Article

Exploring the Antioxidant, Antidiabetic, and Antimicrobial Capacity of Phenolics from Blueberries and Sweet Cherries

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Featured Application: This work analyses the biological potential of blueberries and sweet cherries in several dimensions of human health, reinforcing the research on the benefits of consumption of these fruits.

Abstract: (1) Background: Nowadays, special attention has been paid to red and purple fruits, including blueberries and sweet cherries, since they are highly attractive to consumers due to their organoleptic properties, standing out due to their vibrant red and purple colours and sweet flavour, and nutritional value. (2) Methods: The present study evaluated the phenolic profile of phenolic-enriched extracts from blueberries and sweet cherries and explored their antioxidant potential against DPPH, superoxide and nitric oxide radicals, and ferric species, and their potential to inhibit the α-glucosidase enzyme. Furthermore, their antimicrobial activity was also determined by microdilution method against four Gram-positive strains (*Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* LMG 16779, and *Staphylococcus aureus* ATCC 25923) and five Gram-negative strains (*Salmonella enterica* subsp. *enterica* ATCC 13311 serovar Typhimurium, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* CECT 170, *Serratia marcescens* CECT 159, and *Acinetobacter baumannii* LMG 1025). (3) Results: By chromatographic techniques, eight anthocyanins were detected in blueberry coloured fraction and total extract, and five anthocyanins were detected in sweet cherry total extract and coloured fraction, while quercetin aglycone and chlorogenic acids were the dominant non-coloured compounds in blueberries and sweet cherries, respectively. All extracts demonstrated significant antioxidant properties, as well as the ability to inhibit the activity of α-glucosidase enzyme and the development of various microorganisms. (4) Conclusion: The obtained data evidence the promising biological potential of blueberries and sweet cherries, being highly correlated with the presence of phenolic compounds.

1. Introduction

Currently, red and purple fruits are the focus of many studies given their health-promoting properties [1–9]. These benefits have been closely linked to phenolic compounds, namely, anthocyanins, whose presence of pyrogallol, methoxy, and catechol groups in their chemical structure turn them into notable antioxidant, antimicrobial, anti-parasitic, anti-inflammatory, anti-cancer, and probiotic agents [5,9–11]. Among fruits, blueberries (*Vaccinium corymbosum*) and sweet cherries (*Prunus avium* Linnaeus) have attracted much attention given their high content of anthocyanins (total anthocyanins content varying from 34.5 to 552.2 mg cyanidin 3-*O*-rutinoside per 100 g of fresh weight (fw) for blueberries, and between 3.7 and 98.4 mg cyanidin 3-*O*-rutinoside per 100 g of fw for sweet cherries) [2,12–16]. However, these amounts depend on genotype, local origin, climate, and agricultural and processing techniques [17,18].

In fact, these fruits are very attractive to consumers mainly due to their nutritional values and organoleptic characteristics, standing out due to their vibrant purple and red colours owing to the presence of anthocyanins, and sweet taste; therefore, it is not surprising that their cultivation and economical value are increasing worldwide [17,19,20]. They are mainly consumed fresh, but can also be processed into juices, concentrates, and jams [21,22]. Focusing on blueberries, malvidin 3-*O*-galactoside is the main anthocyanin detected (12.11–67.45 mg per 100 g of fw), followed by peonidin and delphinidin 3-*O*-glucoside (12–54.37 and 1.21–53.62 mg per 100 g fw, respectively), and delphinidin 3-*O*-galactoside (2.29–53.29 mg per 100 g fw) [22–25]. Recent evidence reported that blueberries possess anti-hypertensive properties and are really effective in normalizing stress oxidative levels and inflammation [26–29], and improving neuronal [3,30], endothelial, and vascular functions in humans [31,32]. Regarding sweet cherries, cyanidin 3-*O*-rutinoside is the main anthocyanin found (0.20–389.9 mg per 100 g of fw), followed by cyanidin 3-*O*-glucoside (0.0 to 142.03 mg per 100 g of fw) [15,33,34]. Additionally, vestigial amounts of cyanidin and malvidin aglycones, and delphinidin, malvidin, pelargonidin, and peonidin derivatives are also detected [16,17,33,35,36]. The daily intake of sweet cherries has been demonstrated to diminish blood pressure levels and inflammatory markers [37,38], improve sleep, oxidative stress levels [39], memory and cognition capacity [40], and exert anti-gout effects in humans [41].

Therefore, considering the potential of natural products to attenuate or even prevent the appearance of many disorders and also the current increasing incorporation of natural molecules in pharmaceuticals, the main goal of this work is to analyse the phenolic profile of phenolic-concentrated fractions of Portuguese blueberries (cv. Legacy and cv. Duke) and sweet cherries (cv. Sweetheart), evaluate the scavenging capacity of these fractions against 1,1-diphenyl-2-picrylhydrazyl (DPPH$^\bullet$), the ferric reducing ability of plasma (FRAP), nitric oxide ($^\bullet$NO), and superoxide ($O_2^{\bullet -}$) radicals, and also explore their potential to inhibit the α-glucosidase enzyme. Additionally, we also tested the antimicrobial capacity of these extracts, together with Saco sweet cherry, on nine bacterial strains, which are either food pathogens or part of the list of priority pathogens according to the World Health Organization given their ability to acquire or present resistance to antibiotics [42]. Antibiotic-resistant pathogens are one of the main threats to human health in recent decades, and new strategies should be implemented with the aim to stop the rise and transmission of these cases [43]. We conducted a comparison between the extracts of two types of berries with strong biological properties. While this approach has been used previously [44], this is the first time that the activity of these berries from the same region has been directly compared.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals used were of analytical grade. Anthocyanins were purchased from Extrasynthese (Genay, France). The non-coloured phenolics, β-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitrotetrazolium blue chloride (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and α-glucosidase from Saccharomyces cerevisiae (type I, lyophilized powder) were obtained from Sigma-Aldrich (St. Louis, MO, USA). N-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilamide, 4-nitrophenylalpha-D-glucopyranoside (pNPG), and sodium nitroprusside dihydrate (SNP) were acquired from Alfa Aesar (Karlsruhe, Germany). Methanol and acetonitrile for HPLC (purity ≥ 99.9%) were from Fisher Chemical (Glenfield, Leicestershire, UK). Water was deionized using a Milli-Q water purification system (Millipore Ibérica, S.A.U., Madrid, Spain).

2.2. Samples Collection

Blueberries cv. Legacy and cv. Duke at full maturity were randomly selected and harvested by hand by the authors of this study in the middle of June 2021 in Covilhã and Guarda, respectively. Sweet cherry samples were collected by hand and provided to us by a local company (Cerfundão) (Figure 1). After being collected, samples were immediately transported at 5 °C to a laboratory of the Health Sciences Research Center at the University of Beira Interior, Covilhã, Portugal (CICS-UBI), and then frozen with liquid nitrogen and maintained at −80 °C before being freeze-dried and powdered. Finally, they were divided into three aliquots, extracted, and analysed separately.
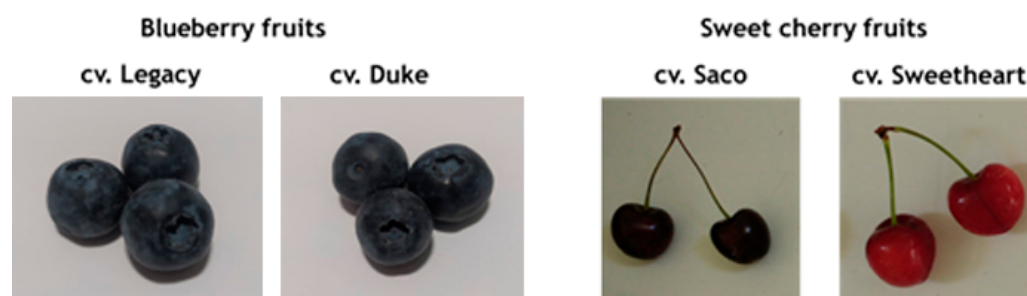

Figure 1. Image of the samples studied in this work (image courtesy of the authors).

2.3. Phenolic Compounds Extraction

Briefly, 1 g of lyophilized extract from each cultivar was firstly stirred with 70% ethanol at 300 rpm for 2 h, protected from light at room temperature, and then centrifugated at 4000 rpm for 15 min. Next, the supernatant was evaporated, dissolved in deionized water, and placed into a C18 solid-phase extraction column (70 mL/10,000 mg; Macherey-Nagel, Düren, Germany) previously conditioned with ethyl acetate, ethanol, and 0.01 mol/L HCl [6]. Then, the sample was passed through the column and the non-coloured fraction was eluted with ethyl acetate and the coloured fraction with ethanol containing 0.1% HCl. This procedure was repeated and the total extract (rich in both phenolic subclasses, i.e., in anthocyanins and non-coloured phenolic compounds) was eluted with ethanol containing 0.1% HCl. Finally, each fraction was evaporated to dryness and frozen at −20 °C until analysis. The extraction yields of non-coloured fraction, coloured fraction, and total extract were 7.3 ± 0.02%, 9.1 ± 0.005%, and 16.76 ± 0.021% for the Legacy blueberry cultivar, respectively, and 6.59 ± 0.0070%, 10.9 ± 0.04%, and 17.9 ± 0.02% for the Duke blueberry cultivar, respectively. Regarding the Sweetheart cherry cultivar, the extraction yields were 6.0 ± 0.004% for the non-coloured fraction, 2.9 ± 0.02% for the coloured fraction, and 8.4 ± 0.02% for the total extract, respectively. The extraction yield concerning the Saco sweet cherry cultivar was already reported in a previous work conducted by our research team and the extraction yields were 8.5 ± 0.02%, 3.6 ± 0.005%, and 13.0 ± 0.01% for the non-coloured, coloured, and total extract, respectively [5].

2.4. Chromatographic Analysis

Each fraction was analysed on a Shimadzu LC-2010A HT Liquid Chromatography system (Shimadzu Corporation, Kyoto, Japan) using a Nucleosil® 100-5 C18 column (25.0 cm × 0.46 cm; 5 μm particle size waters; Macherey-Nagel, Düren, Germany). Detection was achieved with a Shimadzu SPD-M20A diode-array detector using the LabSolutions software (Shimadzu Corporation, Kyoto, Japan). The quantification of the known compounds was carried out by comparing their retention times and ultraviolet–visible spectra with those of authentic standards at 500 nm for anthocyanins, 280 nm for hydroxybenzoic acids, 320 nm for hydroxycinnamic acids, and 350 nm for flavonols. The volume of injection was 20 μL [35]. Each analysis was carried out in triplicate. The total phenolics, non-coloured phenolics, and total anthocyanins (Σ) were the result of the sum of each determined compound belonging to non-coloured phenolics or anthocyanins, respectively.

2.5. Antioxidant Assays

The capacity of blueberry and sweet cherry fractions and total extract to scavenge DPPH, $NO^{\bullet}$, and $O_2^{\bullet-}$ radicals was determined spectrophotometrically at 515 nm, 560 nm, and 562 nm, respectively, according to a previous work [5], using 96-well plates. The absorbances were measured in a microplate reader Bio-Rad Xmark spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). For each assay, seven different concentrations of each fraction were tested. Three experiments were performed in triplicate. Ascorbic acid was used as the positive control. The results were expressed as the concentration required to reduce half of the radicals (IC_{50}).

2.6. α-Glucosidase Inhibitory Activity

The capacity of each fraction and total extract to inhibit the activity of α-glucosidase enzyme was based on Ellmans method and determined spectrophotometrically at 405 nm, using 96-well plates. The absorbances were measured in a microplate reader Bio-Rad Xmark spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). Seven different concentrations of each fraction were tested. Three experiments were performed in triplicate. Acarbose was used as the positive control. The results were expressed as the concentration required to inhibit half of the activity of this enzyme (IC_{50}).

2.7. Antibacterial Activity—Evaluation of the Minimum Inhibitory Concentration (MIC)

Nine bacterial strains were used in this work acquired from the American Type Culture Collection (ATCC, Manassas, VT, USA), the BCCM/LMG Bacteria Collection (Belgian Co-Ordinated Collections of Micro-organisms, Gent, Belgium), and the Spanish Type Culture Collection (Valencia, Spain): four Gram-positive strains (*Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* LMG 16779, and *Staphylococcus aureus* ATCC 25923) and five Gram-negative strains (*Salmonella enterica* subsp. *enterica* ATCC 13311 serovar Typhimurium, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* CECT 170, *Serratia marcescens* CECT 159, and *Acinetobacter baumannii* LMG 1025). Strains were routinely grown on Mueller Hinton Agar (MHA) and Tryptic Soy Agar (TSA).

The susceptibility of bacteria to the coloured and non-coloured fractions and total extract from blueberries and sweet cherries was determined following the broth microdilution method [45], with some modifications. Briefly, inoculums were prepared by suspension in saline solution (NaCl 0.85% (*w/v*)) and turbidity was adjusted to 0.5 McFarland to obtain a final concentration of approximately 5×10^6 CFU mL^{-1}. Extracts were resuspended in saline solution. Positive growth control was conducted in similar conditions replacing tested fractions for saline solution. Gentamicin was employed as the negative control with concentrations ranging between 0.016 and 0.00003 mg mL^{-1}. Experiments were performed with concentrations ranging from 4 to 0.015 mg mL^{-1}, and posteriorly incubated at 37 °C for 24 h. Then, 30 μL of a resazurin solution (0.01%) was added to each well and incubated for 2 h at 37 °C. The assay was performed in triplicate, and results were reported as modal values.

2.8. Statistical Analysis of Results

All data were recorded as mean ± standard deviation of triplicate determinations. The HPLC-DAD statistical phenolic comparison was conducted using two-way ANOVA and the Bonferroni test. Regarding the biological potential, the statistical comparison was assured by one-way ANOVA, and the means were classified by Tukey's test at a 95% level of significance. To determine the correlation between the antioxidant activity methods and the contribution of the total phenols, Pearson's correlation coefficients were calculated. All analyses were performed using GraphPad Prism Version 6.01 (San Diego, CA, USA).

3. Results

3.1. Chromatographic Analysis

Phenolic compounds extracted from natural sources have been a target of intensive studies since they offer notable antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects, as well as neurological and cardiovascular protection. The determination of the phenolic profiles of such natural products is necessary and, as expected, considerable differences were found regarding phenolics present in blueberries and sweet cherries, as described in Tables 1 and 2.

Focusing on anthocyanins from blueberries, eight anthocyanins were found, and the presence of petunidin 3-*O*-galactoside ($C_{22}H_{23}O_{12}$) and malvidin 3-*O*-arabinoside ($C_{22}H_{23}ClO_{11}$) stood out in both total extract and coloured fraction of cv. Legacy. Additionally, higher levels of malvidin 3-*O*-galactoside ($C_{22}H_{25}O_{12}Cl$) were detected in its coloured fraction. Regarding cv. Duke, petunidin 3-*O*-arabinoside was the main compound found in both total extract and coloured fraction. Its total extract also showed higher levels of malvidin 3-*O*-arabinoside, while the coloured fraction was richer in malvidin 3-*O*-galacoside. The presence of these anthocyanins in blueberry fruits is in accordance with the literature [46–48].

On the other hand, four anthocyanins were detected in Sweetheart sweet cherry. As expected considering other published works [17,35], Sweetheart is poor in anthocyanin compounds. However, it was possible to quantify the presence of delphinidin 3-*O*-rutinoside ($C_{27}H_{31}ClO_{16}$) in its total extract. Saco cv possesses higher amounts of anthocyanins, with cyanidin 3-*O*-rutinoside ($C_{27}H_{31}O_{15}$) being one of the most predominant anthocyanins found [5].

Concerning non-coloured phenolic compounds, quercetin aglycone ($C_{15}H_{10}O_7$) was the main one detected in both blueberry cultivars. The presence of the detected compounds has been reported in other works [48–51].

On the other hand, in Sweetheart sweet cherry, the main compound found was 5-*O*-caffeoylquinic acid, also known as *cis* chlorogenic acid ($C_{16}H_{18}O_9$), while Saco extracts showed higher levels of 3-*O*-caffeoylquinic acid (*trans* chlorogenic acid; $C_{16}H_{18}O_9$) [5]. The richness of chlorogenic acids in sweet cherries is in agreement with other research [17,33,52].

Compared with other fruits, higher levels of quercetin were found in blueberry extracts than in tart cherries (292.6 μg/g of dried weight (dw)) [53]. Furthermore, blueberries are richer in anthocyanins than methanolic elderberry extracts (6500 μg/g of dw) [54]. Peaches showed lower levels of phenolic compounds than both blueberry and cherry extracts [55].

These differences were expected since phenolic levels depend on genotype, time of harvest and maturity stage, origin, climate, and agricultural and processing methods [17,18,52].

Table 1. Main anthocyanins found in blueberries (cv. Legacy and Duke) and Sweetheart sweet cherry (µg/g dry weight). The information regarding Saco sweet cherry (µg/g dry weight) is provided for the purpose of comparison and was retrieved from Gonçalves et al. [5].

	Blueberry Fruits				Sweet Cherry Fruits			
	cv. Legacy		cv. Duke		cv. Sweetheart		cv. Saco [5]	
Anthocyanins	Total extract	Coloured fraction	Total extract	Coloured fraction	Total extract	Coloured fraction	Total extract	Coloured fraction
Unknown 1	nd	nd	nd	nd	nd	nd	2.99 ± 0.24	nd
Delphinidin 3-*O*-galactoside	nq	3016.39 ± 37.27	18,692.91 ± 6.13	1662.34 ± 25.72 [a]	nd	nd	nd	nd
Peonidin 3-*O*-rutinoside	nd	nd	nd	nd	nq	nq	nd	nd
Delphinidin 3-*O*-arabinoside	2147.51 ± 7.68	949.45 ± 8.50 [a]	4444.20 ± 5.51	99.56 ± 12.37 [a]	nd	nd	nd	nd
Unknown 2	nd	nd	nd	nd	nd	nd	341.16 ± 2.82	nd
Cyanidin 3-*O*-rutinoside	nd	nd	nd	nd	nd	nq	3865.64 ± 2.95	15,656.18 ± 25.71 [a]
Cyanidin 3-*O*-galactoside	nq	nq	nq	nq	nd	nd	nd	nd
Petunidin 3-*O*-galactoside	3609.50 ± 63.70	3927.97 ± 4.76 [a]	19,654.66 ± 240.64	1899.83 ± 31.56 [a]	nd	nd	nd	nd
Pelargonidin 3-*O*-rutinoside	nd	nd	nd	nd	nq	nq	337.464 ± 20.19 [5]	130.39 ± 1.22 [a]
Cyanidin 3-*O*-arabinoside	nq	915.07 ± 9.97	396.21 ± 23.31	nq	nd	nd	nd	nd
Cyanidin 3-*O*-glucoside	nd	nd	nd	nd	nd	nd	193.48 ± 0.54	3427.93 ± 4.39 [a]
Petunidin 3-*O*-arabinoside	nq	17,729.35 ± 165.12	32,401.38 ± 254.78	12,474.90 ± 149.46 [a]	nd	nd	nd	nd
Malvidin 3-*O*-galactoside	nq	4756.33 ± 55.57	19,631.59 ± 48.43	3011.97 ± 44.56 [a]	nd	nd	nd	nd
Malvidin 3-*O*-arabinoside	2249.05 ± 41.03	1741.46 ± 17.90 [a]	3020.86 ± 22.62	1878.27 ± 56.85 [a]	nd	nd	nd	nd
Delphinidin 3-*O*-rutinoside	nd	nd	nd	nd	nq	22.03 ± 2.46	nd	nd
Σ anthocyanins	8006.05	33,036.02	98,241.80	21,026.88	nq	22.03	4740.73	19,214.50

nd: not detected; nq: not quantified; Σ: sum of total non-coloured phenolics content. [a] Significant result ($p < 0.05$) is indicated vs. total extract of each matrix.

Table 2. Main non-coloured phenolics found in blueberries (cv. Legacy and Duke) and Sweetheart sweet cherry (µg/g dry weight). The information regarding Saco sweet cherry (µg/g dry weight) is provided for the purpose of comparison and was retrieved from Gonçalves et al. [5].

	Blueberry Fruits				Sweet Cherry Fruits			
	cv. Legacy		cv. Duke		cv. Sweetheart		cv. Saco [5]	
Non-coloured phenolics	Total extract	Non-coloured fraction	Total extract	Non-coloured fraction	Total extract	Non-coloured fraction	Total extract	Non-coloured fraction
Hydroxybenzoic acid derivative 1	nd	nd	nd	nd	nd	nd	1337.85 ± 68.16	1839.54 ± 5.09 [a]
Hydroxycinnamic acid derivative 1	nd	nd	nd	nd	nd	nd	494.32 ± 51.66	679.69 ± 71.03
Hydroxycinnamic acid derivative 2	nd	nd	nd	nd	nd	nd	143.45 ± 21.30	197.24 ± 29.28
3-O-Caffeoylquinic acid	810.87 ± 3.95	1405.08 ± 8.46 [a]	nq	184.22 ± 2.92 [a]	nq	nq	1482.97 ± 54.15	2039.09 ± 74.45 [a]
Hydroxybenzoic acid derivative 2	nd	nd	nd	nd	nd	nd	25.08 ± 0.92	34.48 ± 1.27
ρ-Coumaric acid derivative 1	nd	nd	nd	nd	nd	nd	50.02 ± 0.55	68.78 ± 0.76
ρ-Coumaroylquinic acid	nd	nd	nd	nd	nd	nd	nq	nq
Hydroxycinnamic acid derivative 3	nd	nd	nd	nd	nd	nd	372.26 ± 35.99	511.86 ± 49.48
5-O-Caffeoylquinic acid	196.29 ± 1.00	208.33 ± 3.19	183.79 ± 2.42	832.08 ± 1.16 [a]	6984.19 ± 28.18	2974.10 ± 61.48	734.38 ± 44.86	1009.77 ± 61.68 [a]
Hydroxycinnamic acid derivative 4	nd	nd	nd	nd	nd	nd	2835.87 ± 143.08	3899.33 ± 196.73 [a]
Caffeic acid derivative	nq	nq	nd	nd	nd	nd	nd	nd
Caffeic acid	nd	nd	nd	nd	nd	nd	1263.49 ± 98.92	1737.30 ± 136.01 [a]
ρ-Coumaric acid derivative 2	nd	nd	nd	nd	nd	nd	528.74 ± 19.83	727.02 ± 27.26
Hydroxycinnamic acid derivative 5	nd	nd	nd	nd	nd	nd	704.96 ± 97.52	969.31 ± 134.08 [a]
Hydroxycinnamic acid derivative 6	nd	nd	nd	nd	nd	nd	196.75 ± 16.19	270.53 ± 22.26
ρ-Coumaric acid	nd	nd	nd	nd	nd	nd	21.07 ± 1.64	28.96 ± 2.26
Hydroxycinnamic acid derivative 7	nd	nd	nd	nd	nd	nd	666.97 ± 67.02	917.089 ± 92.15 [a]
Hydroxycinnamic acid derivative 8	nd	nd	nd	nd	nd	nd	175.97 ± 16.59	241.95 ± 22.80
Quercetin 3-O-glucoside	nd	nd	nd	nd	nd	nd	nq	nq
Myricetin 3-O-glucoside	nd	nd	nd	nd	nd	nd	nd	nd
Kaempferol 3-O-rutinoside	nd	nd	nd	nd	nd	nd	nq	nq
Quercetin aglycone	6962.43 ± 49.94	7478.30 ± 37.44 [a]	7521.47 ± 12.80	nq	nd	nd	35.58 ± 3.73	48.93 ± 5.13
Σ non-coloured phenolics	7969.59	9091.72	7705.47	1016.29	6984.19	2974.10	11,069.73	15,220.88

nd: not detected; nq: not quantified; Σ: sum of total anthocyanins content. [a] Significant result ($p < 0.05$) is indicated vs. total extract of each matrix.

3.2. Antioxidant Assays

Higher levels of oxidative stress are intimately linked to the increased risk of the appearance of several diseases and, therefore, attenuating their levels are mandatory. Evidence suggests that a colourful diet, rich in fruits and vegetables is an added value and really effective in promoting a healthy status. In fact, natural products are rich in several antioxidants, particularly phenolics whose biological potential is increased due to the chemical structure of carboxyl, hydroxyl, and methoxy groups [7,56]. Without surprise, both blueberry and sweet cherry extracts showed effectiveness in scavenging ferric species and DPPH$^\bullet$. Regarding FRAP, the total extract of Duke blueberry was the most active (IC$_{50}$ value of 18.18 ± 0.38 µg/mL of dw), followed by the coloured fraction of Legacy blueberry fruit (IC$_{50}$ value of 40.60 ± 1.37 µg/mL of dw). Even so, they were 2.9 and 6.4 times less effective than ascorbic acid control. Concerning Sweetheart sweet cherry, the coloured fraction was the one that showed the most significant action (IC$_{50}$ = 62.53 ± 0.74 µg/mL of dw). Compared with other fruits, ethyl acetate extracts of dried tomatoes and Saco cherry non-coloured fraction showed similar capacity to the Legacy coloured extract (IC$_{50}$ value of 46.9 µg/mL and 50.1 µg/mL of dw, respectively) [2,57]. Both berry fractions and total extract were more active in scavenging ferric species than ethyl acetate and butanol fractions of *Myrtus communis* (IC$_{50}$ scores of 4250.0 µg/mL and 5510.0 µg/mL of dw, respectively) [58].

In DPPH$^\bullet$ assay, the coloured fraction of Duke blueberries was the most active (IC$_{50}$ scores of 30.87 ± 0.67 µg/mL of dw); however, it was about 4.3 times less effective than the ascorbic acid control. Additionally, the activity of the non-coloured fraction of Duke showed similar activity as the coloured fraction of cv. Legacy (IC$_{50}$ scores of 49.11 ± 0.68 µg/mL and 44.32 ± 0.75 µg/mL of dw, respectively). Regarding Sweetheart cherry, the coloured fraction was also the most effective (IC$_{50}$ = 84.15 ± 1.46 µg/mL of dw); however, it was about 2.6 times less active than the Saco coloured fraction (IC$_{50}$ = 31.39 ± 0.60 µg/mL of dw) [5]. Ethanolic extracts of peach showed similar activity to the total extract of cv. Legacy (IC$_{50}$ value of 146.7 µg/mL of dw) [55], while the non-coloured fraction of this cv. showed similar activity as butanol and ethyl acetate fractions of *Myrtus communis* fruits (IC$_{50}$ scores of 84.4 µg/mL and 85.6 µg/mL of dw, respectively) [58].

Concerning $^\bullet$NO, the coloured fractions of cv. Duke and Legacy showed the most potential (IC$_{50}$ values of 19.92 ± 0.54 µg/mL and 39.44 ± 0.45 µg/mL of dw) and were around 14 and 7 times more active than the ascorbic acid control, respectively. The non-coloured extract of Sweetheart (IC$_{50}$ score of 167.96 ± 0.92 µg/mL of dw) was the most active, displaying similar activity to the coloured fraction (IC$_{50}$ = 170.74 ± 2.02 µg/mL of dw). Compared with other fruits, both berry extracts showed higher activity than ethanolic extracts of peach fruits (IC$_{50}$ >256.7 µg/mL of dw) [55], while the non-coloured fraction of cv. Duke showed similar activity to ethanolic extracts of strawberry fruits (IC$_{50}$ value of 118.0 µg/mL of dw) [59].

Furthermore, the coloured fraction of blueberry fruits displayed similar $O_2^{\bullet-}$ scavenging activity (IC$_{25}$ values of 0.69 ± 0.16 µg/mL and 0.74 ± 3.15 µg/mL of dw for Legacy and Duke, respectively), showing more activity than the ascorbic acid control (IC$_{25}$ = 8.43 ± 0.38 µg/mL of dw). In Sweetheart cherry, the coloured fraction showed the most significant capacity (IC$_{25}$ value of 3.06 ± 0.34 µg/mL of dw), being five times more effective in scavenging $O_2^{\bullet-}$ than the coloured fraction of Saco cherry (IC$_{25}$ value of 16.58 ± 0.27 µg/mL of dw) [5]. Regarding other fruits, grapes and strawberry ethanolic extracts showed higher activity to scavenge $O_2^{\bullet-}$ than both berry extracts (IC$_{50}$ scores of 3.7 µg/mL and 3.5 µg/mL of dw) [59] as well as blackberry methanolic extracts (IC$_{50}$ value of 72.5 µg/mL of dw) [60].

Other studies have described the antioxidant potential of blueberries and sweet cherries. In particular, Johnson and colleagues [28] reported that a daily intake of 22 g of freeze-dried blueberry powder, which is equivalent to 1 cup of fresh blueberries, for 8 weeks reduces 8-hydroxy-2′-deoxyguanosine, a marker of oxidative damage, and increases the activity of endogenous antioxidant superoxide dismutase, glutathione reductase, and glu-

tathione peroxidase enzymes. Regarding sweet cherries, it was previously verified that the ingestion of 280 g of cherries after an overnight fast is effective in reducing nitric oxide concentrations [41].

Using Pearson's test, positive correlations (r > 0.8689) were found between the antioxidant potential of cv. Legacy and the content of malvidin and delphinidin 3-*O*-arabinoside and the total non-coloured phenolic compounds (r > 0.7057). Additionally, negative correlations were obtained between petunidin 3-*O*-galactoside and DPPH$^\bullet$ (r = −0.9231) and between $^\bullet$NO (r = −0.9826), $O_2^{-\bullet}$ (r = −0.8454), and FRAP (r = −0.8942) assays. Regarding cv. Duke, a positive correlation was obtained between its activity against DPPH$^\bullet$, $^\bullet$NO, and $O_2^{-\bullet}$ and its total coloured phenolic compounds (r > 0.8440), and also between 5-*O*-caffeoylquinic acid and DPPH$^\bullet$ and $^\bullet$NO assays (r = 0.9280). Focusing on Sweetheart cherry, a positive and strong correlation was obtained between $^\bullet$NO and $O_2^{-\bullet}$ assays (r = 0.9907 and r = 0.9958, respectively) and the levels of 5-*O*-caffeoylquinic acid, while negative correlations were obtained regarding its antioxidant potential and the presence of delphinidin 3-*O*-rutinoside (r < −0.9974). In a general way, the antioxidant abilities are strongly related to the chemical structure of these compounds, particularly with the number and type of sugar moieties attached to the aglycone, the degree of methylation position and the number of the hydroxyl groups, and the position of carboxylated and/or aromatic aliphatic acids on the sugar residue [56,61–65]. Normally, the increase in hydroxyl groups increases the radical scavenger capacity; therefore, it is not surprising that the presence of anthocyanins increases this capacity [5,7,66].

3.3. Glucosidase Inhibitory Capacity

The prevalence of diabetes *mellitus* is increasing worldwide and since the current approved pharmaceutical drugs exhibit several undesirable side effects, it is urgent to find new alternatives to attenuate its progress [67]. Within the various existing possibilities, the incorporation of phenolic compounds has been largely studied mainly owing to their ability to compete with carbohydrate enzymes substrates by creating bonds, and in this way, preventing the digestion of these compounds [5,68,69]. Additionally, most phenolics can also protect pancreatic *β*-cells from oxidative damage given their chemical structure [68,70,71].

Keeping these facts in mind, the capacity of blueberries and sweet cherry phenolic-rich fractions to inhibit the activity of *α*-glucosidase was evaluated. The obtained results are described in Table 3. Comparing the results, the activity of blueberry was more effective, with the activity of the coloured extract standing out (IC_{50} values of 65.96 ± 5.07 µg/mL and 83.88 ± 1.49 µg/mL of dw for Legacy and Duke, respectively), exhibiting 6.81 and 5.35 times more efficacy than the acarbose control. The total extract was also the most active in Sweetheart sweet cherry (IC_{50} value score of 449.16 ± 2.49 µg/mL of dw); however, it was about 1.7 times less effective than the acarbose control. Once again, the present results reinforce the positive interaction between coloured and non-coloured phenolic compounds. In fact, both subclasses interact together, increasing the biological potential of the extracts.

Comparing with other published studies, it is possible to see that Saco sweet cherry total extract exhibits similar capacity as blueberry Legacy total extract (IC_{50} values of 53.15 ± 1.32 µg/mL and 51.49 ± 2.54 µg/mL of dw, respectively) [5]. Furthermore, the antidiabetic potential of blueberry is already known. In fact, Stull and colleagues [72] reported that the daily consumption twice a day of 22.5 g of blueberries for 6 weeks can improve insulin sensitivity in obese, nondiabetic, and insulin-resistant women and men participants [72]. More recently, it was reported that 1 cup of blueberries can reduce insulinemia and glucose and cholesterol, and increase Apo-A1, HDL-C, and HDL-P parameters [73]. Similar effects were observed by Basu et al. [74].

Table 3. IC_{25} and IC_{50} (µg/mL of dried weight) values regarding the antioxidant capacity against synthetic ferric species (FRAP), and synthetic DPPH$^\bullet$, nitric ($^\bullet$NO), and superoxide ($O_2^{\bullet-}$) radicals, and α-glucosidase inhibitory effects of phenolic-enriched fractions from blueberry fruits (cv. Duke and cv. Legacy) and sweet cherry fruits (cv. Saco and Sweetheart).

| | Blueberry Fruits | | | | | | Sweet Cherry Fruits | | | | | | Control |
| | cv. Legacy | | | cv. Duke | | | cv. Sweetheart | | | cv. Saco | | | |
Biological potential	Total extract	Coloured fraction	Non-coloured fraction	Total extract	Coloured fraction	Non-coloured fraction	Total extract	Coloured fraction	Non-coloured fraction	Total extract	Coloured fraction	Non-coloured fraction	
Antioxidant assays													
FRAP	87.47 ± 1.46	40.60 ± 1.37	137.59 ± 1.03	18.18 ± 0.38	66.65 ± 0.74	111.14 ± 1.71	145.65 ± 1.37	62.53 ± 0.74	322.64 ± 1.86	27.22 ± 0.23 [2]	9.43 ± 0.43 [2]	50.09 ± 0.77 [2]	6.36 ± 0.35 (acid ascorbic control)
DPPH$^\bullet$	144.68 ± 1.04	44.32 ± 0.75	86.03 ± 1.53	208.06 ± 2.70	30.87 ± 0.67	49.11 ± 0.68	397.84 ± 2.74	84.15 ± 1.46	225.36 ± 1.04	21.88 ± 0.32 [5]	31.39 ± 0.60 [5]	210.86 ± 0.86 [5]	7.18 ± 0.28 (acid ascorbic control)
$^\bullet$NO	50.34 ± 1.12	39.44 ± 0.45	63.91 ± 1.39	69.53 ± 1.55	19.92 ± 0.54	115.11 ± 1.80	358.64 ± 2.40	170.74 ± 2.02	167.96 ± 0.92	33.72 ± 0.89 [5]	47.44 ± 0.67 [5]	167.96 ± 0.92 [5]	279.03 ± 1.71 (acid ascorbic control)
$O_2^{\bullet-}$	1.13 ± 0.21 (IC_{25})	0.69 ± 0.16 (IC_{25})	1.42 ± 0.18 (IC_{25})	1.02 ± 10.33 (IC_{25})	0.74 ± 3.15 (IC_{25})	1.14 ± 15.46 (IC_{25})	39.07 ± 0.77 (IC_{25})	3.06 ± 0.34 (IC_{25})	3.11 ± 0.39 (IC_{25})	41.68 ± 0.72 [5]	16.58 ± 0.27 (IC_{25}) [5]	69.40 ± 1.22 [5]	39.69 ± 0.66 8.43 ± 0.38 (IC_{25}) (acid ascorbic control)
α-Glucosidase inhibitory assay													
α-Glucosidase	65.96 ± 5.07	267.64 ± 4.04	180.36 ± 2.13	83.88 ± 1.45	298.79 ± 2.01	78.05 ± 1.23	449.16 ± 2.49	2349.78 ± 4.18	1325.90 ± 4.69	53.15 ± 1.32 [5]	142.02 ± 1.17 [5]	456.19 ± 3.74 (IC_{25}) [5]	287.51 ± 4.32 (acarbose control)

Focusing on cherries, the capacity of *Prunus* vegetal parts to inhibit the α-glucosidase enzyme was also published, with the activity of stems infusions (IC_{50} score of 3.18 ± 0.23 μg/mL of dw) and hydroethanolic extracts of stems and leaves (IC_{50} values of 3 7.67 ± 0.23 μg/mL and 15.61 ± 0.48 μg/mL of dw, respectively) standing out [75]. Regarding in vivo studies, Noratto and colleagues [76] reported that non-anthocyanin sweet cherry extracts can modulate IL-6, liver lipids, and expression of PPARδ and LXRs in obese diabetic (db/db) mice.

Compared with other fruits, peach ethanolic total extracts showed greater activity in inhibiting the α-glucosidase enzyme than berry extracts (IC_{50} values ranging from 11.7 to 35.8 μg/mL of dw) [55]. On the other hand, higher activities were found in total extracts of Legacy and Duke than in phenolic-rich extracts from Australian plums (IC_{50} value of 130.0 μg/mL of dw) [77].

Positive and strong correlations were found between the inhibitory potential displayed by cv. Legacy and their content in malvidin and delphinidin 3-*O*-arabinoside (r > 0.9840) and the total non-coloured phenolic compounds (r = 0.9018). In contrast, a negative correlation was obtained between this activity and the content of petunidin 3-*O*-galactoside (r = −0.9970). Focusing on cv. Duke, a positive and strong correlation was found regarding its activity and 5-*O*-caffeoylquinic acid content (r = 0.9961), while negative correlations were obtained relative to the presence of anthocyanins (r < −0.5742). Concerning Sweetheart cherry, a negative correlation was obtained between delphinidin 3-*O*-glucoside and 5-*O*-caffeoylquinic acid levels and its inhibitory potential (r = −0.9890 and r = −0.9918, respectively). Generally, the linkage of the B-ring in position three in phenolic compounds, as well as the unsaturated C-ring, the presence of either 3-OH and 4-CO groups, and the hydroxyl substitution on the B ring, increase the inhibitory effects on the α-glucosidase enzyme exhibited by phenolics [5,78].

3.4. Antibacterial Activity

The antibacterial activity of the different blueberry and cherry extracts was tested using several Gram-positive and Gram-negative bacteria. The evaluation of the activity of various extracts revealed varying behaviour among the analysed extracts and a diverse response among the examined strains (Table 4). In the case of the blueberry extract of the non-coloured fraction, *B. cereus* ATCC 11778 was the most resistant strain, with a MIC value of more than 4 mg mL^{-1} for both cultivars, whereas the most susceptible strain was *K. pneumoniae* ATCC 13883 of cv. Legacy with a concentration of 0.12 mg mL^{-1} inhibiting growth. The coloured fraction of blueberry fruits extract exhibited higher inhibitory activity, with *L. monocytogenes* LMG 16779 and *S. aureus* ATCC 25923 having the maximum susceptibility to an inhibitory concentration of 0.12 mg mL^{-1}, followed by *K. pneumoniae* ATC13883 and *A. baumannii* LMG 1025 exhibiting susceptibility to a concentration of 0.25 mg mL^{-1}. However, the strain *B. cereus* ATCC 11778 was not inhibited by the doses tested. The total extract did not suppress the growth of *B. cereus* ATCC 11778 at any concentration; however, *E. faecalis* ATCC 29212 and *P. mirabilis* CECT 170 were inhibited at concentrations of 2 mg mL^{-1}. In terms of sweet cherry extracts, the acquired findings revealed that *S. aureus* ATCC 25923 was the most sensitive strain to the coloured fraction of cherries from cv. Sweetheart and cv. Saco, with MIC values of 1 mg and 2 mg mL^{-1}, respectively. However, all other tested extracts had MIC values greater than 2 mg mL^{-1} for all studied strains (Table 4). Moreover, Gram-positive bacteria are more sensitive to blueberry extracts, exhibiting inhibition in lower concentrations than Gram-negative but not in cherry extracts, where observed activities were similar or higher in Gram positive bacteria.

Table 4. Minimum inhibitory concentration (MIC) values (mg mL^{-1}) of phenolic-enriched fractions from blueberry fruits (cv. Duke and cv. Legacy) and sweet cherry fruits (cv. Saco and Sweetheart).

| | Blueberry Extracts | | | | | | Sweet Cherry Extracts | | | | | | Control |
| | cv. Legacy | | | cv. Duke | | | cv. Sweetheart | | | cv. Saco | | | |
	Non-Coloured Fraction	Coloured Fraction	Total Extract	Non-Coloured Fraction	Coloured Fraction	Total Extract	Non-Coloured Extract	Coloured Extract	Total Extract	Non-Coloured Extract	Coloured Extract	Total Extract	Gentamycin
Gram-positive													
E. faecalis ATCC 29212	2	1	1	2	0.5	0.5	nd	nd	nd	nd	nd	nd	0.016
B. cereus ATCC 11778	>4	4	4	4	4	4	>2	>2	>2	>2	>2	>2	0.00003
L. monocytogenes LMG 16779	0.5	0.25	0.5	1	0.12	2	nd	nd	nd	nd	nd	nd	0.016
S. aureus ATCC 25923	1	0.25	1	1	0.12	2	>2	1	>2	>2	2	>2	0.00003
Gram-negative													
Salmonella enterica subsp. *enterica* ATCC 13311 serovar Typhimurium	2	2	1	2	2	0.5	>2	>2	>2	>2	>2	>2	0.00006
K. pneumoniae ATCC 13883	0.12	0.5	1	0.5	0.25	1	>2	>2	>2	>2	>2	>2	0.016
P. mirabilis CECT 17	2	2	2	2	2	2	>2	>2	>2	>2	>2	>2	0.00013
S. marcescens CECT 159	>4	2	2	4	2	4	>2	>2	>2	>2	>2	>2	0.00025
A. baumannii LMG 1025	1	0.5	0.5	1	0.25	1	>2	>2	>2	>2	>2	>2	0.00006

nd: not detected.

The use of berry extracts has been previously studied mainly due to their phenolic compound contents [79]. The search for new molecules with antibacterial activity is required owing to the increase in antibiotic resistance in pathogenic bacteria, such as *A. baumanni*, *L. monocytogenes*, and *K. pneumoniae*, according to the most recent reports of the World Health Organization [42]. Plants secondary metabolites have shown interesting antibacterial activities against several multi-drug-resistant strains; however, the application of such compounds is limited by effective concentrations in in vivo models being unobtainable, toxicity to the host, or inappropriate pharmacodynamics [80]. The antimicrobial activity of bioactive compounds from plants is commonly evaluated by the microdilution method to determine MIC due to the reliability, cost efficiency, and flexibility of the method employed [81–83]. In this way, phenolic compounds are one of the most studied molecules derived from plants [84] and their activity is associated with the ability to interact with lipid bilayers and perturb plasma membrane functionality [85] or chelate iron [86]. *V. corymbosum* crude extracts from different varieties have been reported to exhibit inhibitory activity against *L. monocytogenes* and *Salmonella enterica* subsp. *enterica* [87]. Thus, ethanolic extracts of *V. corymbosum* fruits are capable of inhibiting the growth of *Vibrio parahaemolyticus* at concentrations between 25 mg and 12.5 mg mL^{-1} [88]. Fruit extracts from other species of the genus *Vaccinium*, such as *V. stenophyllum*, have presented similar results of inhibition against foodborne pathogenic bacteria such as *Salmonella* [89]. In addition, the crude extract of *V. angustifolium* fruits showed activity (MIC = 1 mg·mL^{-1}) against *Fusobacterium nucleatum,* similar to those determined for some of the extracts and species studied, indicating that its activity may be linked to the capacity of the phenolic compounds to chelate iron [86]. This mode of action would determine the variance in responses based on the susceptibility of the different strains to the available concentration of iron in the medium.

In this work, sweet cherry extracts had lower antibacterial activity against the tested strains. However, the antibacterial effect of cherry by-products has already been shown, with these by-products demonstrating inhibitory activity against Gram-positive bacteria [90]. The presence of phenolic compounds, such as sakuranetin, neochlorogenic, chlorogenic acids, and anthocyanins, might explain the observed effects against *S. aureus.* In this way, the antimicrobial effectiveness of the antibiotic used as the negative control was much higher than that shown by the extracts, as other authors describe [85]. These weak results limit the use of these kinds of extracts in pharmacological treatments; however, there is a need for the evaluation of their activities in synergy with commercial antibiotics or their application in other industrial uses and their dynamics in the human body. The lack of results in identifying antimicrobial active plant compounds is an issue of concern as the increase in the number of multi-drug-resistant bacterial strain outbreaks and incidences pose a significant threat to modern human health [91]. However, some phenolic compounds, such as resveratrol, have shown relevant antibacterial activity and could be employed in synergic use with commercial antibiotics [80].

4. Conclusions

The findings of this work show a more complex profile of phenolic compounds in blueberries than in sweet cherries, mainly dominated by anthocyanins. Without surprise, this composition is clearly related to the radical scavenger and antidiabetic activities observed. Among the obtained results, blueberry and sweet cherry extracts were several times more active in reducing NO$^\bullet$ and in inhibiting the α-glucosidase enzyme than the positive controls ascorbic acid and acarbose, respectively. These results reinforce the idea that the consumption of berries is an excellent choice for basal health maintenance, reducing the incidence of chronic diseases, such as diabetes. Additionally, blueberry extracts present activity against bacterial pathogens. Among the alternatives, phenolic compounds derived from natural products appear to be promising alternatives since they have significant health-promoting effects. The current study adds to the evidence that phenolics from red and purple fruits, including blueberries and sweet cherries, have a high potential for

incorporation into pharmaceuticals, food supplements, and nutraceuticals. Nonetheless, further research is required to fully understand their potential and determine safe dosage in the control of bacterial development.

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