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Phenolic Profile and Bioactive Potential of Stems and Seed Kernels of Sweet Cherry Fruit

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Abstract: Every year, large quantities of stems and pits are generated during sweet cherry processing, without any substantial use. Although stems are widely recognized by traditional medicine, detailed and feasible information about their bioactive composition or biological value is still scarce, as well as the characterization of kernels. Therefore, we conducted a study in which bioactivity potential of extracts from stems and kernels of four sweet cherry cultivars (Early Bigi (grown under net cover (C) and without net cover (NC)), Burlat, Lapins, and Van) were examined. The assays included antioxidant (by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid bleaching assays), and antibacterial activities against important Gram negative and Gram positive bacterial human isolates. Profile and individual phenolic composition of each extract were determined by High-performance liquid chromatography (HPLC) analysis. Extracts from stems of cv. Lapins and kernels of Early Bigi NC presented high levels of total phenolics, flavonoids, ortho-diphenols and saponins. Excepting for cv. Early Bigi NC, major phenolic compounds identified in stems and kernels were sakuranetin and catechin, respectively. In cv. Early Bigi NC the most abundant compounds were ellagic acid for stems and protocatechuic acid for kernels. In all extracts, antioxidant activities showed a positive correlation with the increments in phenolic compounds. Antimicrobial activity assays showed that only stems’ extracts were capable of inhibiting the growth of Gram positive isolates. This new data is intended to provide new possibilities of valorization of these by-products and their valuable properties.

Keywords: prunus avium; HPLC; by-products; antioxidant; antimicrobial

1. Introduction

Sweet cherry is one of the fresh fruits most appreciated by consumers in the temperate areas of Europe [1,2], not only due to its organoleptic characteristics, such as color, brightness, flavor, aroma and texture, but also for consumers’ awareness of its benefits for human health [3–5]. Rich in vitamins and minerals, cherries contains different phytochemicals that are closely related to its antioxidant power [3]. This fruit has been recognized as having beneficial medicinal properties such as anticancer, dietary, antioxidant and anti-inflammatory, due to its content of antioxidant compounds [3,5]. The world cherry production has increased from 2.15 to 2.54 million tonnes from 2009-2018, with Turkey, the USA, and Uzbekistan as the main producers, responsible for 50% of the total world production, while Chile, Hong Kong, and the USA are the main exporters [6]. Portugal supplies some of the first cherries in
Europe, and has increased its production by about 46% (2009–2018), with recent data referring to more than 17,500 tons of cherries annually produced [6]. Although sweet cherry is mainly commercialized as fresh fruit, a considerable quantity is used after processing as jam, jelly or juice that generates large amounts of by-products, namely stems and pits [7]. There is no substantial use of this waste, which increase environmental and managements costs each year to deal with the excess of such residues. Therefore, a solution to achieve valorization of the excess of this material is urgent [8].

Although cherry stems are known by traditional medicine and widely used in infusions and decoctions, due to their claimed sedative, diuretic, and anti-inflammatory properties [9], their study, as well as the characterization of pits, commonly used for therapeutic pillows, only recently have received attention. Various hydroxycinnamic acids, such as p-cumaric, ferulic, caffeic, chlorogenic and neochlorogenic acids, were reported in stems of P. avium [9,10], and important anthocyanins were found in kernels [11], whose oil is rich in lipophilic compounds, such as tocopherols and carotenoids [12] (Figure 1). In this context, the objective of the current work is to evaluate the biological potential of such less used residues, providing a detailed study on their chemical composition, antioxidant, and antimicrobial activities.

![Unvalued sweet cherry by-products: view of sweet cherry stem and pit (A) and view of sweet cherry pit (B).](image)

**Figure 1.** Unvalued sweet cherry by-products: view of sweet cherry stem and pit (A) and view of sweet cherry pit (B).

## 2. Materials and Methods

### 2.1. Plant Material

Samples were collected from a 6-year old cherry orchard, planted at low elevation, located in Alufinha, S. João de Fontoura, municipality of Resende, Portugal (latitude 41°06’ N, longitude 7°54’ W, altitude 140 m). Fruit from four cherry cultivars, in the commercial ripeness stage, were selected for testing: **Early Bigi** and **Burlat** (cultivars of early maturity), **Van** (cultivar of intermediate maturity) and **Lapins** (cultivar of late maturity). Cultivar **Early Bigi** was grown in two different modes: using net cover over the trees (C) and without net cover (NC). After selecting ten homogeneous trees, three replicates of about 2 kg of cherry per cultivar were collected (May 2019). Stems and pits were removed, with kernels removed from the pits, and both stems and kernels were freeze-dried and then ground to a fine powder used in all analysis.

### 2.2. Chemical Composition and Antioxidant Extracts

#### 2.2.1. Extraction Procedure

For chemical composition and antioxidant activities assays, extracts were prepared by mixing 40 mg dry weight (DW) of samples of each sweet cherry cultivar with 1 mL of 70% methanol, thoroughly in a vortex. The mixtures were heated at 70 °C during 30 min, and then centrifuged at 13,000 rpm, 1 °C for 15 min (Eppendorf Centrifuge 5804 R, Hamburg, Germany). The supernatants were collected and filtered with Spartan filters (0.2 µm) to HPLC amber vials.
2.2.2. Total Phenolics and Flavonoids

The methodology of Singleton and Rossi [13] was used for the quantification of total phenolics, with minor modifications: 20 µL of extract were added with 100 µL of Folin Ciocalteu’s phenol reagent (1:10 in bidistilled H₂O) and 80 µL of 7.5% Na₂CO₃ in a 96-well microplate (Multiskan™ FC Microplate Photometer, Waltham, MA, USA). The microplate was incubated for 15 min at 45 °C, in the dark. Afterward, the absorbance values against a blank were recorded at 765 nm in a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland). A standard curve with gallic acid at different concentrations was performed and total phenolics results were expressed as mg gallic acid equivalent (GAE)/g DW as the mean ± standard deviation (SD) of three replicates.

The total flavonoid content was determined using the colorimetric method described in Dewanto et al. [14], with some modification. In a 96-well microplate, 25 µL of extract was mixed with 100 µL of ultra-pure water, and 10 µL of 5% NaNO₂. The microplate was then placed in the dark at room temperature. After 5 min, 15 µL of 10% AlCl₃ was added and the microplate incubated again at room temperature in the dark for 6 min. Then, 50 µL of NaOH 1M, and 50 µL of ultra-pure water were added. The absorbance values were measured against a blank at 510 nm. A standard curve with catechin at different concentrations was performed to quantify total flavonoid content, which values were expressed as mg catechin equivalent (CE)/g DW as the mean ± standard deviation (SD) of three replicates.

2.2.3. Quantification of Ortho-Diphenols

The method used for the determination of ortho-diphenols was adapted from Garcia et al. [15]. First, 20 µL of extract were mixed with 100 µL of ultra-pure water. Then 80 µL of phosphate buffer (pH 6.5, 0.1 M) was added, followed by 160 µL of 5% sodium molybdate (Na₂MoO₄·2H₂O) solution. The microplate was left to stand in the dark for 15 min and the absorbance was measured at 370 nm against a blank reagent. Caffeic acid was used as standard to prepare a calibration curve and ortho-diphenolic content was expressed as caffeic acid equivalents per g of sample (mg CAE/g DW) as the mean ± standard deviation (SD) of three replicates.

2.2.4. Saponins

For saponin quantification, extractions were performed as referred by Chen et al. [16], with modifications. Briefly, 200 mg of plant material was extracted with 5 mL of 95% (v/v) ethanol and sonicated for 1 h at room temperature. After extraction, the contents were filtered and stored in amber vials. In 96-well plate, 43 µL of sample was mixed with 34 µL perchloric acid and 9 µL of 5% vanillic acid. Plates were incubated at 60 °C for 20 min, and 214 µL of glacial acetic acid were added. Absorbances were read at 554 nm, and results are expressed using a calibration curve of diosgenin, as diosgenin equivalents per g of sample (mg DE/g DW) as the mean ± standard deviation (SD) of three replicates.

2.2.5. Determination of Chlorophyll a, b and Total Carotenoids

The determination of pigments (only for stems) was performed according to an adaptation of the methods from Lichtentaler and Wellburn [17], Warren [18] and Pompelli et al. [19]. Briefly, 100 mg of each stems’ samples was extracted with 5 mL of 80% acetone with 0.01% of CaCO₃, homogenized and centrifuged at 4000 rpm, 20 °C, during 5 min. The absorbance of 200 µL of each sample was recorded at 470, 645 and 662 wavelengths against a blank (pure solvent used in pigments extraction instead of sample). The concentration of each pigment is calculated from 1 cm corrected pathlength using the following formulas:

\[
\text{Chlorophyll a (µg/mL): Chl a} = (11.75 \times \text{Abs662}) - (2.350 \times \text{Abs645})
\]

\[
\text{Chlorophyll b (µg/mL): Chl b} = (18.61 \times \text{Abs645}) - (3.960 \times \text{Abs662})
\]
2.2.6. Antioxidant Activity

**ABTS Method**

The 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) radical scavenging activity was evaluated in a 96-well microplate using the method of Re et al. [20]. An ABTS radical solution was prepared by mixing 7 mM of ABTS at pH 7.4 (5 mM NaH$_2$PO$_4$, 5 mM Na$_2$HPO$_4$, and 154 mM NaCl) with 2.5 mM potassium persulfate, and storing the mixture in the dark at room temperature for 16 h. The mixture was then diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm. In each microplate well, 15 µL of extract was mixed with 285 µL of the freshly prepared ABTS solution, and incubated at room temperature in the dark for 10 min. Absorbance values were measured at 734 nm, with ABTS radical scavenging activity expressed from a linear calibration curve of Trolox as µg Trolox equivalent/g DW as the mean ± standard deviation (SD) of three replicates.

**DPPH Method**

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity assay was performed by spectrophotometry as described by Siddhraju and Becker [21]: in a 96-well microplate, 15 µL of extract was combined with 285 µL of freshly prepared methanolic radical DPPH solution (60 µM). The microplates were covered with foil paper and left for 30 min at room temperature. The reduction in absorbance was measured at 517 nm. DPPH was expressed from the linear calibration curve of Trolox as µg Trolox equivalent/g DW as the mean ± standard deviation (SD) of three replicates.

**FRAP Method**

The Ferric Reducing Activity Power (FRAP) assay was performed as described by Stratil et al. [22]. Briefly, a volume of 10 mM solution of 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ) in 40 mM HCl was mixed with the same volume of 20 mM FeCl$_3$·6H$_2$O and 10 times volume of acetate buffer pH 3.6 (3.1 g of sodium acetate and 16 mL of glacial acetic acid 100% per L). Afterwards, 275 µL of the Fe$^{3+}$-TPTZ mixture was combined with 25 µL extract, and incubated. After 5 min, the absorbances were recorded at 593 nm, and FRAP was expressed from the linear calibration curve of Trolox as µg Trolox equivalent/g DW as the mean ± standard deviation (SD) of three replicates.

**β-Carotene-Linoleic Acid Bleaching Assay**

The β-carotene-linoleic acid bleaching assay described by Salleh et al. [23] was used, with minor modifications. A mixture of β-carotene and linoleic acid was prepared by adding 0.5 mg β-carotene with 1 mL chloroform (HPLC grade), 25 µL linoleic acid and 200 mg Tween 40. After complete evaporation of the chloroform under vacuum at 40 °C, 100 mL of distilled water was added to the residue, which was gently mixed to form a yellowish emulsion. Then, 50 µL of extract was combined with 0.25 µL of the yellowish emulsion and mixed thoroughly. The mixture was then incubated in a water bath at 50 °C for 2 h, followed by the measurement of absorbance values at 470 nm against a blank. Percentage inhibitions (I%) of the extracts were calculated using the following equation:

\[
\text{I\%} = \left( \frac{A_{\text{β-carotene after 2 h}}}{A_{\text{initial β-carotene}}} \right) \times 100
\]

where $A_{\text{β-carotene after 2 h}}$ is the absorbance value of β-carotene after 2 h of incubation and $A_{\text{initial β-carotene}}$ is the absorbance value of β-carotene before incubation. All results were presented as the mean ± standard deviation (SD) of three replicates.
2.2.7. Phenolic Composition by HPLC-DAD

The phenolic composition was assessed by high performance liquid chromatography (HPLC)-diode-array detector (DAD) [24], using a Gilson HPLC (Villers-le-bel, France) and a Finnigan/Surveyor DAD (Thermo Electron, San Jose, CA, USA). The eluent consisted of water with 0.1% trifluoroacetic acid (solvent A) and acetonitrile with 1% trifluoroacetic acid (solvent B) with a gradient starting with 0% solvent B, at a flow rate of 1 mL/min, 0% solvent B at 5 min, 20% solvent B at 15 min, 50% B at 30 min, 100% solvent B at 45 min, 100% solvent B at 50 min, 0% solvent B at 55 min and 0% solvent B at 60 min. The extract was injected into the HPLC (10 µL) and the compounds were separated on a C18 column (250 × 4.6 mm, 5 µm) (ACE, Aberdeen, Scotland). The chromatograms were recorded at wavelengths ranging from 200 to 520 nm. Phenolics were identified by their peak retention times, UV spectra and UV max absorbance bands in comparison with commercial standards (purchased from Extrasynthese, Genay, France) and data reported in the literature. Phenolics were quantified using the internal standard method, and the results expressed in mg/100 g DW as the mean ± standard deviation (SD) of three replicates.

2.3. Antimicrobial Assays

2.3.1. Extraction and Bacterial Strains

For each sweet cherry cultivar sample, 800 mg of dry weight (DW) was weighed and mixed with 10 mL of methanol 80%, thoroughly in a vortex. The mixtures were heated at 70 °C for 20 min, and then centrifuged at 13,000 rpm, 1 °C for 15 min (Eppendorf Centrifuge 5804 R, Hamburg, Germany). The supernatants were collected, filtered with Spartan filters (0.2 mm) and solvent removed under vacuum. The extracts were dissolved in 10% dimethylsulfoxide (DMSO) (Sigma-Aldrich, Steinheim, Germany) to a concentration of 1 mg/mL to be used in disk diffusion assay. Bacterial isolates of Gram negative (Klebsiella pneumoniae MJ5281 and Enterobacter aerogenes MJH813) were collected from urine and Gram positive (Staphylococcus aureus MJ5241 and Enterococcus faecalis MJ5257) were collected from fecal samples from patients hospitalized in various departments of Hospital Centre of Trás-os-Montes and Alto Douro (CHTMAD, Vila Real, Portugal)—these are located in the cities of Lamego, Peso da Régua, Chaves, and Vila Real, Portuguese north province of Trás-os-Montes and Alto Douro. Ethical approval for this study was granted by the Ethics Committee of Hospital Vila Real (CHTMAD, Vila Real, Portugal), according to a research collaboration protocol established in 2003. Bacteria was isolated and identified by standard biochemical classification technique followed by genetic identification over 16S rRNA sequencing. American Type Culture Collection (ATCC) of E. coli and S. aureus were also used in the antimicrobial assays. Isolates were freshly prepared and inoculated in Petri dishes with Brain Heart Infusion (BHI) agar medium and incubated overnight at 37 °C.

2.3.2. Disk Diffusion Assay

Disk diffusion assay [25] was used to screen the antibacterial effect of plant extracts. Test isolates were grown on nutrient agar plates and later inoculated on nutrient broth to log phase. Test bacterial suspensions turbidity was adjusted to 0.5 MacFarland units. Petri dishes were prepared with 20 mL of Mueller-Hinton Agar (Oxoid, UK) and seeded with bacterial suspensions. Sterile paper disks were placed on an agar plate and impregnated with 15 µL of each previous extract solution in DMSO. Plates were incubated at 37 °C for 24 h. Disks of gentamicin (CN10–Oxoid CT0024B, UK) were used as positive control and disks impregnated with DMSO were used as negative control. After incubation, the diameter (mm) of the inhibitory zone around the disk was recorded. All the tests were performed in triplicate and antibacterial activity was expressed as the mean of inhibition diameters (mm) produced. The percentage of relative inhibition zone diameter (%RIZD) of each extract was calculated, according to Aires et al. [26] by the application of the following equation:

\[
%\text{RIZD} = \left(\frac{\text{IZD}_{\text{sample}} - \text{IZD}_{\text{negative control}}}{\text{IZD}_{\text{antibiotic standard}}}\right) \times 100
\]
where IZD is the inhibition zone diameter (mm).

2.4. Statistical Analysis

Data is presented as mean ± standard deviation of three replicates, and the results presented by dry weight (DW). Differences among means were determined by analysis of variance (ANOVA), using SPSS (Statistical Package for Social Sciences) software, version 24.0 (IBM Corporation, New York, NY, USA) software. The fulfillment of the ANOVA requirements, namely the normal distribution of the residuals and the homogeneity of variance, were evaluated by means of the Shapiro–Wilk’s test (n < 50), and the Levene’s tests, respectively. All dependent variables were analyzed using a one-way ANOVA with or without Welch correction, depending if the requirement of the homogeneity of variances was fulfilled. If statistically significant effect was found, comparison of means was performed using Tukey’s multiple comparison test. All statistical tests were performed at a 5% significance level. Pearson correlations and regression analysis were also calculated using SPSS, but, in the latter situation, only those presenting a R² value > 0.5 are referred.

3. Results and Discussion

3.1. Total Phenolics, Flavonoids, Photosynthetic Pigments and Antioxidant Activity

3.1.1. Extracts from Stems

Regarding stem’s extracts, significant differences were observed in several of the analyzed parameters, being the exception the ortho-diphenols and the antioxidant activity measured by the DPPH and β-carotene methods (Table 1).

The total phenolic content ranged from 32.49 mg/g in cv. Lapins, to 23.59 mg/g recorded in stems of cv. Early Bigi C. Similarly, total flavonoids presented the same trend, with higher values in cv. Lapins (24.75 mg/g) and lower in stems of cv. Early Bigi C (13.06 mg/g). Although these values are hard to compare, due to the lack of previous works, the total phenolic and flavonoids content can be considered similar to the ones reported by Bastos et al. [10]. Other reports show higher amount of total phenolics and similar content of flavonoids [27], or comparable values [28]. Saponins, a group of glycosides, known to have a bitter taste, are widely distributed in plants and known by their pharmacological properties [29] that makes of them the main constituents of many plant drugs, folk medicines and are responsible for most of the observed biological effects [30,31], were present in higher amount in stems of cv. Lapins (181.12 mg/g), with similar amount recorded for cv. Early Bigi NC (101.79 mg/g). For cvs. Early Bigi C and Burlat, the content of saponins were lower, with 42.45 and 38.05 mg/g, respectively. No previously data is available regarding saponin content in sweet cherry stems, being these the first data about the presence of those compounds in this samples. To provide same comparison values, works done with tea leaves, one of the most studied plant species regarding biochemical and bioactive properties, showed similar values of saponins content [32].
Table 1. Bioactive content and antioxidant activity of sweet cherry stems from four different cultivars.

| Cultivar  | Total Phenolic (mg GAE/g) | Total Flavonoid (mg CE/g) | Ortho-Diphenols (mg CAE/g) | Saponins (mg DE/g) | Cla (µg/mL) | Clb (µg/mL) | Total Carotenoids (µg/mL) | DPPH (µg Trolox/g) | FRAP (µg Trolox/g) | β-Carotene (% Inhibition) |
|-----------|---------------------------|---------------------------|----------------------------|-------------------|-------------|-------------|--------------------------|-------------------|------------------|-----------------------------|
| Burlat    | 26.63 ± 1.65ab            | 14.67 ± 3.23b             | 3.75 ± 0.85                | 38.05 ± 3.94c     | 3.70 ± 0.44b| 2.61 ± 0.25ab| 1.25 ± 0.05b              | 26.28 ± 1.15      | 15.15 ± 1.40b   | 90.13 ± 1.26                      |
| Early Bigi C | 23.59 ± 0.14b     | 13.06 ± 1.97b             | 3.88 ± 0.15                | 42.45 ± 2.97c     | 4.18 ± 0.42a| 2.60 ± 0.10ab| 1.42 ± 0.16b              | 25.98 ± 1.15      | 12.66 ± 2.48b   | 88.37 ± 0.94                      |
| Early Bigi NC | 31.30 ± 2.15a   | 19.71 ± 3.43ab            | 5.15 ± 0.97                | 101.79 ± 8.35a    | 4.91 ± 0.34a| 2.89 ± 0.18a| 1.58 ± 0.11a              | 27.48 ± 0.60      | 18.15 ± 2.24b   | 90.36 ± 4.17                      |
| Lapins    | 32.49 ± 5.23a            | 24.75 ± 1.14a             | 5.65 ± 0.75                | 181.12 ± 6.92a    | 2.76 ± 0.08c| 2.04 ± 0.02c| 1.25 ± 0.06b              | 28.06 ± 0.13      | 26.66 ± 2.26a   | 91.82 ± 0.69                      |
| Van       | 30.56 ± 1.29ab           | 21.18 ± 4.75ab            | 4.24 ± 0.70                | 98.66 ± 13.44b    | 3.64 ± 0.16b| 2.43 ± 0.09bc| 1.29 ± 0.07b              | 27.02 ± 0.58      | 18.21 ± 2.19b   | 94.96 ± 3.23                      |

*p value 0.012 0.006 0.113 0.000 0.001 0.008 0.060 0.000 0.070

Abbreviations: Early Bigi C—grown under net cover; Early Bigi NC—grown without net cover. Values are means ± SD (n = 3). Different small letters (a–c) in the same row are significantly different at p < 0.05 according to the analysis of variance (ANOVA) and multiple range test (Tukey’s test).
Overall, content in the photosynthetic pigments (chlorophyll a, b, and carotenoids) was found to be higher in samples of cv. Early Bigi NC, although with other samples showing similar amounts of Cla (Early Bigi C) and Clb (Burlat and Early Bigi C). The antioxidant activity only differed between cultivars for the FRAP methodology, with enhanced results when using cv. Lapins stems. For this methodology, results were found to correlate with the content in flavonoids (y = 0.8899x + 1.5521, \(R^2 = 0.781\)) and saponins (y = 0.0874x + 10.085, \(R^2 = 0.824\)). There was also a correlation between DPPH radical scavenging activity and the FRAP assay, but with low \(R^2 (0.54)\), probably due to the fact that compounds that react with the DPPH radical might not be the same as those reacting with the Fe \(^{3+}\)-TPTZ complex. In the DPPH method, the radical is neutralized when receiving H\(^+\) and/or electrons from antioxidants, but for the FRAP assay the Fe \(^{3+}\)-TPTZ complex is reduced to TPTZ-Fe\(^{2+}\) only by an electron transfer mechanism, by compounds with redox potential below 0.7 V [33]. Furthermore, the measure of reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols [34], and the FRAP assay is not able to detect compounds that act by radical quenching (H\(^+\) transfer), particularly thiols and proteins [35]. The use of other methodologies to access the antioxidant activity of extracts could also provide more information about the underlying mechanisms and help to explain these differences among spectrophotometric methods. One approach could be the use of cyclic voltammetry (CV), an electrochemical methodology that is fast and cheap [36]. This technique is commonly employed to investigate the reduction and oxidation processes of molecular species, to which good correlations between the oxidation potentials of various antioxidant and their antioxidant efficiency have been found [37]. Indeed, strong correlations have been found between DPPH, ABTS, FRAP and reducing power assays and CV [38]. However, correlations of CV to metal chelation ability and advanced glycation end products formation are weak, implying that different mechanisms are behind the antioxidant effects [39].

Significant antioxidant activity was already found for several types of extracts of cherry stems [7–9,27,28], with correlations recorded between phenolic and flavonoids in one or more antioxidant evaluation tests performed [7–9,28].

3.1.2. Extracts from Kernels

For kernel’s extracts, statistically significant differences were observed in all tested parameters, with the exception of \(\beta\)-carotene method (Table 2).

### Table 2. Bioactive content and antioxidant activity of sweet cherry kernels from four different cultivars.

| Cultivar       | Total Phenolic (mg GAE/g) | Total Flavonoid (mg CE/g) | Ortho-Diphenols (mg CAE/g) | Saponins (mg DE/g) | DPPH (mg Trolox/g) | FRAP (mg Trolox/g) | \(\beta\)-Carotene (% Inhibition) |
|----------------|--------------------------|---------------------------|---------------------------|-------------------|-------------------|-------------------|----------------------|
| urlat          | 2.46 ± 0.09a             | 1.81 ± 0.18b              | 0.09 ± 0.01b              | 41.83 ± 0.46b     | 1.05 ± 0.06a      | 0.58 ± 0.01a      | 93.46 ± 1.12         |
| Early Bigi NC  | 2.76 ± 0.14a             | 2.59 ± 0.44a              | 0.16 ± 0.02a              | 23.53 ± 6.05c     | 1.17 ± 0.06a      | 0.59 ± 0.04a      | 93.95 ± 0.47         |
| Lapins         | 1.29 ± 0.09bc             | Nd                        | Nd                        | 8.34 ± 0.76d      | 0.30 ± 0.08c      | 0.20 ± 0.03c      | 89.13 ± 2.25         |
| Van            | 1.17 ± 0.13c             | Nd                        | Nd                        | 13.18 ± 1.27d     | 0.30 ± 0.01c      | 0.19 ± 0.01c      | 91.31 ± 0.50         |
| \(p\) value    | 0.000                    | 0.000                     | 0.000                     | 0.000             | 0.000             | 0.000             | 0.062                |

Abbreviations: Early Bigi C—grown under net cover; Early Bigi NC—grown without net cover. Nd—not detected. Values are means ± SD (\(n = 3\)). Different small letters (a–d) in the same row are significantly different at \(p < 0.05\) according to the analysis of variance (ANOVA) and multiple range test (Tukey’s test).

The content of total phenolics was higher in cvs. Early Bigi NC (2.76 mg/g) and Burlat (2.46 mg/g), with the former presenting also increased content in total flavonoids and ortho-diphenols. These two groups of compounds were not recorded in cvs. Lapins and Van, and Early Bigi C, Lapins and Van, respectively. Saponin content ranged from 8.34 mg/g in cv. Lapins to 49.62 mg/g in cv. Early Bigi C, results opposed to those verified for stems, with regard to their content in both cultivars. As for stems, there is no previously data available regarding kernels saponin content, being this work the first one concerning saponins content in kernels of sweet cherry, although some works performed with plants
also belonging to the genus *Prunus* (*Prunus armeniaca* L.) kernel extracts have been performed and showed similar saponin content [40].

The recorded antioxidant activity was also significantly different when comparing samples, with the exception of the β-carotene assay. For DPPH and FRAP methods, higher antioxidant activity was found for samples of cvs. *Early Bigi NC* and *Burlat*. Again, all these data are difficult to compare, as this is one of the first reports concerning this type of samples. However, phenolic content of cherry kernels has been reported earlier, ranging from 601 to 1648 µg/g [41] for cvs. *Grace Star*, *Black Star*, *Vigred* and *Vera* or as low as about 320 µg/g, for an unidentified cultivar grown in Slovenia [42]. In both works, the flavonoid group corresponds to about one third of the quantified phenolics, considerably different of the flavonoid content recorded in the present work. The antioxidant activity of sweet cherry kernels has been poorly studied, but it has been recorded previously, with about 90% of inhibition of DPPH radicals [43]. In the present work, the antioxidant activity, either using the DPPH ($y = 1.7147x + 0.6952, R^2 = 0.958$) or the FRAP ($y = 3.55x + 0.5148, R^2 = 0.950$) method was found to correlate positively with the phenolic content of samples. There was also a strong correlation between DPPH and FRAP methods, which can indicate that the compounds responsible for the antioxidant activity are the same [28]. This is further confirmed by the positive correlations also found for individual compounds. The results for the DPPH method were also found to correlate strongly with values of ortho-diphenols ($y = 5.314 + 0.4090, R^2 = 0.8674$) and flavonoid content ($y = 0.3257x + 0.3754, R^2 = 0.9079$).

### 3.2. Identification of Phenolic Compounds

#### 3.2.1. Extracts from Stems

The phenolic profile of sweet cherry stem’s extracts is showed in The total amount of phenolic compounds ranged from 434.02 mg/100 g to 589.99 mg/100 g in cvs. *Van* and *Early Bigi NC* respectively, and 16 individual compounds were identified in extracts of stems. The content of each phenolic compound varied significantly when comparing cultivars, with only syringic acid present in similar amounts in all cultivars. In all samples, the most abundant compound was sakuranetin (Figure 3), with the exception of stems of cv. *Early Bigi NC*, where the most abundant compound was the ellagic acid. Of the identified compounds, the majority have already been described to be present in cherry stems [9,10]. However, there are some differences in the number and identification of the compounds with those authors, since in the present work epicatechin and hydroxycinnamic, ellagic, chlorogenic, and syringic acids are reported and absent in the work of Bastos et al. [10]. Some works have recorded a strong relationship between the content of some phenolics present in cherry stems and antioxidant activity [9]. In the present work, only the amount of neochlorogenic acid and its isomer was strongly and positively correlated with the recorded antioxidant activity in the FRAP method ($Y = 0.600x + 0.789, R^2 = 0.879$). Neochlorogenic acid, as well as other phenolic compounds, show their antioxidant properties by chelating metal ions, inhibiting radical forming enzymes and lipid oxidation, eliminating free radicals, diminishing oxidative stress levels, and thus protecting cell membranes against damage [3,8].

#### 3.2.2. Extracts from kernels

Regarding kernel’s extracts, only four compounds were found in the present work (Table 4 and Figure 4). The total amount of phenolic compounds in kernel’s extracts ranged from 16.58 mg/100 g to 75.58 mg/100 g in cvs. *Van* and *Early Bigi NC*, respectively. The most abundant phenolic compound was catechin (Figure 3), in four of the five samples, with protocatechuic acid being the major one in kernels of cv. *Early Bigi NC*, again showing the effect of tree cover in chemical parameters of sweet cherries. Previous works have reported the presence of anthocyanins, but also epicatechin, procyanidins, and flavonol glycosides [11]. Similarly, Senica et al. [41] have also identified hydroxycinnamic and
hydroxybenzoic acids, including catechin and $p$-coumaric acid. Variations in the number and quantity of compounds can be related to extractions and analysis methodologies, as well as genotypic diversity, as previously reported for another Prunus [44]. Interestingly, for kernels, all identified compounds strongly correlate with the recorded antioxidant activity, namely for FRAP and DPPH, with $R^2$, for all correlations, above 0.81. For the $\beta$-carotene assay, although positive correlations were found, $R^2$ was always below 0.5.

### Table 3. Phenolic composition of sweet cherry stems (mg/100 g DW).

|                   | Early Bigi C | Early Bigi NC | Lapins | Buralt | Van | $p$ Value |
|-------------------|--------------|---------------|--------|--------|-----|-----------|
| Catechin          | 15.02 ± 2.12b| 22.26 ± 0.51a| 17.45 ± 0.53b| 15.64 ± 0.71b| 17.86 ± 0.31b| 0.000    |
| Epicatechin       | 59.05 ± 4.19b| 74.88 ± 5.05a| 50.23 ± 0.99c| 61.82 ± 0.72b| 49.39 ± 1.63c| 0.000    |
| Naringenin-7-O-glucoside | 22.78 ± 2.42bc| 27.52 ± 1.11a| 26.61 ± 0.40ab| 26.37 ± 0.44ab| 20.77 ± 1.75c| 0.000    |
| Hydroxycinnamic acid | 18.16 ± 3.89a| 19.14 ± 0.20a| 8.51 ± 0.08b| 18.09 ± 0.75a| 9.52 ± 0.40b| 0.000    |
| Sakuranetin       | 139.73 ± 11.06ab| 128.31 ± 7.79b| 140.88 ± 0.58ab| 158.30 ± 10.08a| 131.63 ± 0.85b| 0.006    |
| Ellagic acid      | 106.87 ± 14.56b| 145.25 ± 4.49a| 96.37 ± 5.99b| 106.42 ± 3.93b| 95.59 ± 5.26b| 0.000    |
| Neochlorogenic acid + isomer | 19.11 ± 2.09d| 25.63 ± 0.28c| 40.62 ± 0.20a| 26.38 ± 0.79c| 33.11 ± 1.25b| 0.000    |
| Chlorogenic acid + isomer | 43.29 ± 4.90b| 51.13 ± 0.57a| 23.77 ± 0.41c| 19.18 ± 0.45c| 25.23 ± 1.60c| 0.000    |
| $p$-Coumaric acid + isomer | 18.81 ± 2.58bc| 22.47 ± 0.71ab| 18.38 ± 0.69c| 22.56 ± 0.85a| 13.46 ± 1.02c| 0.000    |
| Ferulic acid      | 14.95 ± 1.82a| 14.28 ± 0.07a| 9.56 ± 0.25bc| 10.99 ± 0.57b| 7.59 ± 0.65c| 0.000    |
| Syringic acid     | 12.63 ± 1.38a| 13.89 ± 0.22a| 9.48 ± 0.29c| 7.37 ± 0.33a| 9.15 ± 0.17b| 0.470    |
| Taxifolin         | 7.07 ± 1.00a| 8.34 ± 0.32a| 4.94 ± 0.29b| 4.09 ± 0.30b| 4.85 ± 0.18b| 0.000    |
| Kaempferol-3-O-rutinoside | 5.94 ± 0.60c| 11.69 ± 0.20a| 7.60 ± 0.17b| 12.92 ± 0.69a| 6.28 ± 0.67bc| 0.000    |
| Kaempferol-3-O-glucoside | 3.20 ± 0.41d| 5.95 ± 0.07a| Nd         | 4.29 ± 0.22b| 2.70 ± 0.28c| 0.000    |
| Genistein         | 4.93 ± 0.74b| 6.63 ± 0.13a| 4.97 ± 0.08b| 1.53 ± 0.07d| 3.25 ± 0.00c| 0.000    |
| Quercetin-3-O-glucoside | 16.17 ± 1.50a| 8.61 ± 0.31b| Nd         | 3.78 ± 0.18c| 0.65 ± 0.12d| 0.000    |
| Total             | 508.57 ± 46.57ab| 589.99 ± 21.20a| 493.02 ± 501.71| 513.0b    | 434.02 ± 9.74b| 0.003    |

Abbreviations: Early Bigi C—grown under net cover; Early Bigi NC—grown without net cover. Nd—not detected. Values are means ± SD ($n = 3$). Different small letters (a–d) in the same row are significantly different at $p < 0.05$ according to the analysis of variance (ANOVA) and multiple range test (Tukey’s test).

### 3.3. Antimicrobial Assays

The antimicrobial activity expressed as% RIZD is showed in Table 5. The negative control (DMSO) used for the extract’s preparation showed no effectiveness against the target Gram positive and Gram negative bacteria, as expected. Extracts from kernels were not able to inhibit the growth of any of the bacterial stains tested, and their use resulted in only a small synergistic effect in three situations. By the other hand, extracts from cherry stems proved to be more effective, namely for Gram positive bacteria.

### Table 4. Phenolic composition of sweet cherry kernels (mg/100 g DW).

|                   | Early Bigi C | Early Bigi NC | Lapins | Buralt | Van | $p$ Value |
|-------------------|--------------|---------------|--------|--------|-----|-----------|
| Gallic acid       | 4.05 ± 0.27c| 13.19 ± 0.78a| 3.12 ± 0.10cd| 8.27 ± 0.55b| 2.74 ± 0.04d| 0.000    |
| Protocatechuic acid | 6.17 ± 0.41c| 30.34 ± 0.86a| 5.92 ± 0.14c| 21.05 ± 0.42b| 4.92 ± 0.06c| 0.000    |
| Catechin          | 10.32 ± 0.41c| 20.06 ± 0.55b| 6.66 ± 0.15d| 22.87 ± 1.39a| 7.06 ± 0.15d| 0.000    |
| $p$-coumaric acid + isomer | 4.24 ± 0.15c| 11.99 ± 0.32a| 1.26 ± 0.05d| 10.27 ± 0.48b| 1.87 ± 0.31d| 0.000    |
| Total             | 24.78 ± 1.18c| 75.58 ± 2.49a| 16.97 ± 0.35d| 62.46 ± 2.73b| 16.58 ± 0.40d| 0.000    |

Abbreviations: Early Bigi C—grown under net cover; Early Bigi NC—grown without net cover. Values are means ± SD ($n = 3$). Different small letters (a–d) in the same row are significantly different at $p < 0.05$ according to the analysis of variance (ANOVA) and multiple range test (Tukey’s test).
Figure 2. Example of a HPLC chromatograms of phenols detected in sweet cherry stem’s extracts, recorded at different wavelengths: 280 nm (A), 320 nm (B), 370 nm (C) and 254 nm (D).

Figure 3. Chemical structure of sakuranetin (A) and catechin (B).
Figure 3. Chemical structure of sakuranetin (A) and catechin (B).

3.2.2. Extracts from kernels

Regarding kernel’s extracts, only four compounds were found in the present work (Table 4 and Figure 4).

Table 4. Phenolic composition of sweet cherry kernels (mg/100 g DW).

|                | Early Bigi C | Early Bigi NC | Lapins | Van  |
|----------------|-------------|---------------|--------|------|
| Gallic acid    | 4.05 ± 0.27c| 13.19 ± 0.78a| 3.12 ± 0.10cd | 8.27 ± 0.55b | 2.74 ± 0.04d | 0.000        |
| Protocatechuic acid | 6.17 ± 0.41c | 30.34 ± 0.86a | 5.92 ± 0.14c | 21.05 ± 0.42b | 4.92 ± 0.06c | 0.000        |
| Catechin       | 10.32 ± 0.41c| 20.06 ± 0.55b | 6.66 ± 0.15d | 22.87 ± 1.39a | 7.06 ± 0.15d | 0.000        |
| p-coumaric acid + isomer | 4.24 ± 0.15c | 11.99 ± 0.32a | 1.26 ± 0.05d | 10.27 ± 0.48b | 1.87 ± 0.31d | 0.000        |
| Total          | 24.78 ± 1.18c| 75.58 ± 2.49a | 16.97 ± 0.35d | 62.46 ± 2.73b | 16.58 ± 0.40d | 0.000        |

Abbreviations: Early Bigi C—grown under net cover; Early Bigi NC—grown without net cover. Values are means ± SD (n = 3). Different small letters (a–d) in the same row are significantly different at p < 0.05 according to the analysis of variance (ANOVA) and multiple range test (Tukey’s test).

Figure 4. Example of a HPLC chromatogram of phenols detected in sweet cherry kernel’s extracts, recorded at different wavelengths: 280 nm (A) and 320 nm (B).

The total amount of phenolic compounds in kernel’s extracts ranged from 16.58 mg/100 g to 75.58 mg/100 g in cvs. Van and Early Bigi NC, respectively. The most abundant phenolic compound was catechin (Figure 3), in four of the five samples, with protocatechuic acid being the major one in

Table 5. Antimicrobial activity (% RIZD) of cherry stems and kernels relatively to antibiotic gentamicin.

|                | Burt                         | Early Bigi C                | Early Bigi NC                  | Lapins                 | Van  | S. aureus ATCC | S. aureus MJS241 | E. faecalis MJS257 | S. aureus MJS241 | S. aureus MJS241 | K. pneumonia MJH812 | E. aerogenes MJH13 |
|----------------|------------------------------|-----------------------------|--------------------------------|------------------------|------|----------------|-------------------|--------------------|-------------------|-------------------|-----------------------|----------------------|
| Gram positive  | Standard Bacterial Strains   | Stems | Kernels | Stems | Kernels | Stems | Kernels | Stems | Kernels | Stems | Kernels | Stems | Kernels | Stems | Kernels | Stems | Kernels | Stems | Kernels |
| S. aureus ATCC |                               | 66.6  | 0      | 58.8  | 0      | 56.3  | 0      | 68.8  | 0      | 62.5  | 0      |         |        |        |        |        |        |        |        |
| S. aureus MJS241 |                            | *     | 0      | *     | 0      | *     | 0      | *     | 0      | *     | 0      | *      | 0      | *     | 0      | 0      | 0      | 0      | 0      |
| E. faecalis MJS257 |                           | *     | 0      | *     | 0      | *     | 0      | *     | 0      | *     | 0      | *      | 0      | *     | 0      | 0      | 0      | 0      | 0      |
| Gram negative  |                              | 0     | 0      | 0      | 0      | 0      | 0      | 0     | 0      | 0     | 0      | 0      | 0      | 0     | 0      | 0     | 0      | 0      | 0      |

Means ± SD (n = 3). 0—extract without effect; 0–100—extract less effective than an antibiotic; *—extract effective and antibiotic without effect; # slight synergistic effect when antibiotic used in combination with extract, with increased inhibition zone compared to the former.

Indeed, for S. aureus ATCC, all samples were able to inhibit its growth, with values of % RIZD above 55%. More interestingly, for the clinical isolates of S. aureus MJS241 and E. faecalis MJS257, all the extracts of stems were able to inhibit the growth of those bacteria that proved to be resistant to the tested antibiotic (Figure 5). Although no significant correlation was found, this ability to inhibit bacterial growth may be due to the presence of sakuranetin, since it was the most abundant phenolic compound in all samples, although cv. Early Bigi NC has it in lower amount. Sakuranetin antibacterial activities have been demonstrated by several studies and subject of reviews [45]. These authors indicate that antibacterial activity might have multiple cellular targets, namely form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation, inactivating microbial adhesins, enzymes, cell envelope transport proteins, among others, or even disrupting microbial membranes. Extracts of cv. Lapins exhibited the strongest growth suppression for the Gram positive S. aureus MJS241 and E. faecalis MJS257 (12.11 and 11.08 mm, respectively). Extracts of cv. Burt presented 10.21 mm of inhibition for S. aureus MJS241 and 9.00 mm for E. faecalis MJS257. Growth inhibition of S. aureus MJS241 was also caused by stem extracts of cv. Van (10.13 mm), while extracts of cv. Early Bigi C showed an inhibitory effect on E. faecalis MJS257.
Antimicrobial effect of cherry stems has already been recorded that has been linked to the content of phenolic compounds [9,27]. Indeed, in the present work, the higher values of total phenolics were found in stems of cv. *Lapins* that presented the higher% RIZD. The stems of this cultivar had the higher content in neochlorogenic acid that might be the reason of the increased antimicrobial activity. This compound presents aromatic hydroxyl groups that have high affinity to bacterial membranes, and are able to interfere with both membrane and cytoplasmic organelles, leading to the bacteria death and it recorded antimicrobial effect [46–48]. Neochlorogenic acid is also known to have activity against Gram positive *E. faecalis* and *S. aureus* [47].

![Figure 5. Clinical isolates of S. aureus MJS241 (A), E. faecalis MJS257 (B) and S. aureus ATCC (C) and the inhibitory effect of sweet cherry stem’s extracts. (1) Sweet cherry stem’s extract; (2) tested antibiotic (Gentamicin); (3) sweet cherry stem’s extract and tested antibiotic.](image)

For the tested Gram negative bacteria, no inhibitory effect was found, although extracts of cvs. *Early Bigi* C and NC and *Lapins* potentiated the antimicrobial effect of the latter when used in combination. The resistance of Gram negative bacteria is linked to the composition of cell wall components (lipopolysaccharides) that makes them less susceptible to the inhibitory effects of some phenolic compounds [49]. The analysis of correlation between the antimicrobial activity and the concentration of the phytochemical profile of sweet cherry stem’s extracts allowed to establish a significant positive correlation between the activity against *S. aureus* MJS241 and their content in neochlorogenic acid (Y = 0.16x + 5.253, R² = 0.887, p < 0.001). Lou et al. [47], Nunes et al. [50] and Taofiq et al. [51] showed the strong antimicrobial activity linked to this polyphenol. A positive correlation was also recorded between the content in saponins and the inhibition of its growth rate (Y= 0.0187x + 8.1595, R² = 0.6093, p < 0.001). Antimicrobial activity of saponins extracted from diverse plants has been already described by several authors (reviewed by [52]).

4. Conclusions

Sweet cherry is one of the most popular fruits, highly appreciated by consumers, not only for its fresh consumption but also as a processed fruit, which leads to an increase on its production. In parallel, the amount of sweet cherry by-products also increases, and the need to their valorization becomes even more important. In the present work, stems and kernels of four different cultivars of sweet cherry were characterized, providing useful information in order to find new alternative uses for such by-products. For the first time, saponins, known by their pharmacological properties, were quantified in both stems and kernels. Out of all the tested cultivars, and regarding stem’s extracts, cv. *Lapins* had overall highest bioactive content, saponins and antioxidant activity, while concerning kernel’s extracts, *Early Bigi* NC was the one with higher bioactive content and antioxidant activity. Stem’s extracts had the biggest polyphenolic compound diversity, with 16 different phenolic compounds identified. The compound present in highest amount was sakuranetin in all cultivars with exception of cv. *Early Bigi* NC, where ellagic acid was the most abundant. Only the amount of neochlorogenic acid and its isomer found in extracts from stems was strongly and positively correlated with the antioxidant activity. Four compounds were identified in sweet cherry kernel’s extracts, being catechin the most
abundant. These extracts were not able to inhibit the growth of the bacterial stains tested, contrary to the observed for stem’s extracts that proved to be effective against Gram positive bacteria, namely for *S. aureus* MJS241 and *E. faecalis* MJS257 that were resistant to the tested antibiotic. The most promising extracts were those from cv. Lapins, as they presented increased% RIZD, probably linked to the higher amount of neochlorogenic acid, known for its recognized antibacterial activity.

This work shows that sweet cherry by-products have very interesting bioactive properties, mainly regarding to the content in phenolics, saponins, antioxidant and antimicrobial activities. However, these by-products should be further explored and more studies are required, in order to explore nutraceutical and pharmacological formulations or antioxidant preservatives for the food industry and their effects on human health. Sweet cherry stem’s extracts may provide valuable solutions to the global problem of antibiotic resistance due to their antimicrobial activity linked to this unvalued by-product, although more research should be performed to test its applicability.

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**References**

1. Di Matteo, A.; Russo, R.; Graziani, G.; Ritieni, A.; Di Vaio, C. Characterization of autochthonous sweet cherry cultivars (*Prunus avium* L.) of southern Italy for fruit quality, bioactive compounds and antioxidant activity. *J. Sci. Food Agric.* 2017, 97, 2782–2794. [CrossRef] [PubMed]

2. Usenik, V.; Fabčič, J.; Štampar, F. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chem.* 2008, 107, 185–192.

3. Ballistreri, G.; Continella, A.; Gentile, A.; Amenta, M.; Fabroni, S.; Rapisarda, P. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chem.* 2013, 140, 630–638. [CrossRef] [PubMed]

4. Pacifico, S.; Di Maro, A.; Petriccione, M.; Galasso, S.; Piccolella, S.; Di Giuseppe, A.M.; Monaco, P. Chemical composition, nutritional value and antioxidant properties of autochthonous *Prunus avium* cultivars from Campania Region. *Food Res. Int.* 2014, 64, 188–199. [CrossRef]

5. Blando, F.; Oomah, B.D. Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits. *Trends Food Sci. Technol.* 2019, 86, 517. [CrossRef]

6. FAOSTAT. Data. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 6 July 2018).

7. Švarc-Gajić, J.; Cerdà, V.; Clavijo, S.; Suárez, R.; Mašković, P.; Cvetanović, A.; Novakov, V. Bioactive compounds of sweet and sour cherry stems obtained by subcritical water extraction. *J. Chem. Technol. Biotechnol.* 2018, 93, 1627–1635.

8. Jesus, F.; Gonçalves, A.C.; Alves, G.; Silva, L.R. Exploring the phenolic profile, antioxidant, antidiabetic and anti-hemolytic potential of *Prunus avium* vegetal parts. *Food Res. Int.* 2019, 116, 600–610. [CrossRef]
9. Aires, A.; Dias, C.; Carvalho, R.; Saavedra, M.J. Analysis of glycosylated flavonoids extracted from sweet-cherry stems, as antibacterial agents against pathogenic *Escherichia coli* isolates. *Acta Biochim. Pol.* 2017, 64, 265–271.

10. Bastos, C.; Barros, L.; Dueñas, M.; Calhelha, R.C.; Queiroz, M.J.R.; Santos-Buelga, C.; Ferreira, I.C. Chemical characterisation and bioactive properties of *Prunus avium* L.: The widely studied fruits and the unexplored stems. *Food Chem.* 2015, 173, 1045–1053.

11. Chaovanalikit, A.; Wrolstad, R.E. Anthocyanin and polyphenolic composition of fresh and processed cherries. *J. Food Sci.* 2004, 69, FCT73–FCT83. [CrossRef]

12. Górnáš, P.; Rudzińska, M.; Raczyk, M.; Mišina, I.; Seglina, D. Impact of cultivar on profile and concentration of lipophilic bioactive compounds in kernel oils recovered from sweet cherry (*Prunus avium* L.) by-products. *Plant Foods Hum. Nutr.* 2016, 71, 158–164. [CrossRef] [PubMed]

13. Singleton, V.; Rossi, J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 1965, 16, 144–158.

14. Dewanto, V.; Wu, X.; Adom, K.; Liu, R. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* 2002, 50, 3010–3014. [CrossRef] [PubMed]

15. Garcia, B.; Coelho, J.; Costa, M.; Pinto, J.; Paiva-Martins, F. A simple method for the determination of bioactive antioxidants in virgin olive oils. *J. Sci. Food Agric.* 2013, 93, 1727–1732. [CrossRef] [PubMed]

16. Chen, Y.; Xie, M.Y.; Gong, X.F. Microwave-assisted extraction used for the isolation of total triterpenoid saponins from *Ganoderma atrum*. *J. Food Eng.* 2007, 81, 162–170. [CrossRef]

17. Lichtenthaler, H.; Wellburn, A. Determination of total carotenoids and chlorophylls a and b of leaves in different solvents. *Biochem. Soc. Trans.* 1983, 11, 59–592. [CrossRef]

18. Warren, C.R. Rapid measurement of chlorophylls with a microplate reader. *J. Plant Nutr.* 2008, 31, 1321–1332. [CrossRef]

19. Pompelli, M.F.; França, S.C.; Tigre, R.C.; Oliveira, M.T.; Scilot, M.; Pereria, E.C. Spectrophotometric determinations of chloroplastic pigments in acetone, ethanol and dimethylsulphoxide. *Rev. Bras. Biociênc.* 2013, 11, 52–58.

20. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 1999, 26, 1231–1237. [CrossRef]

21. Siddhrai, P.; Becker, K. Antioxidant properties of various solvents extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam) leaves. *J. Agric. Food Chem.* 2003, 51, 2144–2155. [CrossRef]

22. Stratil, P.; Klejdus, B.; Kubáň, V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. *J. Agric. Food Chem.* 2006, 54, 607–616. [CrossRef] [PubMed]

23. Salleh, W.; Ahmad, F.; Yen, K.; Sirat, H. Chemical compositions, antioxidant and antimicrobial activity of the essential oils of *Piper officinarum* (Piperaceae). *Nat. Prod. Commun.* 2012, 7, 1659–1662. [CrossRef] [PubMed]

24. Aires, A.; Carvalho, R.; Rosa, E.; Saavedra, M.J. Phytochemical characterization and antioxidant properties of organic baby-leaf watercress produced under organic production system. *CYTA J. Food.* 2013, 11, 343–351. [CrossRef]

25. Jorgensen, J.H.; Turnidge, J.D.; Washington, J. Antibacterial susceptibility tests: Dilution and disk diffusion methods. In *Manual of Clinical Microbiology*, 7th ed.; Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R., Eds.; ASM Press: Washington, DC, USA, 2015; pp. 1526–1543.

26. Aires, A.; Mota, V.R.; Saavedra, M.J.; Rosa, E.A.S.; Bennett, R.N. The antimicrobial effects of glucosinolates and their respective enzymatic hydrolysis products on bacteria isolated from the human intestinal tract. *J. Appl. Microbiol.* 2009, 106, 2086–2095. [CrossRef] [PubMed]

27. Ademović, Z.; Hodžić, S.; Halilić-Zahirović, Z.; Husejnagić, D.; Džananović, J.; Šarić-Kundalić, B.; Suljagić, J. Phenolic compounds, antioxidant and antimicrobial properties of the wild cherry (*Prunus avium* L.) stem. *Acta Period. Technol.* 2017, 48, 1–13. [CrossRef]
28. Peixoto, J.; Álvarez-Rivera, G.; Alves, R.C.; Costa, A.S.; Andrade, N.; Moreira, A.; Ibáñez, E. Cherry stem infusions: Antioxidant potential and phenolic profile by UHPLC-ESI-QTOF-MS. Food Funct. 2020, 11, 3471–3482. [CrossRef] [PubMed]
29. Sparg, S.; Light, M.; Van Staden, J. Biological activities and distribution of plant saponins. J. Ethnopharmacol. 2004, 94, 219–243. [CrossRef]
30. Estrada, A.; Katselis, G.S.; Laarveld, B.; Barl, B. Isolation and evaluation of immunological adjuvant activities of saponins from *Polygona senega* L. Comp. Immunol. Microbiol. Infect. Dis. 2000, 23, 27–43. [CrossRef]
31. Liu, J.; Henkel, T. Traditional Chinese medicine (TCM): Are polyphenols and saponins the key ingredients triggering biological activities? Curr. Med. Chem. 2002, 9, 1483–1485. [CrossRef]
32. Chen, D.; Chen, G.; Sun, Y.; Zeng, X.; Ye, H. Physiological genetics, chemical composition, health benefits and toxicology of tea (*Camellia sinensis* L.) flower: A review. Food Res. Int. 2020, 137, 109584. [CrossRef]
33. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 2005, 53, 4290–4302. [CrossRef] [PubMed]
34. Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 2000, 48, 3396–3402. [CrossRef] [PubMed]
35. Ou, B.; Huang, D.; Hampsch-Woodill, M.; Flanagan, J.; Deemer, E. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. J. Agric. Food Chem. 2002, 50, 3122–3128. [CrossRef] [PubMed]
36. Arteaga, J.F.; Ruiz-Montoya, M.; Palma, A.; Alonso-Garrido, G.; Rodrigo, D.; Manolovici, D.D.; Stankovic, D.M. Use of cyclic voltammetry to determine the antioxidant capacity of berry fruits: Correlation with spectrophotometric assays. Eur. J. Hortic. Sci. 2019, 84, 152–160. [CrossRef]
37. Amidi, S.; Moghaddam, F.; Moghaddam, A.B.; Tabib, K.; Kobarfard, F. A simple electrochemical method for the rapid estimation of antioxidant potentials of some selected medicinal plants. Iran. J. Pharm. Res. 2012, 11, 117.
38. Nikolić, M.D.; Pavlović, A.N.; Mitić, S.S.; Tošić, S.B.; Mitić, M.N.; Kaličanin, B.M.; Manolovici, D.D.; Stanković, D.M. Use of cyclic voltammetry to determine the antioxidant capacity of berry fruits: Correlation with spectrophotometric assays. Eur. J. Hortic. Sci. 2019, 84, 152–160. [CrossRef]
39. Giménez-Bastida, J.A.; Zielinski, H.; Piskula, M.; Zielinska, D.; Szawara-Nowak, D. Buckwheat bioactive compounds, their derived phenolic metabolites and their health benefits. Mol. Nutr. Food Res. 2017, 61, 1600475. [CrossRef]
40. Tanwar, B.; Modgil, R.; Goyal, A. Effect of detoxification on biological quality of wild apricot (*Prunus armeniaca* L.) kernel. J. Sci. Food Agric. 2019, 99, 517–528. [CrossRef]
41. Senica, M.; Stampar, F.; Veberic, R.; Mikulic-Petkovsek, M. Fruit seeds of the Rosaceae family: A waste, new life, or a danger to human health? J. Agric. Food Chem. 2017, 65, 10621–10629. [CrossRef]
42. Senica, M.; Stampar, F.; Veberic, R.; Mikulic-Petkovsek, M. Transition of phenolics and cyanogenic glycosides from apricot and cherry fruit kernels into liqueur. Food Chem. 2016, 203, 483–490. [CrossRef]
43. Yi˘git, D.; Yi˘git, N.; Mavi, A. Antioxidant and antimicrobial activities of bitter and sweet apricot (*Prunus armeniaca* L.) kernels. Braz. J. Med. Biol. Res. 2009, 42, 346–352. [CrossRef] [PubMed]
44. Korekar, G.; Stobdan, T.; Arora, R.; Yadav, A.; Singh, S.B. Antioxidant capacity and phenolics content of apricot (*Prunus armeniaca* L.) kernel as a function of genotype. Plant Foods Hum. Nutr. 2011, 66, 376–383. [CrossRef] [PubMed]
45. Kumar, S.; Pandey, A.K. Chemistry and biological activities of flavonoids: An overview. Sci. World J. 2013, 2013, 162750. [CrossRef] [PubMed]
46. Ulltee, A.; Kets, E.P.W.; Smid, E.J. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 1999, 65, 4606–4610. [CrossRef]
47. Lou, Z.; Wang, H.; Zhu, S.; Ma, C.; Wang, Z. Antibacterial activity and mechanism of action of chlorogenic acid. J. Food Sci. 2011, 76, 398–403. [CrossRef]
48. Tsai, S.J.; Huang, C.S.; Meng, M.C.; Kam, W.Y.; Huang, H.Y.; Yin, M.C. Anti-inflammatory and antifibrotic effects of naringenin in diabetic mice. J. Agric. Food Chem. 2012, 60, 514–521. [CrossRef]
49. Cardona, F.; Andrés-Lacueva, C.; Tulipani, S.; Tinahones, F.J.; Queipo-Ortuño, M.I. Benefits of polyphenols on gut microbiota and implications in human health. J. Nutr. Biochem. 2013, 24, 1415–1422. [CrossRef]
50. Nunes, R.; Pasko, P.; Tyszka-Czochara, M.; Szewczyk, A.; Szlósarczyk, M.; Carvalho, I.S. Antibacterial, antioxidant and anti-proliferative properties and zinc content of five south Portugal herbs. Pharm. Biol. 2017, 55, 114–123. [CrossRef]
51. Taofiq, O.; González-Paramás, A.M.; Barreiro, M.F.; Ferreira, I.C. Hydroxycinnamic acids and their derivatives: Cosmeceutical significance, challenges and future perspectives, a review. *Molecules* 2017, 22, 281. [CrossRef]

52. Cheok, C.Y.; Salman, H.A.K.; Sulaiman, R. Extraction and quantification of saponins: A review. *Food Res. Int.* 2014, 59, 16–40. [CrossRef]

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