J. ISSAAS Vol. 30, No. 1:159-174 (2024)

PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF *Vaccinium barandanum* LEAF EXTRACT

Regina Lourdes Broñola-Hipol*[1,3], Hilda S. Wayas[1], Florence Mae S. Bacuyag[2], Jedida F. Cabanlong[3], Madonna C. Daquigan[2] and Roland M. Hipol[2]

[1] Department of Pharmacy, School of Nursing, Allied Health and Biological Sciences, Saint Louis University, Bonifacio St., Baguio City, 2600, Philippines

[2]Department of Biology, College of Science, University of the Philippines Baguio, Gov. Pack Road, Baguio City, 2600, Philippines

[3]School of Advanced Studies, Saint Louis University, Bonifacio St., Baguio City, 2600, Philippines

*Corresponding author: rlbhipol@slu.edu.ph

(Received: August 28, 2023; Accepted: May 12, 2024)

ABSTRACT

Vaccinium species are valuable resources for bioactive compounds with various applications. This study investigated the phytochemical composition and TLC profile, and antioxidant and antibacterial activities of the ethanolic extract of *Vaccinium barandanum* Vidal, a species endemic to the Philippines. The study was conducted in the Research Laboratories of Saint Louis University, Philippines and University of the Philippines Baguio from November 2021 to August 2023. The ethanolic extract of the leaves was analyzed for its phenolic content (TPC) and flavonoid content (TFC). Phytochemical analysis identified tannins, polyphenols, flavonoids, saponins, steroids, and triterpenoids. TPC and TFC were at 96.62 ± 0.77 mg GAE/g DE and 39.99 ± 2.45 mg QE/g DE, respectively. The DPPH antioxidant assay showed that *V. barandanum* IC_{50} was at 144.09 µg/mL and the extract showed a radical scavenging effect in a concentration-dependent manner. Activities against bacterial pathogens with antibiotic resistance genes, namely *S. aureus*, *E. cloacae, E. faecium, and K. pneumoniae*, were determined using a resazurin-based assay. The extract showed better antibacterial activity against the gram-positive pathogens *E. faecium* and MRSA, with MIC of 6,000 ppm (6 mg/mL), compared to the gram-negative pathogens, *E. cloacae* and *K. pneumoniae*, with MIC of 12,000 ppm (12 mg/mL). This study is a pioneering work which revealed the bioactivities of the extract of the leaves against bacteria with antibiotic resistance genes. *V. barandanum* has the potential to be developed as a functional food and a raw material for nutraceutical and pharmaceutical products.

Key words: antibacterial, antioxidant, flavonoids, phenolics, TLC profile

INTRODUCTION

Vaccinium is a genus belonging to the tribe Vacciniae of the subfamily Vaccinoiodae of the family Ericaceae (Ballington 2001). Ericaceae has approximately 450 species with a wide geographic distribution, mainly in the northern hemisphere (Song and Hancock 2010). It is notable that Southeast Asia is the origin of almost 40% of the *Vaccinium* species (Song and Hancock 2010). Representatives of this genus are known as blueberries, cranberries, and bilberries. One *Vaccinium* species in the Philippines is the endemic *Vaccinium barandanum* Vidal, in the section Barandanum (Vander Kloet and Dickinson 2009). In the Cordillera, it is known by several names, such as *lusong, dusong, loso, losong* (Igorot); *alimomosong* (Bontok)*;* and *ladew* (Kankanaey) (Barcelo and Barcelo 2020).

Leaves and fruits are the most cited useful parts of *Vaccinium* species (Abreu et al. 2014). The berries are consumed as food and used as a rich source of polyphenol antioxidants (Tundis et al. 2021). As such, the berries are popular targets for functional foods or nutraceuticals research due to their benefits in promoting health and reducing the risks of chronic diseases, including diabetes and atherosclerosis (Martau et al. 2023). Both leaves and berries have also been used in traditional medicine (Tundis et al. 2021). The medicinal applications that were most frequently reported are urinary antiseptic, antidiabetic, antidiarrheal, diuretic, antipyretic, and astringent effects (Abreu et al. 2014). The decoction and infusion of leaves are used as remedies to treat diabetes and inflammation (Martau et al. 2023; Tundis et al. 2021). The leaves, which are abundant throughout the year, have markedly higher phenolic composition and higher antioxidant activity than the fruits (Bujor et al. 2019).

Discovering new antioxidant and antibacterial leads is an ongoing and significant field of study in the light of nutraceutical and functional food research. Currently, alternative sources of such products are required to meet the high demand for these products (Awuchi and Okpala 2022; Nwosu and Ubaoji 2020). Natural antioxidants play a crucial role in preventing the rancidity caused by the oxidation of unsaturated fats, stabilizing food colors, and participating in the chemoprevention of various diseases (Pires et al. 2020). Antimicrobial compounds naturally produced by plants, including *V. barandanum*, for their defense against pathogens may be applied for food processing and preventing spoilage and proliferation of pathogenic microorganisms (Juneja et al. 2012). Moreover, this plant may also contribute toward the mitigation of antibiotic resistance, one of the most important public health issues in recent decades (Begum et al. 2021). More than 50% of the bacterial isolates from water, soil, and vegetables grown in gardens watered with polluted surface waters in Metro Manila, Philippines, have antibiotic resistance (Vital et al. 2018).

Two of the most studied species of the genus *Vaccinium*, namely bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.) were studied for antioxidant properties (Bujor et al. 2019; Ștefănescu et al. 2020), antimicrobial, and antibiofilm activity (Kryvtsova et al. 2020). On the other hand, there are very limited studies on *V. barandanum.* The fruits of this plant were examined for its ethnobotanical uses and antioxidant properties (Barcelo 2014, 2015) while the leaves were investigated for antibiofilm activity (Mirghani et al. 2019). However, its bioactivity against antibiotic-resistant strains of bacteria has not yet been investigated. To address this, the chemical constituents of this plant were evaluated for its phytochemical profile, its antioxidant properties, and its bioactivity against bacteria with antibiotic resistance genes, specifically *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus, Enterococcus faecium,* and *Enterobacter cloacae.* This research aimed to document and evaluate the health properties of *V. barandanum* for possible nutraceutical or pharmaceutical product development.

MATERIALS AND METHODS

Plant materials. Leaves of *V. barandanum* Vidal were collected in November 2021 from Topdac, Atok, Benguet Province, Philippines (Fig. 1). Mature leaves without any visible signs of disease were selected. The collected plants are deposited at the University of the Philippines Baguio Herbarium, Department of Biology, College of Science, University of the Philippines Baguio, with accession number UPBH-2024-Eric-002-1.

The leaves were carefully cleaned, washed, and oven-dried (60 °C) for 3 days. After drying, these were homogenized using Retsch Ultra-centrifugal Mill ZM 200 to a fine powder (0.2mm) and stored securely in sealed plastic containers (Escueta-De Cadiz and Almeria 2019).

Figure 1. *Vaccinium barandanum* leaves taken *in-situ* in Topdac, Atok, Benguet, Philippines

Extraction. Crude leaf extracts were prepared following the protocol of Cruz (2019) with some modifications. Powdered leaf samples (50g) were placed in beakers with 500 mL of 80% ethanol. The sample was macerated and occasionally stirred for 48 h, then filtered into an Erlenmeyer flask. The plant ethanolic extract was concentrated using a rotary evaporator set at 50 ℃ (Cruz 2019), then evaporated to dryness as needed per assay using the Biotage V10-Touch solvent evaporation system at 48 ℃. The dried extract was then stored at 2 – 8 ℃ (Escueta-De Cadiz and Almeria 2019).

Phytochemical screening. The phytochemical contents were determined using standard colorimetric methods as preliminary qualitative analysis. All assays were performed in triplicates with one negative control.

Test for tannins. (Gelatin test). Powdered leaf sample (50g) was macerated in 500 mL distilled water for 48 h with occasional stirring. The extract was filtered then concentrated using a rotary evaporator at 50 °C (Cruz 2019). The aqueous extract (1 mL) was placed in a test tube, and four drops of gelatin-salt solution, containing 1% gelatin solution and 10% sodium chloride (NaCl), were added to the tube. Tannins are present when white precipitate formed (Ben et al. 2013; Shaikh and Patil 2020).

Test for polyphenols (Ferric chloride test). Powdered leaf sample (50g) was macerated in 500 mL ethanol (80%) for 48 h with occasional stirring. The ethanolic extract (1 mL) and 10% ferric chloride solution (four drops) were mixed in a test tube. Polyphenols are present when a dark green or bluish-black color formed (Shaikh and Patil 2020).

Tests for flavonoids. The procedures below were performed for the detection of flavonoids.

Alkaline reagent test. Two drops of 2% sodium hydroxide (NaOH) were added to 1 mL of the ethanolic extract in a test tube. The formation of an intense yellow color was observed. One mL of 2 M hydrochloric acid (HCl) was added dropwise until the solution became colorless. This color change indicated the presence of flavonoids (Gul et al. 2017; Shaikh and Patil 2020).

Lead acetate test. In a test tube, 1 mL of the ethanolic extract and three drops of 10% lead acetate solution were mixed. The formation of a yellow precipitate confirmed the presence of flavonoids (Shaikh and Patil 2020).

Shinoda's test. Dried ethanolic extract was dissolved in distilled water (10 mL) and in 95% ethanol (5 mL). The sample dissolved in ethanol was mixed, filtered, and heated in a water bath, while the sample in distilled water was filtered and mixed. One mL of the sample solutions in distilled water and in ethanol were then added with three magnesium ribbon fragments and a few drops of concentrated HCl. Flavonol glycosides are detected when a pink to crimson color forms; flavanones are present when an intense cherry red color is observed; and flavonols are detected when an orange-red color was formed (Gul et al. 2017; Shaikh and Patil 2020).

Tests for saponins (Froth test). Two mL of ethanolic extract was added to 10 mL distilled water in a test tube. The solution was mixed vigorously for 1 min. Saponins were detected with the formation of a 1-cm foam layer (Ben et al. 2013).

Test for alkaloids. Five grams of dried ethanolic extract were dissolved in 5 mL of 1% aqueous HCl and then filtered (Ben et al. 2013). The filtrate was distributed among 4 test tubes to determine the presence of alkaloids using 4 procedures: Mayer's test, Wagner's test, Dragendorff's test, and Hager's test (Ben et al. 2013; Shaikh and Patil 2020).

Mayer's test. One mL of the filtrate was added with 2 mL of Mayer's reagent. The appearance of a creamy white/yellow precipitate signified the presence of alkaloids.

Wagner's test. One mL of the filtrate was treated with 2 mL of Wagner's reagent. A positive result is indicated by the presence of a brownish/reddish precipitate.

Dragendorff's test. One mL of the filtrate was added with 1 mL of Dragendorff's reagent. The occurrence of alkaloids is confirmed by a red/reddish-brown precipitate.

Hager's test. One mL of the filtrate was treated with 1 mL of Hager's reagent. A cream white/yellow precipitate indicated alkaloid presence.

Test for steroids and triterpenoids (Liebermann Burchard Test). The dried ethanolic extract (1 g) was dissolved in 1 mL chloroform in a test tube. Glacial acetic acid (3 mL) and acetic anhydride (3 mL) were then added. The solution was heated in a water bath and cooled in running water. Sulfuric acid (three drops) was added along the side of the tube. A range of colors including blue to green, violet, and red confirmed the presence of steroids and triterpenoids (Shaikh and Patil 2020).

Quantification of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). The dried ethanolic extract was dissolved in distilled water for the determination of total phenolics and total flavonoids, at concentrations of 2 mg/mL and 1 mg/mL, respectively.

Total Phenolic Content. The Folin-Ciocalteu method (Al-Dhabi et al. 2017) was employed to estimate the total phenolic content. Briefly, 100 µL of the diluted plant extract (2 mg/mL), 150 µL Folin & Ciocalteu's phenol reagent (Sigma-Aldrich, USA), and 1 mL distilled water were mixed in a 2-mL microtube and vortexed for 1 min. After adding 600 µL sodium carbonate (10% w/v), the solution was vortexed for another 1 min and incubated at dark room temperature for 2 h. The absorbance was read at 760 nm using the BMG Labtech FLUOstar Omega microplate reader. TPC was quantified as Gallic acid equivalents (mg GAE/g of dry extract (DE) and converted to dry weight (DW) and fresh weight (FW) basis) using a gallic acid reference curve. The experiment was performed in three trials with three replicates per trial.

Total Flavonoid Content. Aluminum chloride assay (Alnajar et al. 2012) was used to analyze the total flavonoid content. Briefly, 200 µL of the diluted plant extract (1 mg/mL), 600 µL of 95% ethanol, 40 µL of 1 M potassium acetate, 40 µL of aluminum chloride, and 1.12 mL of distilled water were mixed in a 2-mL microtube. After incubation at room temperature for 30 min, the absorbance was read at 415 nm using the BMG Labtech FLUOstar Omega microplate reader. TFC was quantified as Quercetin equivalents (mg QE/g of dry extract (DE) and converted to dry weight (DW) and fresh weight (FW) basis) using a quercetin reference curve. The experiment was done in three trials with three replicates per trial.

Antioxidant activity assay. The antioxidant activity of the *V. barandanum* extract was determined on the basis of its 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (Masuku et al. 2020). Prior to the assay, a 0.051 mM DPPH solution in methanol was prepared. The dried ethanolic extract and the ascorbic acid standard were dissolved separately in distilled water at a concentration of 400 µg/mL and serially diluted to 12.5 µg/mL.

In a 96-well microplate, 100 µL of each plant extract and ascorbic acid (12.5 to 400 µg/mL) were dispensed in separate wells in triplicate. Then, 100 µL of DPPH radical solution was added to each well. DPPH in methanol was used as the negative control, while 200 µL methanol was used as the blank. After all samples and controls were dispensed in triplicate, the microplate was incubated at ambient temperature for 30 min. The absorbance was measured at 517 nm using the BMG Labtech FLUOstar Omega microplate reader, and the percentage inhibition was determined using the formula:

$$\% \, Inhibition = [1 - (Abs_{sample}/Abs_{control})] \times 100$$

Where: Abs_{sample} is the absorbance of the sample
$Abs_{control}$ is the absorbance of the negative control.

Antioxidant activity was also determined in terms of IC_{50}, or the sample concentration required to inhibit 50% of the DPPH free radicals. The IC_{50} of the plant extract and the ascorbic acid were calculated based on linear regression plots.

Thin-layer chromatography profiling. TLC plate preparation and development, visualization of spots, and calculation of the retention factor (R_f) values were conducted following the protocol of Punzalan and Villaseñor (2019). The solvent system of 70% hexane:30% ethyl acetate (n-hexane (Duksan Pure Chemicals, South Korea) and ethyl acetate (Scharlau ACS Basic, Spain/European Union)) resulted in the best resolution of the components of the extract. A plate measuring 4.5 cm by 9 cm from aluminum sheets of TLC silica gel (Merck TLC Silica gel 60 F254) was heated for 20 min at 55 °C in an incubator (Tryte Technologies Electrothermal Thermostatic Incubator TNP-9082-11) and was carefully spotted with the sample in two replicates using a capillary tube (Kimble Chase 34500-99 Borosilicate Glass Melting Point Capillary Tube).

After plate development and air-drying, it was viewed at short (254 nm) and long (365 nm) wavelengths of UV light (Analytik Jena UVP UVGL-58, Analytik Jena UVP Chromato-Vue® Cabinet C-10), and the spots were marked. Vanillin-sulfuric acid (4 g of vanillin in 25 mL of concentrated sulfuric acid) was sprayed onto the TLC plate and then heated at 110 °C, until coloration was observed on the chromatogram. The retention factor (R_f) values were calculated by dividing the distance traveled by the sample spots from the origin by the distance traveled by the solvent.

Antibacterial assay. Test pathogens *Klebsiella pneumoniae* (NCTC 13440), methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33592), *Enterococcus faecium* (NCTC 12204), and *Enterobacter cloacae* (ATCC BAA-2341) were inoculated separately into a cation-adjusted Mueller

Hinton Broth (CAMHB) and incubated overnight. All bacterial suspensions were adjusted to an optical density equivalent to 0.5 McFarland Standard prior to the bioassay (Table 1).

Table 1. Strain characteristics of the bacterial pathogens used.

Bacteria	**Catalogue Number**	**Strain Characteristics**
Klebsiella pneumoniae	NCTC 13440	MecA positive SCCmec type III positive Methicillin and gentamicin resistant
Methicillin-resistant *Staphylococcus aureus* (MRSA)	ATCC 33592	Metallo-beta-lactamase positive, VIM-1 Beta-lactam antibiotic resistant
Enterococcus faecium	NCTC 12204	VanA-type glycopeptide resistance Vancomycin resistant
Enterobacter cloacae	ATCC BAA-2341	BlaKPC positive Beta-lactam antibiotic resistant

The minimum inhibitory concentration (MIC) of the dried plant ethanolic extract was determined using the broth microdilution method and the resazurin microtiter test (Elshikh et al. 2016; Sarker et al. 2007). The dried plant ethanolic extract was dissolved in 2% dimethyl sulfoxide (DMSO) and prepared at different concentrations (40, 32, 24, 20, 16, 12, 8, 4, and 2 mg/mL). The antibiotic used for positive control was ciprofloxacin, which was dissolved in sterile distilled water with a stock concentration of 200 ppm.

Diluted plant extracts and bacterial suspensions were dispensed in the 96-well microplate accordingly. Sample wells contained 50 µL bacterial suspension and 50 µL plant extract (final well concentrations of 20,000, 16,000, 12,000, 10,000, 8,000, 6,000, 4,000, 2,000, and 1,000 ppm). Sample blank wells contained 50 µL CAMHB and 50 µL plant extract (final well concentrations of 20,000, 16,000, 12,000, 10,000, 8,000, 6,000, 4,000, 2,000, and 1,000 ppm). Positive control wells contained 50 µL bacterial suspension and 50 µL ciprofloxacin (a final well concentration of 100 ppm). Positive blank wells contained 50 µL CAMHB and 50 µL ciprofloxacin (a final well concentration of 100 ppm). Negative control wells contained 50 µL bacterial suspension and 50 µL CAMHB. Sterility control wells contained 100 µL CAMHB. All samples, controls, and blanks have triplicate wells, and each well had a total of 100 µL final volume. Microplates were sealed and incubated at 37 °C for 22 h.

After incubation, 20 µL of 300 ppm resazurin were dispensed into each well, then re-incubated at room temperature for 15 min. A color change from blue to pink signifies the reduction of resazurin, an indication of bacterial growth. After 15 min re-incubation, fluorescence (in RFU or relative fluorescence units) was measured using a BMG Labtech FLUOstar Omega microplate reader with 544 nm excitation and 590 nm emission filters and gain at 700 nm. The concentration that inhibited the growth of 100% of the pathogen was recorded as the MIC. Percent inhibition was calculated using the equation shown below:

$$\%\ Inhibition = \left[1 - \frac{RFU_{sample} - RFU_{blank}}{RFU_{negative\ ctrl} - RFU_{sterility\ ctrl}}\right] \times 100\%$$

To determine the minimum bactericidal concentration (MBC), the contents of the wells were inoculated into nutrient broth and incubated for 24 h at 37 ºC. Turbidity signified bacterial growth. The experiment was performed in three trials, three replicates per trial, and results were expressed as mean ± standard deviation (n=3).

RESULTS AND DISCUSSION

Phytochemical analysis. Tannins, polyphenols, flavonoids, saponins, steroids and triterpenoids were detected in ethanolic extracts of *V. barandanum* leaves, while alkaloids were not detected (Table 2). Flavonoids, saponins, and tannins were also detected on *V. barandanum* fruits in a previous study (Barcelo 2015). Phytochemicals are often studied in *Vaccinium* berries for their application in human health (Debnath and Goyali 2020), as this genus is a commonly consumed fruit.

Table 2. Phytochemical screening of *V. barandanum* leaf extract.

Test	Positive Result	Result
Tannins		
Gelatin test	white precipitate	+
Polyphenols		
Ferric chloride test	dark green/bluish-black color	+
Flavonoids		
Alkaline reagent test	colorless	-
Lead acetate test	yellow precipitate	-
Shinoda's test	pink to crimson color (flavonol glycosides)/ intense cherry red color (flavanones)/ orange red color (flavonols)	+
Saponins		
Froth test	formation of 1 cm layer of foam	+
Alkaloids		
Mayer's test	creamy white/yellow precipitate	-
Wagner's test	brown/reddish precipitate	-
Dragendorff's Test	red/reddish-brown precipitate	-
Hager's Test	creamy white/yellow precipitate	-
Steroids and Triterpenoids		
Liebermann Burchard test	colors ranging from blue to green, violet and red	+

(-) not detected, (+) detected

The leaves, roots, and stems of this species are also valuable sources of antioxidants. Specific phenolics had been previously studied. About 30 times more chlorogenic acid (a phenolic acid derivative) concentrated were detected in leaf extracts than in fruits of *V. angustifolium* and ten-fold less in glycoside species (Harris et al. 2007).

Total phenolic content (TPC) and total flavonoid content (TFC). Total phenolic and total flavonoid contents of the leaf ethanolic extract were quantified using standard calibration curves of gallic acid (R^2 = 0.994) and quercetin (R^2 = 0.9788), respectively (Table 3).

Table 3. Total phenolic content (TPC) and total flavonoid content (TFC) of *V. barandanum* leaf per g of dry extract (DE), leaf dry weight (DW), and leaf fresh weight (FW), presented as mean ± standard deviation (n = 3).

TPC		
mg GAE/g DE ± SD	**mg GAE/g DW ± SD**	**mg GAE/g FW ± SD**
96.62 ± 0.77	3.67 ± 0.77	1.25 ± 0.77
TFC		
mg QE/g DE ± SD	**mg QE/g DW ± SD**	**mg QE/g FW ± SD**
39.99 ± 2.45	1.52 ± 2.45	0.52 ± 2.45

Comparing the results of the current study with those of other *Vaccinium* species, the TPC of *V. barandanum* leaf extract is within the range of previous results for *V. formosum* (67.15 to 349.17 mg GAE/g DE) (Deng et al. 2014) and *V. ashei* (75.4 to 222 mg GAE/g DE) (Cezarotto et al. 2017), but lower compared to reported values for *V. myrtillus* (90.5 mg GAE/g DW) and *V. vitis-idaea* (96.68 mg GAE/g DW) (Vrancheva et al. 2020). Meanwhile, the TFC observed in this study is higher than the TFC value reported for *V. arctostaphylos* (26.1 mg QE/g DE) (Mahboubi et al. 2013), but lower than those reported for *V. myrtillus* (34.96 mg QE/g DW) and *V. vitis-idaea* (21.2 mg QE/g DW) (Vrancheva et al. 2020).

When compared with the previously reported findings for *V. barandanum* fruit (Barcelo 2015), with TPC ranging from 84 to 90 mg GAE/100 g FW (or 0.84 to 0.90 mg GAE/g FW) and TFC ranging from 11 to 18 mg QE/100 g FW (or 0.11 to 0.18 mg QE/g FW), the TPC and TFC of the leaf extract were found to be considerably higher. The results are in agreement with previous comparative studies on TPC and TFC of other *Vaccinium* species, where higher values were recorded in leaves and stems than in fruits (Bujor et al. 2019; Mahboubi et al. 2013; Silva et al. 2013; Vucic et al. 2013).

Antioxidant activity. The radical scavenging activity of the *V. barandanum* extract increased with increasing concentrations, reaching 78.85% at 400 µg/mL, but has lower activity than the standard ascorbic acid (Table 4).

Table 4. The DPPH radical scavenging activity of *V. barandanum* leaf ethanolic extract expressed as a percentage inhibition at a given concentration and as IC_{50} value.

	Concentration (µg/mL)						**IC_{50} Extract (µg/mL)**	**IC_{50} AA* (µg/mL)**
	12.5	**25**	**50**	**100**	**200**	**400**		
Inhibition	14.30%	15.06%	21.26%	36.14%	67.04%	78.85%	144.09	23.31
SD	2.77	2.73	2.26	3.94	0.59	5.16	5.77	0.9

*AA - Ascorbic acid

The antioxidant activity of *V. barandanum* leaf is lower than those of other *Vaccinium* species recorded earlier, with IC_{50} values ranging from 5.80 to 105 µg/mL for *V. ashei* (Cezarotto et al. 2017) and 25 µg/mL for *V. arctostaphylos* (Mahboubi et al. 2013). Despite the wide variation, *Vaccinium* species, including *V. barandanum*, remain recognized as rich sources of antioxidants and other bioactive molecules (Tundis et al. 2021). TPC and TFC values were directly correlated with antioxidant activities

of *Vaccinium* extracts in previous studies (Mahboubi et al. 2013; Tian et al. 2018). The observed antioxidant activity of the *V. barandanum* leaf extract may therefore be attributed to the phenolics and flavonoids detected.

TLC profile. After development using 70% hexane:30% ethyl acetate as the mobile phase system, the chromatogram showed 11 spots under long UV, 4 under short UV, and 14 under vanillin-sulfuric acid spray. Under UV_{365}, the spots fluoresced red, some lighter in intensity. Four spots exhibited fluorescence quenching and appeared as dark spots under UV_{254}. The majority of the spots displayed a violet or purple color in different intensities while one spot was blue, and another was yellow green-gray under vanillin spray.

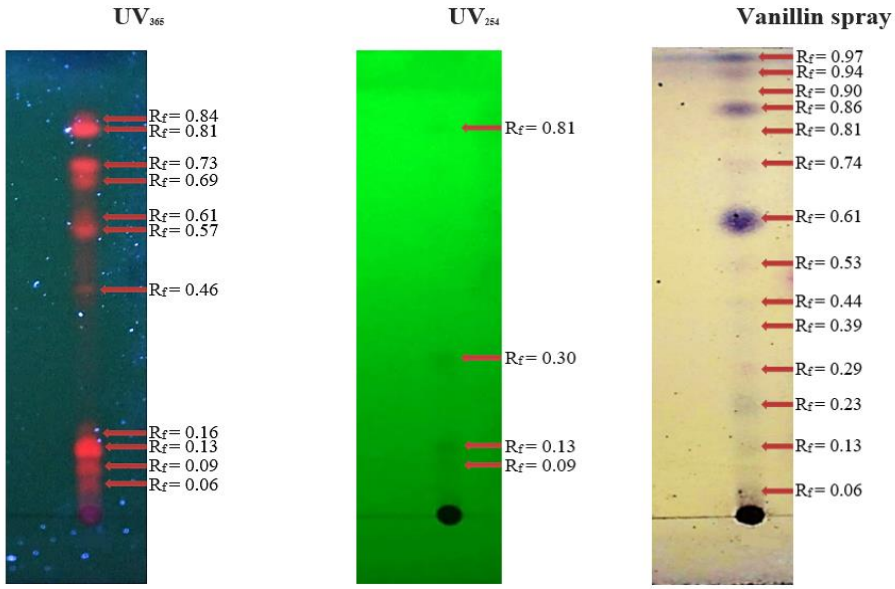

Figure 2. TLC chromatograms of *V. barandanum* leaf ethanolic extract visualized under UV at 365 nm, UV at 254 nm and after spraying with vanillin-sulfuric acid. Mobile phase: hexane: ethyl acetate (7: 3 v/v).

Table 5 presents the summary of the TLC profile of the leaf ethanolic extract of *V. barandanum*. Most of the spots appeared under UV_{365} and vanillin-sulfuric acid staining.

Table 5. Summary of *V. barandanum* leaf ethanolic extract TLC profile.

R_f value	**UV_{365} nm**	**UV_{254} nm**	**Vanillin spray**	**Phytochemical group**
0.06	Red	-	Violet	Phenolics
0.09	Red	Quenching	-	Phenolics
0.13	Red	Quenching	Yellow Green-Gray	
0.16	Red	-	-	
0.23	-	-	Blue	Sterol
0.29	-	-	Light Violet	

R_f value	UV_{365} nm	UV_{254} nm	Vanillin spray	Phytochemical group
0.30	-	Quenching	-	Flavonoid
0.39	-	-	Light Violet	
0.44	-	-	Light Violet	
0.46	Red	-	-	
0.53	-	-	Light Violet	
0.57	Red	-	-	
0.61	Red	-	Dark Violet	Phenolics
0.69	Red	-	-	
0.73	Red	-	-	
0.74	-	-	Light Violet	
0.81	Red	Quenching	Light Violet	Phenolics
0.84	Red	-	-	
0.86	-	-	Dark Violet	
0.90	-	-	Light Violet	
0.94	-	-	Violet	
0.97			Dark Violet	

The presence of phenolics was confirmed under UV (Harborne 1998; Martin-Puzon et al. 2015). The dark spots observed in *V. barandanum* extract under UV_{254} indicate the presence of phenolics and further suggest that of a few flavonoids, which are known to cause fluorescence quenching (Medic-Saric et al. 2008; Skorek et al. 2016). The application of vanillin-sulfuric acid reagent corroborates the presence of phenolic compounds (violet/dark violet) (Napiroon et al. 2017), triterpenes or terpenoids (purple), sterols (blue) (Akpalo et al. 2020), and tannic acid (purple) (Sharma et al. 1998). Overall, the characteristic TLC profile of *V. barandanum* leaf ethanolic extract validates this study's phytochemical screening results.

The R_f value can be utilized to tentatively identify a compound by comparing the value of the unknown to that of a reference (Kumar et al. 2013). Therefore, the observed coloration of the spots in the chromatograms of *V. barandanum* extract and their respective R_f values can aid in the identification of these phytochemicals when compared with standards.

Antibacterial activity. The ethanolic extract was tested against four bacterial pathogens with antibiotic resistance genes. Comparing the MIC values of the extract against the different pathogens, the lower MIC values of the extract against gram-positive bacteria, *E. faecium* and MRSA (Table 6) indicate greater sensitivity of these bacteria to the extract. In contrast, the higher MIC values against gram-negative bacteria, *E. cloacae* and *K. pneumoniae*, indicate that they are less sensitive to the extract. The MIC of ciprofloxacin against the test pathogens was significantly lower than that of the extract (MIC of 50-100 ppm concentration). While the positive control had a better MIC value, the observed MIC of the *V. barnandanum* leaf extract is still notable, especially that it was tested against antibiotic-resistant bacteria. This result demonstrates the plant's potential to combat infections caused by these test pathogens.

Mechanistically, antibacterial compounds destroy and penetrate the cell walls of gram-positive bacteria more effectively compared to the cell walls of gram-negative bacteria because the latter have hydrophilic outer membranes that are more resistant to antibacterial agents. The observed inhibition of

the gram-negative bacteria, however, may be partially due to some organic acids found in berry plants that weaken this outer membrane (Tian et al. 2018).

Table 6. Antibacterial activity of *V. barandanum* leaf ethanolic extract against bacterial pathogens with antibiotic resistance genes.

Test Pathogen	MIC of the Extract (ppm)	MIC of Ciprofloxacin (ppm)	MBC (ppm)
E. faecium	6,000	≤100	>20,000
MRSA	6,000	80	>20,000
E. cloacae	12,000	≤100	>20,000
K. pneumoniae	12,000	50	>20,000

As to the MBC determination, there was growth in all of the test pathogens when these were re-inoculated in nutrient broth and incubated for 24 h. Thus, the reduction in the bacterial population at all the concentrations used was not enough to be considered a bactericidal activity.

Plant secondary metabolites are crucial for their inherent defensive mechanisms and are known to have antimicrobial effects *in vitro* (Jurikova et al. 2018). These natural compounds, such as the flavonoid phenolics that were detected in the *V. barandanum* extract, may explain the observed antibacterial activities in the present study. In a previous report, the polyphenols found in the *Vaccinium corymbosum* extract were found related to the inhibition of biofilm formation by clinically obtained antibiotic-resistant pathogens, including MRSA and carbapenem-resistant *K. pneumoniae* (Gato et al. 2021). Earlier studies have also reported the significant contribution of phenolics, and their synergistic effects, to the antimicrobial property of plant extracts against bacterial and fungal pathogens (Deng et al. 2014). The phenolic compounds detected in the *V. barandanum* extract may have resulted in a combination of mechanisms that is crucial in minimizing bacterial resistance (Gato et al. 2021). In this regard, formulations using crude extract of the plant may have its advantages over using isolated and purified compounds.

The MIC value from this study (6,000 ppm; 6 mg/ml) against MRSA suggests that *V. barandanum* leaf extract has better antibacterial property against this bacterial pathogen compared to *V. corymbosum*. An MIC value of 12,500 ppm (12.5 mg/mL) was previously reported for *V. corymbosum* against MRSA and methicillin sensitive *S. aureus* (MSSA) (Silva et al. 2013, 2015). Meanwhile, a lower MIC value of 3,750 ppm (3.75 mg/ml) was obtained for clinically acquired MRSA using *V. vitis-idaea* leaf extract (Kryvtsova et al. 2020). Earlier studies, using MSSA strains as test pathogens, also reported MIC values of 3,200 ppm (3.2 mg/mL) for *V. arctostaphylos* (Mahboubi et al. 2013), 1,500 ppm (1.5 mg/mL) for *V. myrtillus* (Sadowska et al. 2014), and 25,000 ppm (25 mg/mL) for *V. formosum* leaf extract (Deng et al. 2014). A lower MIC value implies better antimicrobial property, however, it should be noted that the better MIC values for *V. vitis-idaea*, *V. arctostaphylos*, and *V. myrtillus* extracts were observed against non-antibiotic resistant strains, while the present study used resistant test bacteria.

To-date, there is no available data on the antibacterial properties of *V. barandanum* leaf extract against antibiotic-resistant pathogens. *V. barandanum* extract showed inhibition of biofilm formation in *P. aeruginosa* and methicillin resistant *S. aureus*, done in combination with the antibiotic oxacillin (Mirghani et al. 2019). There are also no minimum inhibitory concentration (MIC) or minimum

bactericidal concentration (MBC) values that have been reported for *V. barandanum* against any test pathogen. Notably, the MIC detected in this study against vancomycin-resistant *E. faecium* (6,000 ppm; 6 mg/ml) is lower than the reported values for *V. arctostaphylos* (MIC value of 12,800 ppm; 12.8 mg/mL) (Mahboubi et al. 2013) and *V. corymbosum* (MIC value of 25,000 ppm; 25 mg/mL) (Silva et al. 2013) leaf extracts against non-resistant strains of *E. faecium*. This result suggests that *V. barandanum* extract has better antibacterial property against *E. faecium* compared to the other *Vaccinium* species.

There are no reports to date on the MIC or MBC values for other *Vaccinium* species specifically against beta-lactam antibiotic-resistant *E. cloacae* and methicillin- and gentamicin-resistant *K. pneumoniae*. There are, however, reports of *Vaccinium* fruit and leaf extracts tested against strains of *E. cloacae* and *K. pneumoniae,* which lack antibiotic resistance genes. Earlier studies reported a MIC of 10,000 ppm (10 mg/mL) for *V. floribundum* (Guevara-Terán et al. 2022) and *V. myrtillus L.* fruit extracts (Vega et al. 2023) against *E. cloacae*. Reports also include an MIC of 6,400 ppm (6.4 mg/mL) for *V. arctostaphylos* leaf extract (Mahboubi et al. 2013) and 126,000 ppm (126 mg/mL) for *V. myrtillus L*. fruit extract (Miljkovic et al. 2017) against *K. pneumoniae.*

The results of this study provide a valuable contribution to the general chemical profile and composition of this native species from the Cordillera region and to its biological activities, including antibacterial and antioxidant properties. *V. barandanum* leaf ethanolic extract has broad antibacterial activity, as shown against antibiotic-resistant *E. faecium*, MRSA, *E. cloacae*, and *K. pneumoniae*, with better inhibition of the growth of the gram-positive bacteria. This is the first study to report the MIC of *V. barandanum* extract against multidrug-resistant bacterial pathogens, and it allowed the comparison of the antimicrobial activity of the extract with other *Vaccinium* extracts published in literature. This shows the potential of *V. barandanum* leaf ethanolic extract as a source of antibacterial drug leads. Further studies are needed to identify the phytochemical components and determine their mechanisms of action to explain their bioactivity against the tested multidrug-resistant bacteria. Moreover, the study established that the leaves of this species possess higher phenolic and flavonoid contents than the fruit.

CONCLUSION

The phytochemical analysis and assays expand on prior works on the Philippine native species, *V. barandanum*. In addition to demonstrating that the leaves are a good source of antioxidants, the activity against antibiotic-resistant strains of *E. faecium*, MRSA, *E. cloacae*, and *K. pneumoniae* is a novel finding, albeit at high MIC concentrations.

This study provides a framework for future studies on nutraceutical and pharmaceutical product development using this species. It is recommended that additional research be conducted to isolate and characterize the specific chemical compounds responsible for the observed antibacterial effects, specifically to determine better MIC values for the purified components. This may result in novel drug leads, as metabolomics studies have not yet been conducted on *V. barandanum*. With the chemical constituents and bioactivities observed in this study, this plant is an attractive candidate for the development of functional foods and nutraceuticals.

ACKNOWLEDGEMENT

The authors are grateful for the support provided by the Department of Science and Technology- Philippine Council for Health Research and Development. We also thank the Tuklas Lunas Laboratory of Benguet State University and the Science Research Center, University of the Philippines Baguio for their valuable assistance.

REFERENCES CITED

Abreu, O.A., G. Barreto and S. Prieto. 2014. *Vaccinium* (Ericaceae): Ethnobotany and pharmacological potentials. Emirates Journal of Food and Agriculture. 26: 577-591.

Akpalo, A.E., I.K. Saloufou, K. Eloh and K. Kpegba. 2020. Wound healing biomolecules present in four proposed soft aqueous extractions of *Ageratum conyzoides* Linn. International Journal of Biological and Chemical Sciences. 14(2): 638-651.

Al-Dhabi, N.A., K. Ponmurugan and P. Maran Jeganathan. 2017. Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds. Ultrasonics Sonochemistry. 34: 206-213.

Alnajar, Z.A.A., M.A. Abdulla, H.M. Ali, M.A. Alshawsh and A.H.A. Hadi. 2012. Acute toxicity evaluation, antibacterial, antioxidant and immunomodulatory effects of *Melastoma malabathricum*. Molecules. 17(3): 3547-3559.

Awuchi, C.G. and C.O. Okpala. 2022. Natural nutraceuticals, especially functional foods, their major bioactive components, formulation, and health benefits for disease prevention - An overview. Journal of Food Bioactives. 19: 97–123.

Ballington, J.R. 2001. Collection, utilization, and preservation of genetic resources in *Vaccinium*. HortScience. 36(2): 213-220.

Barcelo, R.C. 2015. Phytochemical screening and antioxidant activity of edible wild fruits in Benguet, Cordillera Administrative Region, Philippines. Electronic Journal of Biology. 11(3): 80-89.

Barcelo, R.C. and J.M. Barcelo. 2020. *Vaccinium barandanum* S. Vidal Ericaceae. Ethnobotany of the Mountain Regions of Southeast Asia. 1-3.

Barcelo, R.T. 2014. Ethno–botanical survey of edible wild fruits in Benguet, Cordillera administrative region, the Philippines. Asian Pacific Journal of Tropical Biomedicine. 4(Suppl1): S525–38.

Begum, S., T. Begum, N. Rahman and R.A. Khan. 2021. A review on antibiotic resistance and ways of combating antimicrobial resistance. GSC Biological and Pharmaceutical Sciences. 14(2): 087-097.

Ben, I.O., E. Woode, W.K.M. Abotsi and E. Boakye-Gyasi. 2013. Preliminary phytochemical screening and *in vitro* antioxidant properties of *Trichilia monadelpha* (Thonn.) JJ De Wilde (Meliaceae). Journal of Medical and Biomedical Sciences. 2(2): 6-15.

Bujor, O.-C., C. Tanase and M.E. Popa. 2019. Phenolic antioxidants in aerial parts of wild *Vaccinium* species: Towards pharmaceutical and biological properties. Antioxidants. 8(12): 1-13.

Cezarotto, V.S., S.R. Giacomelli, M.H. Vendruscolo, A.S. Vestena, C.S. Cezarotto, R.C. Da Cruz, L.H. Maurer, L.M. Ferreira, T. Emanuelli, and L. Cruz. 2017. Influence of harvest season and cultivar on the variation of phenolic compounds composition and antioxidant properties in *Vaccinium ashei* leaves. Molecules. 22(10): 1-11.

Cruz, P.S. 2019. Spray drying of plant extracts, pp 13-15. In A.P. Guevara and, R.G.Y. Alvero (eds.). Tuklas Lunas Protocols for Drug Discovery and Development Manual 3. Philippine Council for Health Research and Development. Taguig City, Philippines.

Debnath, S.C. and J.C. Goyali. 2020. *In vitro* propagation and variation of antioxidant properties in micropropagated *Vaccinium* berry plants—A review. Molecules. 25(4): 1-26.

Deng, Y., G. Yang, J. Yue, B. Qian, Z. Liu, D. Wang, Y. Zhong and Y. Zhao. 2014. Influences of ripening stages and extracting solvents on the polyphenolic compounds, antimicrobial and antioxidant activities of blueberry leaf extracts. Food Control. 38: 184-191.

Elshikh, M., S. Ahmed, S. Funston, P. Dunlop, M. McGaw, R. Marchant and I.M. Banat. 2016. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnology Letters. 38(6): 1015-1019.

Escueta-De Cadiz, A. and A.D. Almeria. 2019. Collection, extraction and purification of terrestrial plant samples, pp 19-24. In A.P. Guevara and, R.G.Y. Alvero (eds.). Tuklas Lunas Protocols for Drug Discovery and Development Manual 1. Philippine Council for Health Research and Development. Taguig City, Philippines.

Gato, E., A. Perez, A. Rosalowska, M. Celeiro, G. Bou and M. Lores. 2021. Multicomponent polyphenolic extracts from *Vaccinium corymbosum* at lab and pilot scale. Characterization and effectivity against nosocomial pathogens. Plants. 10: 1-16.

Guevara-Terán, M., K. Padilla-Arias, A. Beltrán-Novoa, A.M. González-Paramás, F. Giampieri, M. Battino, W. Vásquez-Castillo, P. Fernandez-Soto, E. Tejera and J.M. Alvarez-Suarez. 2022. Influence of altitudes and development stages on the chemical composition, antioxidant, and antimicrobial capacity of the wild Andean blueberry (*Vaccinium floribundum* Kunth). Molecules. 27(21): 1-23.

Gul, R., S.U. Jan, S. Faridullah, S. Sherani and N. Jahan. 2017. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. Scientific World Journal. 2017: 1-7.

Harborne, J.B. 1998. Phenolic compounds, pp 40-106. In Phytochemical Methods: A guide to modern techniques of plant analysis (3rd ed). Chapman & Hall. London, U.K.

Harris, C.S., A.J. Burt, A. Saleem, P.M. Le, L.C. Martineau, P.S. Haddad, S.A.L. Bennett and J.T. Arnason. 2007. A single HPLC-PAD-APCI/MS method for the quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium*. Phytochemical Analysis. 18(2): 161-169.

Juneja, V.K., H.P. Dwivedi and X. Yan. 2012. Novel natural food antimicrobials. Annual Review of Food Science and Technology. 3: 381-403.

Jurikova, T., S. Skrovankova, J. Mlcek, S. Balla and L. Snopek. 2018. Bioactive compounds, antioxidant activity, and biological effects of European cranberry (*Vaccinium oxycoccos*). Molecules. 24(1): 1-21.

Kryvtsova, M.V., I. Salamon, J. Koscova and M.Y. Spivak. 2020. Antibiofilm forming, antimicrobial activity and some biochemical properties of *Vaccinium vitis-idaea* leaf and berry extracts on *Staphylococcus aureus*. Biosystems Diversity. 28(3): 238-242.

Kumar, S., K. Jyotirmayee and M. Sarangi. 2013. Thin layer chromatography: A tool of biotechnology for isolation of bioactive compounds from medicinal plants. International Journal of Pharmaceutical Sciencies Review and Research. 18(1): 126-132.

Mahboubi, M., N. Kazempour and M. Taghizadeh. 2013. *In vitro* antimicrobial and antioxidant activity of *Vaccinium arctostaphylos L.* extracts. Journal of Biologically Active Products from Nature. 4: 241-247.

Martau, G.A., T. Bernadette-Emoke, R. Odocheanu, D.A. Soporan, M. Bochiş, E. Simon and D.C. Vodnar. 2023. *Vaccinium* species (Ericaceae): Phytochemistry and biological properties of medicinal plants. Molecules. 28(4): 1-32.

Martin-Puzon, J.J.R., D.L. Valle and W.L. Rivera. 2015. TLC profiles and antibacterial activity of *Glinus oppositifolius L*. Aug. DC. (Molluginaceae) leaf and stem extracts against bacterial pathogens. Asian Pacific Journal of Tropical Disease. 5(7): 569-574.

Masuku, N.P., J.O. Unuofin and S.L. Lebelo. 2020. Phytochemical content, antioxidant activities and androgenic properties of four South African medicinal plants. Journal of HerbMed Pharmacology. 9(3): 245-256.

Medic-Saric, M., I. Jasprica, A. Mornar, and Z. Maleš. 2008. Application of TLC in the isolation and analysis of flavonoids, pp 405–423. In M. Waksmundzka-Hajnos, J. Sherma, and T. Kowalska (eds.). Thin Layer Chromatography in Phytochemistry. CRC Press. Boca Raton, Florida, USA.

Miljkovic, V.M., G.S. Nikolic, J. Zvezdanovic, T.M. Mihajlov-Krstev, B.B. Arsic and M. Miljkovic. 2017. Phenolic profile, mineral content and antibacterial activity of the methanol extract of *Vaccinium myrtillus L*. Notulae Botinicae Horti Agrobotanici Cluj-Napoca. 46: 122-127.

Mirghani, M.E.S., J.I. Daoud and A.A.M. Elnour. 2019. Wild species of *Vaccinium* composition, nutritional value and utilization, pp 523–537. In A. Mariod (eds.). Wild Fruits: Composition, Nutritional Value and Products. Springer Cham. Switzerland.

Napiroon, T., D. Sookchaloem and S. Vajrodaya. 2017. Thin layer chromatography screening and profiling of terrestrial aroids (Araceae) lipophilic extracts from Saiyok Forest, Thailand. Journal of Tropical Forest Research. 1(1): 1-10.

Nwosu, O.K. and K.I. Ubaoji. 2020. Nutraceuticals: History, classification and market demand, pp 13-22. In C. Egbuna and G. Dable-Tupas (eds.). Functional Foods and Nutraceuticals: Bioactive Components, Formulations and Innovations. Springer Cham. Switzerland.

Pires, T.C., C. Caleja, C. Santos-Buelga, L. Barros, and I.C. Ferreira. 2020. *Vaccinium myrtillus* L. fruits as a novel source of phenolic compounds with health benefits and industrial applications-a review. Current Pharmaceutical Design. 26(16): 1917-1928.

Punzalan, C.V. and I.M. Villaseñor. 2019. Thin-layer chromatography (TLC), pp 38-43. In A.P. Guevara and, R.G.Y. Alvero (eds.). Tuklas Lunas Protocols for Drug Discovery and Development Manual 1. Philippine Council for Health Research and Development. Taguig City, Philippines.

Sadowska, B., M. Paszkiewicz, A. Podsędek, M. Redzynia and B. Różalska. 2014. *Vaccinium myrtillus* leaves and *Frangula alnus* bark derived extracts as potential antistaphylococcal agents. Acta Biochimica Polonica. 61(1): 163-169.

Sarker, S.D., L. Nahar, and Y. Kumarasamy. 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 42(4): 321-324.

Shaikh, J.R. and M. Patil. 2020. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 8(2): 603-608.

Sharma, O.P., T.K. Bhat and B. Singh. 1998. Thin-layer chromatography of gallic acid, methyl gallate, pyrogallol, phloroglucinol, catechol, resorcinol, hydroquinone, catechin, epicatechin, cinnamic acid, p-coumaric acid, ferulic acid and tannic acid. Journal of Chromatorgraphy A. 822(1): 167-171.

Silva, S., E.M. Costa, M.F. Pereira, M.R. Costa and M.E. Pintado. 2013. Evaluation of the antimicrobial activity of aqueous extracts from dry *Vaccinium corymbosum* extracts upon food microorganism. Food Control. 34(2): 645-650.

Silva, S., E.M. Costa, M.R. Costa, M.F. Pereira, J.O. Pereira, J.C. Soares and M.M. Pintado. 2015. Aqueous extracts of *Vaccinium corymbosum* as inhibitors of *Staphylococcus aureus*. Food Control. 51: 314-320.

Skorek, M., K. Jurczyk, M. Sajewicz and T. Kowalska. 2016. Thin-layer chromatographic identification of flavonoids and phenolic acids contained in cosmetic raw materials. Journal of Liquid Chromatography & Related Technologies. 39(5-6): 286–291.

Song, G.Q. and J.F. Hancock. 2010. *Vaccinium*, pp 197-221. In Wild Crop Relatives: Genomic and Breeding Resources Temperate Fruits. Springer. Berlin Heidelberg, Germany.

Ștefănescu, B.-E., L.F. Călinoiu, F. Ranga, F. Fetea, A. Mocan, D.C. Vodnar, and G. Crișan. 2020. Chemical composition and biological activities of the nord-west Romanian wild bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.) leaves. Antioxidants. 9(6): 1-22.

Tian, Y., A. Puganen, H.-L. Alakomi, A. Uusitupa, M. Saarela, and B. Yang, 2018. Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. Food Research International. 106: 291–303.

Tundis, R., M.C. Tenuta, M.R. Loizzo, M. Bonesi, F. Finetti, L. Trabalzini, and B. Deguin. 2021. *Vaccinium* species (Ericaceae): From chemical composition to bio-functional activities. Applied Sciences. 11: 1-19.

Vander Kloet, S.P. and T.A. Dickinson. 2009. A subgeneric classification of the genus *Vaccinium* and the metamorphosis of V. section *Bracteata Nakai:* more terrestrial and less epiphytic in habit, more continental and less insular in distribution. Journal of Plant Research. 122: 253-268.

Vega, E.N., P. García-Herrera, M. Ciudad-Mulero, M.I. Dias, M.C. Matallana-González, M. Cámara, J. Tardío, M. Molina, J.C.S.P. Pinela, T. Pires, L. Barros, V. Fernández-Ruiz, and P. Morales. 2023. Wild sweet cherry, strawberry and bilberry as underestimated sources of natural colorants and bioactive compounds with functional properties. Food Chemistry. 414: 1-12.

Vital, P.G., E.S. Zara, C.E.M. Paraoan, M.A.Z. Dimasupil, J.J.M. Abello, I.T.G. Santos, and W.L. Rivera. 2018. Antibiotic resistance and extended-spectrum beta-lactamase production of *Escherichia coli* isolated from irrigation waters in selected urban farms in Metro Manila, Philippines. Water. 10(5): 1-11.

Vrancheva, R., I. Ivanov, I. Badjakov, I. Dincheva, V. Georgie, and A. Pavlov. 2020. Optimization of polyphenols extraction process with antioxidant properties from wild *Vaccinium myrtillus L.* (bilberry) and *Vaccinium vitis idaea* L. (lingonberry) leaves. Food Science and Applied Biotechnology. 3(2): 149-156.

Vucic, D.M., M.R. Petković, B.B. Rodić-Grabovac, O.D. Stefanović, S.M. Vasić, and L.R. Čomić. 2013. Antibacterial and antioxidant activities of bilberry (*Vaccinium myrtillus L.*) *in vitro.* African Journal of Microbiology Research. 7(45): 5130-5136.