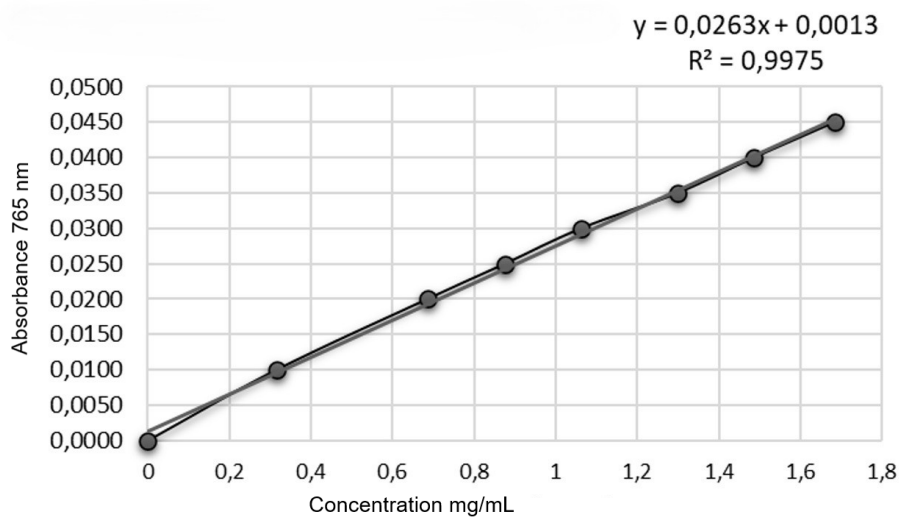


Figure 1. Calibration curve for gallic acid (mg/mL)

Figure 2 shows the total phenolic content in the *Vaccinium floribundum* samples at 20 % and 50 % ethanol.

Based on parametric statistical tests, a significant difference can be identified between the phenolic compound content of the 20 % ethanolic extract and the 50 % ethanolic extract of *Vaccinium floribundum*, with a p-value < 0.0001. Therefore, it can be stated that the 50 % ethanolic extract has a higher content of phenolic compounds than the 20 % ethanolic extract, as evidenced in the bar graph shown in Figure 2.

The phenolic compound content of both the 20 % and 50 % ethanolic extracts of *Vaccinium floribundum* can be corroborated with the results reported by Aaby et al. (2013) in their study on blueberries, which reported 564 mg GA/100 g dry fruit of total phenolic content. Grace et al. (2015) analyzed several species of the genus *Vaccinium*, including *Vaccinium uliginosum* (bog bilberry), in which 504.5 mg GA/100 g of fruit was found. Meanwhile, Llivisaca et al. (2018) reported a content of 146.10 mg GA/100 g fruit in samples collected in Pichincha province and 107.37 mg GA/100 g fruit in samples from Chimborazo province. They also mention that the total polyphenol content in the leaves of *Vaccinium floribundum* is significantly higher than that present in the fruit (Espinosa et al., 2016).

3.2 Quantification of anthocyanins

By applying Equations 2 and 3, different anthocyanin concentrations expressed in mg/L were obtained, as detailed in Table 3.

Table 3. Quantification of total anthocyanins in *Vaccinium floribundum* extracts at concentrations of 20 % and 50 %

Samples	Absorbance Reading by Differential pH				Anthocyanin Concentration		
Ethanolic extract	pH 1		pH 4,5		Average Abs	mg/L	mg/mL
E20	0.1447	0.0383	0.1447	0.0393	0.0697	11.6391	0.0116
E50	0.4203	0.0413	0.1630	0.0437	0.2613	43.6342	0.0436

Note. The concentrations correspond to results obtained from Equation 3 and are expressed in mg/L. Abs Prom: average absorbance.

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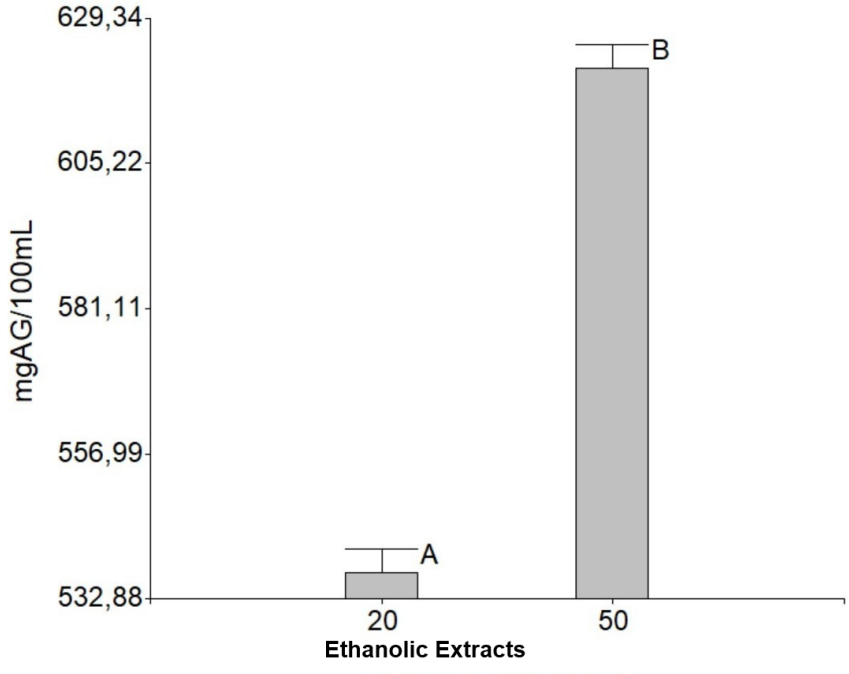


Figure 2. Comparison between 20 % and 50 % extracts in terms of total phenolic content.

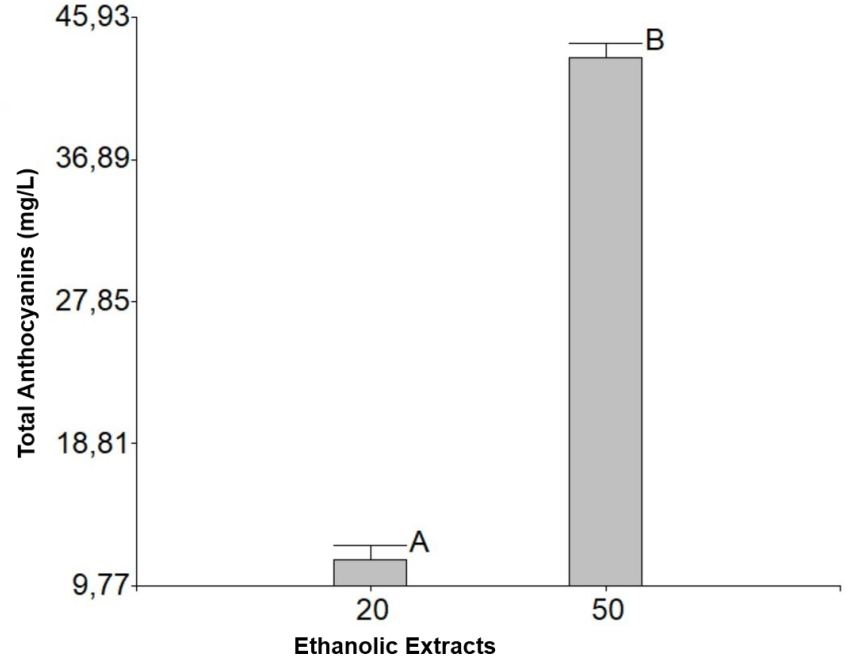


Figure 3. Comparison between 20 % and 50 % extracts in terms of total anthocyanin content.

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Figure 3 shows the total anthocyanin content in *Vaccinium floribundum* samples at 20 % and 50 % ethanol. From the values obtained using the differential pH method, the anthocyanin concentration in the 20 % ethanolic extract was 11.6391 mg/L (Table 3), which is considered similar to that reported in *Vaccinium myrtillus* (blueberry) (Medina, 2016). In contrast, the 50 % ethanolic extract showed a significant difference ($p < 0.05$) and a concentration of 43.63 mg/L (Figure 3). This extract presented a higher number of anthocyanins obtained from mortiño, while also providing a good yield.

This may be explained by the fact that extraction processes are related to the maturity stage of the berry during harvest, which may change color due to the influence of temperature, oxygen, and light. High temperatures increase acid breakdown, while acidity is related to the pH found in mortiño (Guerrero, 2019). On the other hand, Pérez et al. (2020) worked with *Vaccinium floribundum* and, regarding anthocyanin quantification, obtained satisfactory results using 60 % ethanol at 40 °C, with a result of 332.63 mg C3G 100 g^{-1} FW. Meanwhile, in the case of anthocyanin extraction from cranberry pomace (*Vaccinium macrocarpon*) using 75 % ethanol, greater extraction efficiency and selectivity were achieved (Constantine and Istrati, 2022).

Finally, species such as *Ficus carica* L., *Syzygium cumini* L., *Lonicera cerulea* L., and *Purple Majesty* potato, when subjected to extraction with ethanol at 100 %, 79.6 %, 79.6 %, and 70 %, respectively, yielded different cyanidin glycosides: 4.32 mg C3R/g fig peel DW; 60.5 mg C3GE/g fruit DW; 22.45 mg C3GE/g berries DW; and 364.3 mg C3G/kg potato FW (Tena and Azuero, 2022). The results obtained in this study are similar to those reported by other authors in different plant species.

3.3 Determination of the Minimum Inhibitory Concentration (MIC)

As shown in Table 4, which corresponds to the antimicrobial activity of the 20 % ethanolic extract of *Vaccinium floribundum* against the bacterium *Staphylococcus saprophyticus*, the minimum inhibitory concentration obtained was 390.6 μg/mL, and the percentage of inhibition was 88.42 %. Meanwhile, Figure 5 illustrates the behavior of the 20 % ethanolic extract against the microorganism *S. saprophyticus*, confirming that higher extract concentrations result in greater inhibition of the microorganism (Varela and Musuña, 2020).

Figure 4 shows the microplate stained red with 1 % TTC dye; this coloration indicates the presence of *Staphylococcus saprophyticus*, while the wells that do not show coloration correspond to those in which microbial growth is being inhibited.

To calculate the percentage of inhibition relative to the concentration of each of the ethanolic extracts of *Vaccinium floribundum*, the equation proposed by Owen et al. (2014) was applied.

Table 5 details the value obtained for the minimum inhibitory concentration and the percentage of inhibition of the 50 % ethanolic extract of *Vaccinium floribundum* against *Staphylococcus saprophyticus*, being 390.6 μg/mL and 88.44 %, respectively.

Figure 5 shows the microplate stained red with 1 % TTC dye; this coloration indicates the presence of *Staphylococcus saprophyticus*, while the wells that do not show coloration correspond to those in which microbial growth is being inhibited.

In Table 6, by comparing the data obtained for the minimum inhibitory concentration and the percentage of inhibition of the ethanolic extracts of *Vaccinium floribundum* at 20 % and 50 % against *Staphylococcus saprophyticus*, it is evident that the 50 % ethanolic extract shows a higher percentage of inhibition compared to the 20 % ethanolic extract.

In the case of the commercial antibiotic nitrofurantoin, the percentage of inhibition and minimum inhibitory concentration were 87.7 % and 0.78 μg/mL, respectively. The obtained value was superior to that of the natural agent tested. This is since uropathogens, in large numbers, are susceptible to this drug because their nitroreductase enzymes possess highly reactive electrophilic intermediates that non-specifically attack proteins in the bacterial ribosome, causing complete inhibition and resulting in high inhibition percentages (Komp et al., 2014). Additionally, it is necessary to mention that the MIC achieved in the experiments is close to the theoretical values reported in studies with nitrofurantoin, which indicate ≤ 16 μg/mL within the sensitivity range (IDEXX, 2022).

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Table 4. Antimicrobial activity of the 20 % ethanolic extract of *Vaccinium floribundum* against *Staphylococcus saprophyticus*

Extract concentration (μg/mL)	Growth Control Abs. (C_c)	Average Abs. at 450 nm	% Inhibition
390.6*	1.229	0.142	88.419**
781.3	1.206	0.135	88.778
1562.5	1.579	0.170	89.234
3125.0	1.462	0.149	89.831
6250.0	1.628	0.164	89.947
12 500.0	1.452	0.138	90.519
25 000.0	1.644	0.143	91.322
50 000.0	1.652	0.143	91.344
100 000.0	1.326	0.112	91.554

Note. The asterisks represent: * Minimum Inhibitory Concentration and ** Percentage of inhibition. Abs.: Absorbance.

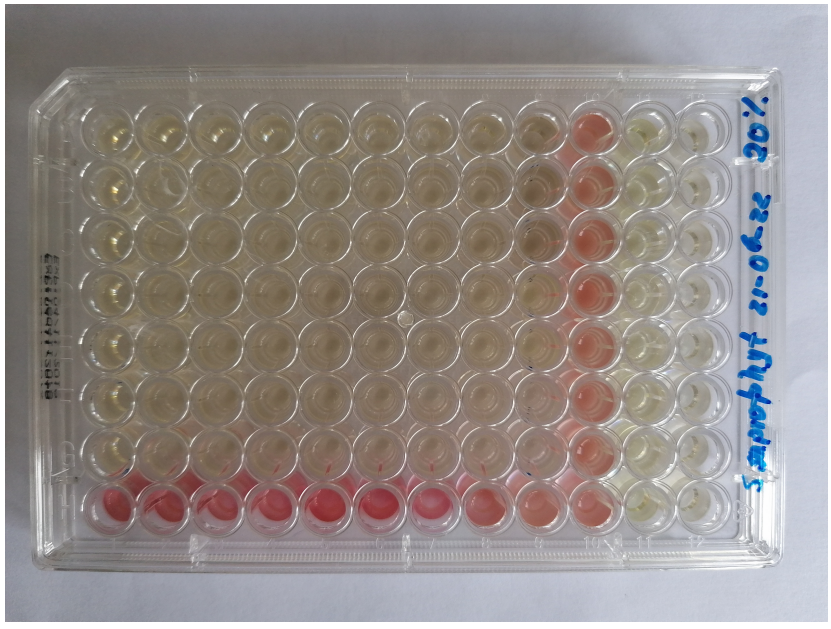


Figure 4. Microplate of *Staphylococcus saprophyticus* with 20 % ethanolic extract stained with 1 % TTC and incubated for 24 hours.

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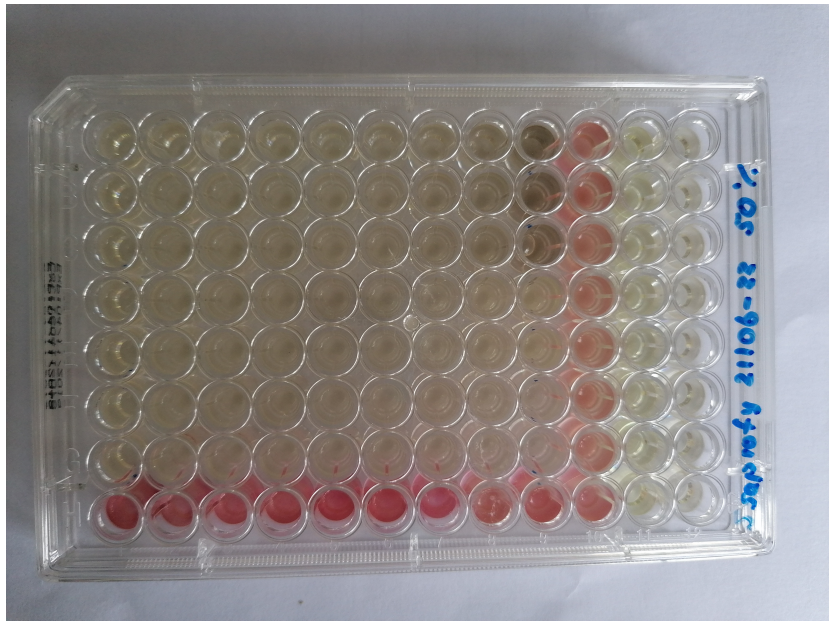


Figure 5. Microplate of *Staphylococcus saprophyticus* with 50 % ethanolic extract stained with 1 % TTC and incubated for 24 hours.

Table 5. Antimicrobial activity of the 50 % ethanolic extract of Vaccinium floribundum against *Staphylococcus saprophyticus*

Extract concentration (μg/mL)	**Growth Control Abs. (C_c)**	**Average Abs. at 450 nm**	**% Inhibition**
390.6*	1.206	0.143	88.439**
781.3	1.229	0.137	88.816
1 562.5	1.579	0.153	88.913
3 125.0	1.462	0.153	88.921
6 250.0	1.628	0.123	88.969
12 500.0	1.452	0.133	89.116
25 000.0	1.644	0.145	89.220
50 000.0	1.652	0.116	89.397
100 000.0	1.326	0.117	89.432

Note. The asterisks represent: * Minimum Inhibitory Concentration and ** Percentage of inhibition. Abs.: Absorbance.

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Table 6. Percentage of inhibition and MIC of the ethanolic extracts of *Vaccinium floribundum* and established controls against *Staphylococcus saprophyticus*

Microorganism	Extract 20 %		Extract 50 %		Thymol		Nitrofurantoin	
	MIC (μg/mL)	% Inh.	MIC (μg/mL)	% Inh.	MIC (μg/mL)	% Inh.	MIC (μg/mL)	% Inh.
S. saprophyticus	390.63	88.42	390.63	88.44	3.91	87.42	0.78	87.73

Note. % Inh: Percentage of inhibition; MIC: Minimum Inhibitory Concentration.

The best result against *S. saprophyticus* was obtained with the 50 % ethanolic extract of *Vaccinium floribundum*, with a MIC of 390.63 μg/mL and an inhibition percentage of 88.44 %. According to Varela and Musuña (2020), who worked with granules of *Vaccinium floribundum* at 150 mg, 200 mg, and 250 mg against the same microorganism, the inhibition percentages were 50.48 %, 91.17 %, and 86.56 %, respectively, data that corroborate the inhibition percentage obtained in this study.

3.4 Separation of compounds by thin-layer chromatography (TLC)

The chromatographic plates obtained after one hour and 15 minutes of development were as follows:

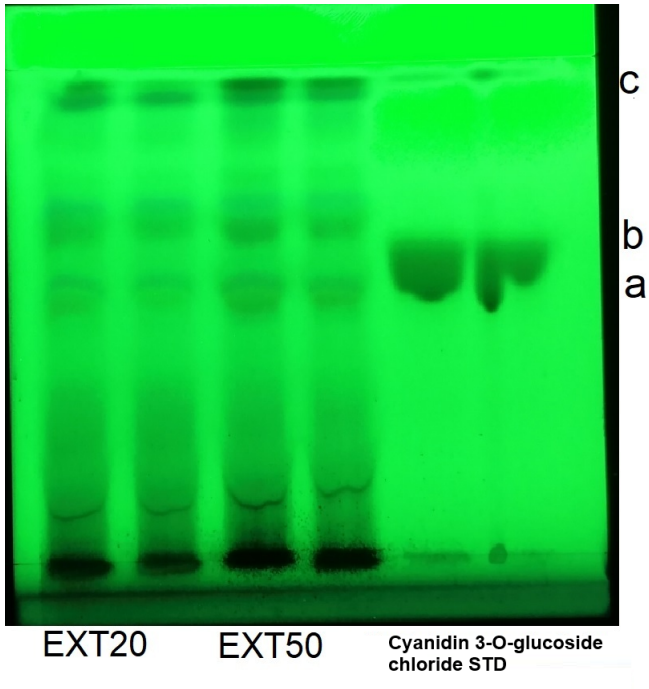


Figure 6. HPTLC plate factor 254 nm 10×10 cm showing separated components of the ethanolic extracts at 20 % (EXT 20) and 50 % (EXT 50) of *Vaccinium floribundum*, respectively. Plate reading wavelength: 254 nm.

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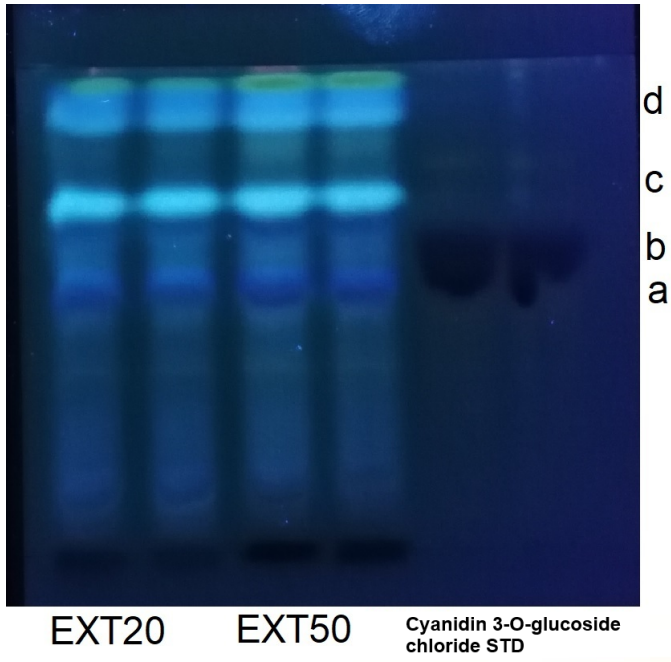


Figure 7. HPTLC plate 254 nm 10×10 cm showing separated components of the ethanolic extracts at 20 % (EXT 20) and 50 % (EXT 50) of *Vaccinium floribundum*, respectively. Plate reading wavelength: 365 nm.

Once the process was completed, the retention factors were determined by relating the distance traveled by the compound to the distance traveled by the solvent (Figure 6). The results are reported in Table 7.

Regarding the results obtained, retention factors may vary due to the different maximum wavelengths at which the compounds are identified. In the case of anthocyanins and anthocyanidins—cyanidin 3-galactoside, cyanidin 3-arabinoside, and malvidin reported in Table 8—these present values similar to those described in studies on the genus *Vaccinium* (blueberries) using methanolic extracts and hydrolyzed compounds (Filip et al., 2012).

According to the results in Table 8, the compounds were identified as cyanidin 3-galactoside, cyanidin 3-arabinoside, malvidin, and cyanidin in the species *Vaccinium floribundum* (mortiño), as also reported by Taco (2017).

Meanwhile, the cyanidin 3-*O*-glucoside standard dissolved in methanol (2 mg/1 mL) presented a retention factor of 0.42, similar to values reported by Narváez (2016) for the species *Vaccinium floribundum* (mortiño).

Other relevant bioactive compounds not detected on the HPTLC plate were delphinidin and pelargonidin, which are found at wavelengths between 520–546 nm, and their respective monoglucosides can be distinguished within a range of 10 to 15 nm from these wavelengths. Additional factors that may affect anthocyanin identification include the nature of the solvent and pH; therefore, acidification of the extracts with HCl is important, as it induces a systematic shift toward higher wavelengths compared to aqueous extracts (Filip et al., 2012). It is also worth noting that the solubility of anthocyanins in alcohol increases when stable hydrochloric acid is added (Eng et al., 2017).

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Table 7. Identification of anthocyanins and anthocyanidins present in the 20 % and 50 % ethanolic extracts of *Vaccinium floribundum* at a maximum wavelength of 254 nm (see Figure 6)

Fraction	Compound	$\lambda_{máx}$ nm	Rf (Retention factor)
A	Cyanidin 3-O-glucoside	254	0.42
B	Malvidin	254	0.62
C	Cyanidin	254	0.86

Table 8. Identification of anthocyanins and anthocyanidins present in the 20 % and 50 % ethanolic extracts of *Vaccinium floribundum* at a maximum wavelength of 365 nm (see Figure 7)

Fraction	Compound	$\lambda_{máx}$ nm	Rf (Retention factor)
A	Cyanidin 3-galactoside	365	0.39
B	Cyanidin 3-arabinoside	365	0.51
C	Malvidin	365	0.67
D	Cyanidin	365	0.86

4 Conclusions

The 50 % ethanolic extract of *Vaccinium floribundum* showed better results in the quantification of phenolic compounds and total monomeric anthocyanins, yielding 621.04 mg GA/mL of extract and 43.63 mg/L, respectively.

It was possible to separate and identify, through thin-layer chromatography (TLC), anthocyanin bioactive compounds in the 20 % and 50 % ethanolic extracts of *Vaccinium floribundum*, which were: cyanidin 3-arabinoside (R_f 0.51), cyanidin 3-galactoside (R_f 0.39), malvidin (R_f 0.67), and cyanidin (R_f 0.86).

In this study, it was validated that the ethanolic extracts of *V. floribundum* at 20 % and 50 % exhibit antimicrobial activity aimed at inhibiting *Staphylococcus* spp., a pathogen associated with urinary tract diseases.

It is recommended to continue studies using effective, sensitive, and reproducible analytical techniques such as HPLC (High-Performance Liquid Chromatography) for each identified chemical component.

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Declaration on the use of artificial intelligence

The authors DECLARE that, during the preparation of the article entitled "Antimicrobial potential of *(Vaccinium floribundum)* extract fluid against *Staphylococcus saprophyticus* causing urinary tract infections", no generative artificial intelligence tools or automated assistance systems were used for the writing, analysis, data interpretation, content generation, translation, or editing of the manuscript.

The authors assume full responsibility for the content, originality, integrity, and final version of the article.

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Author's contribution

J.G.M.M.: Conceptualization, investigation, methodology, supervision, writing – original draft, writing – review and editing. **S.A.M.P.:** Investigation, methodology. **M.E.M.R.:** Funding acquisition, resources. **R.D.A.V.:** Data curation.

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