Cornus mas (L.) Fruit as a Potential Source of Natural Health-Promoting Compounds: Physico-Chemical Characterisation of Bioactive Components

Cornus mas (L.) fruit as a potential source of natural health-promoting compounds: physico-chemical characterisation of bioactive components

Marta De Biaggi[1], Dario Donno[1], Maria Gabriella Mellano[1], Isidoro Riondato[1], Ernest N. Rakotoniaina[2] , Gabriele L. Beccaro[1].

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Largo Braccini 2, 10095 Grugliasco (TO), Italy

[2] Département de Biologie et Écologie Végétales, Faculté des Sciences. Université d'Antananarivo, BP 566, Antananarivo 101, Madagascar.

Corresponding author: marta.debiaggi@unito.it

Abstract

Interest in new sources of anti-inflammatory and antioxidant compounds has recently become a major research issue, with the cornelian cherry (*Cornus mas* L.) receiving particular attention for its significant amounts of phenolic compounds and vitamins, which exhibit a wide range of biological and pharmacological properties. This study was aimed at increasing knowledge regarding the cornelian cherry in Italy through the analysis of biologically active substances in the locally available genotype "Chieri". Spectrophotometric methods were applied to evaluate antioxidant activity, total anthocyanin content and total polyphenolic content. Identification and quantification of the main phytochemical compounds (polyphenols, monoterpenes, organic acids and vitamin C) was performed via high performance liquid chromatography coupled to a diode array detector. *C. mas* extracts showed high levels of total soluble solids and low acidity. High amounts of phenolic secondary metabolites were observed, with particular reference to anthocyanins (134.71 $mg_{C3G}/100\ g_{FW}$), which confer remarkable nutraceutical properties to the analysed samples. These results highlight the potential of *C. mas* fruits as a good source of natural antioxidants, suggesting their use as a functional food. Future studies should focus on identifying other

specific phytochemical compounds and the genetic traits of local varieties in order to improve cornelian cherry cultivars for food and medicine production.

Key words: Cornelian cherry, phytochemicals, anthocyanins, antioxidant activity, functional food.

Abbreviations

C3G cyanidin-3-O-glucoside

FRAP ferric reducing antioxidant power

FW fresh weight

GAE gallic acid equivalent

TA titratable acidity

TAC total anthocyanin content

TBCC total bioactive compound content

TPC total polyphenolic content

TSS total soluble solids

Introduction

The consumption of vegetables and fruits is crucial to improving human intake of critical nutrients, as well as protecting against several chronic and degenerative diseases. Indeed, the intake of exogenous antioxidants in the diet is known to play a positive role in enhancing the endogenous antioxidant defence of the human body against disease development[1]. Thus, the search for new sources of anti-inflammatory and antioxidant compounds is now a major research issue [2], pushing food supplement producers towards the investigation of fruits with a high content of bioactive compounds with the aim of formulating new commercial products. Berries and other superfruits are rich in sugars but low in calories, and contain high quantities of both dietary fibre (cellulose, hemicellulose, pectin) and phytochemicals such as phenolic compounds, organic acids and some vitamins (e.g. vitamin C and folic acid) [3].

Phenolic compounds in particular belong to a heterogeneous group of chemical components that are mostly responsible for health benefits and are associated with various antioxidant and antimicrobial properties [3]. In addition to the better-known superfruit candidates such as bilberries, chokeberries, elderberries, tart cherries, cranberries, blueberries, strawberries and lingonberries [4], the cornelian cherry (*Cornus mas* L.) is now receiving growing attention since being reported as containing significant amounts of phenolic compounds and vitamins that exhibit a wide range of biological and pharmacological properties, including antimicrobial, anti-inflammatory, anticancer, antidiabetic and antiatherosclerotic actions [5-7]. Among the 65 *Cornus* species that are widely distributed across southwest Asia and the inland European continent, the cornelian cherry is one of the few species grown not only for ornamental purposes but also for their edible fruits [8], which are used in the production of jam, stewed fruit, syrup, yoghurt, liquor, wine, soft drinks and cosmetics [9]. *C. mas* is highly tolerant not only to different environmental and pedoclimatic conditions, but also to pests and diseases. As a result it grows successfully in undisturbed natural conditions without pesticides, making it suitable for production according to the principles of organic agriculture [9,10]. Despite its biological and economic potential, the species is still underutilised and insufficiently studied [10], and is mainly considered in the Italian peninsula as a landscape plant. The objective of this study was to increase the knowledge of the cornelian cherry in Italy, through a preliminary analysis of the biologically active substances contained in the locally available *C. mas* genotype "Chieri", applying spectrophotometric methods to evaluate antioxidant activity, total anthocyanin content (TAC) and total polyphenolic content (TPC). The main phytochemical compounds (polyphenols, monoterpenes, organic acids and vitamin C) were quantified via high performance liquid chromatography (HPLC). Indeed, a better understanding of local cultivars is essential for the selection of fruitful and high quality cornelian cherry genotypes if they are to be exploited successfully for nutraceutical purposes.

Materials and Methods

Plant material and sample preparation

Fully ripe *C. mas* fruits (500 g for each replication) were harvested manually in August 2016 from the genotype "Chieri" located in the germplasm repository of the Department of Agricultural, Forest and Food Sciences of the University of Turin in Chieri (45°1'0"N, 7°49'0"E, at 305 m a.s.l.), Piedmont (north-western Italy). The area is characterised by a temperate climate and average precipitation of approximately 810 mm/year; the soil is loam–clay. Immediately after harvest, samples were subdivided into two equal portions, one of which was used to determine fruit physicochemical parameters on the same day of harvest, and the other stored for one day at 4 °C and 95% relative humidity until the extraction of bioactive compounds.

Analytical methods

A more detailed description of the methods used for the extraction and analysis of quality properties, as well as spectrophotometric and chromatographic traits, is reported in the Supplementary material (see Online resource 1).

Determination of quality properties

Fruit width and length were measured using a 0.01 mm sensitive digital calliper (Traceable Digital Caliper-6″, VWR International, Milano, Italy), and fruit weight determined to the nearest 0.01 g (Mettler, Greifensee, Switzerland). The fruit was then homogenised in a blender and centrifuged (4000 rpm, 10 min), with pH, total soluble solids (TSS) and titratable acidity (TA) then evaluated.

Spectrophotometric analysis

TPC was determined following the Folin–Ciocalteu colorimetric method [11], with the results expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW). Extract TAC was determined using the pH-differential method [12] and expressed as milligrams of

cyanidin-3-O-glucoside (C3G) per 100 g of FW (mg_{C3G}/100 g_{FW}). Antioxidant activity was evaluated via ferric reducing antioxidant power (FRAP) assay [13], with the results expressed as millimoles of ferrous iron (Fe^{2+}) equivalents per kilogram (solid food) of FW.

Chromatographic analysis

Chromatographic analysis was carried out using an Agilent 1200 High-Performance Liquid Chromatograph coupled to an Agilent UV-Vis diode array detector (AgilentTechnologies, Santa Clara, CA, USA), based on HPLC methods previously tested and validated for herbal medicines and food supplements [14].

Identification and quantification of bioactive compounds

Total bioactive compound content (TBCC) was determined as the sum of selected biomarkers playing a positive role in human health ("multi-marker approach") [15].

Statistical analysis

All samples were prepared and analysed in triplicate. Phytochemical composition data were subjected to analysis of variance (ANOVA) for mean comparison (SPSS 22.0 Software), followed by the HSD Tukey multiple range test ($P < 0.05$) to highlight any significant statistical differences among the respective quantities of detected phytochemicals.

Results and discussion

Morphological and quality parameters

The analysed dark red cornelian cherry fruits were slightly ovoid in shape (15.04 mm ± 0.31 mm in width and 12.37 mm ± 0.38 mm in length), with a mean weight of 2.19 g ± 0.13 g. Quality analysis produced a mean TSS value of 17.70 ± 2.48 °Brix, falling within the range reported for this species (from 12.5% to 21.2%) [10] and higher than those of other superfruits, including blackcurrant (14.00 °Brix), blackberry (12.81 °Brix), raspberry (10.70 °Brix) and strawberry (8.00 °Brix) [16]. As also reported by Demir and Kalyoncu [17], such high intraspecific variability in TSS values could be a result of the strong influence of

different genotypes and the environmental conditions in which the plants grow. TA ranged from 56.40 meq · L^{-1} to 65.12 meq · L^{-1}, with a mean pH value of 3.01 ± 0.04 pH units. These results are in accordance with previous studies [18,19]. Compared to berry fruits (e.g. raspberry 413.57 meq · L^{-1}, strawberry 184.65 meq · L^{-1}) [16], the tested *C. mas* extracts were less acidic, a characteristic which makes the cornelian cherry highly appreciated by both consumers and the food industry, since it provides a longer shelf life [20]. Moreover, the average TSS/acid ratio observed in this study (6.50) was similar or higher than what reported in previous researches that [18,17,10], highlighting once more the large intraspecific variability of its fruit.

Nutraceutical properties

The remarkable nutraceutical properties of the cornelian cherry mainly reflect its high content of phenolic secondary metabolites [2]. TPC, TAC and antioxidant activity values of the fruits examined in this study are reported in Table 1. TPC value (196.68 ± 24.68 $mg_{GAE}/100g_{FW}$) was determined using the widely applied Folin-Ciocalteu reagent, thereby allowing a preliminary comparison with other studies on *C. mas* and other fruits [21-23]. This result was also confirmed by HPLC analysis, as reported below. The observed TAC values were comparable and in some cases higher than those observed for other small fruits [24,16], indicating that the analysed cornelian cherries are a good source of anthocyanins among fruit species. FRAP assay was applied to assess the antioxidant activity of *C. mas* samples as it is a low-cost method that is widely used for routine analysis, offers a high throughput and yields an index value useful for the comparison of natural products [25,26]. Although no single method can fully evaluate the antioxidant capacity of foods, this *in vitro* chemical-based assay may provide preliminary information regarding fruit characteristics, given that different techniques (DPPH, ORAC, TEAC, ABTS, FRAP or β-carotene bleaching assays) applied to the same fruit samples have produced overall similar antioxidant activity patterns, with

comparable and non-contradictory results [21,27,2]. The analysed fruits exhibited a mean FRAP value of 20.41 ± 0.50 mmol Fe^{+2}/kg.

Correlation of antioxidant activity was carried out against TPC, TAC and the main detected bioactive compounds are indicated in Table S2 (see Online resource 1). Positive correlation was observed with polyphenols and TPC (Pearson's correlation index: 0.77 and 0.63, respectively), underlining the importance of phenolic compounds from a functional point of view. The TAC correlation coefficient was also positive, indicating that anthocyanins in *C. mas* extracts are the main component responsible for the antioxidant activities, as observed in the phytochemical analysis. Vitamin C exhibited a positive correlation with antioxidant capacity, even though weaker than that observed for phenolic compounds, while monoterpenes and organic acids presented a negative correlation (R: -0.20 and -0.34, respectively).

Phytochemical fingerprint

Phytochemical composition analysis of the *C. mas* samples carried out via HPLC-DAD revealed 23 biomarkers (Table 2), with a mean TBCC value of 451.73 ± 16.50 mg/100 g_{FW}. Among the analysed compounds, isoquercitrin, quercetin, castalagin, quinic acid and sabinene were not detected.

In order to evaluate the contribution of each class to the total fruit phytocomplex composition, the health-promoting agents were grouped in the following classes: polyphenols (as the sum of anthocyanins, cinnamic acids, flavonols, benzoic acids, catechins and tannins), monoterpenes, organic acids and vitamin C (mean values considered) (Fig. 1). The analysis results revealed a prevalence of polyphenols (37.36%), followed by monoterpenes and organic acids in similar proportions (26.26% and 25.91%, respectively) and vitamin C (10.47%). In the polyphenolic group, anthocyanins were, as expected, the most important bioactive class (61.49%), with catechins the second most abundant (16.49%), followed by

benzoic acids (10.78%), cinnamic acids, tannins and flavonols, which accounted for 11.25% of the group total. As reported in previous studies [28,29,22], among the flavonoids, anthocyanins are present in significant amounts in cornelian cherry fruits, underlining their potential as a good source of natural antioxidants [6].

The vitamin C content of the examined fruits was 61.43 ± 3.31 mg/100 g_{FW}, in line with values observed in previous studies (from 31.70 to 99.52 mg/100 g_{FW}), as reported by Demir and Kalyoncu [17], and higher than those found in more common superfruits such as mulberry (2.97 ± 0.23 mg/100 g_{FW}), blueberry (12.60 ± 2.79 mg/100 g_{FW}), blackberry (45.07 ± 5.82 mg/100 g_{FW}) and strawberry (57.95 ± 2.60 mg/100 g_{FW}) [16]. Thus, *C. mas* fruits may represent a promising source of vitamin C, contributing to the recommended daily intake (60-90 mg/d for adults) [30].

In the analysed samples, monoterpenes and organic acids are important components of the *C. mas* fruit composition, together accounting for half of the bioactive compound pattern. Monoterpenes have recently been attracting research interest due to their anti-inflammatory properties and potential application in pharmacological therapies [31]. Citric acid was the predominant acid in the examined cornelian cherry fruits (58.24 ± 0.87 mg/100 g_{FW}) accounting for 38% of the total organic acid content, followed by malic acid (32%) and tartaric acid (27%); very low quantities of oxalic and succinic acids were observed. Despite the growing attention paid to these chemical classes, very few analyses have examined their content on *C. mas* [32], with the interesting nutraceutical aspects of this species essentially unstudied.

Previous studies have reported high phytochemical variability among small fruits [27,33], and the cornelian cherry is no exception considering its wide intraspecific variation [24]. The *C. mas* extracts analysed here also exhibited high variability in bioactive compound content when compared to previous studies, thus providing additional information regarding the

species diversity. This high variability results in a wide range of interesting agronomic traits, and depends on several biotic and abiotic factors, including the fruit maturity, genotype, environmental conditions, time of harvest and subsequent storage conditions [28,34]. Since commercial cornelian cherry cultivars have yet not been developed [6], this unexploited variability could be applied to well-focused breeding programmes in order to produce specifically selected varieties for improved nutraceutical potential [35] and resistance to pests and diseases.

Conclusions

In the last decade, wild edible and lesser-known plant species have received more attention as sources of biologically active substances, with the cornelian cherry an interesting example. This preliminary research revealed the presence of considerable amounts of bioactive compounds in the analysed fruits, which are particularly rich in anthocyanins, underlining the benefits of this plant species as a source of antioxidant and anti-inflammatory agents. The fruits also contain a high percentage of vitamin C, monoterpenes and organic acids, further contributing to their health-protection potential. The differences in the phytochemical content and composition of the analysed samples compared to those reported in previous studies confirm the species' high variability and breeding potential. Additional specific genetic and phytochemical analysis of selected cultivars and wild plants is thus crucial to evaluate polymorphism among genotypes. The results of this study highlight the necessity of popularising lesser-known fruits such as the cornelian cherry, in order to improve their propagation in the Italian peninsula as new promising fruit species for use the preparation of various high quality nutritional and pharmaceutical ingredients.

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Table 1 Total polyphenol content (TPC), total anthocyanin content (TAC) and antioxidant activity data of cornelian cherry samples

	Mean value	SD
TPC ($mg_{GAE}/100g_{FW}$)	196.68	24.68
TAC ($mg_{C3G}/100g_{FW}$)	134.71	7.10
Antioxidant activity (mmol Fe^{2+}/kg)	20.41	0.50

Mean value and standard deviation (SD) for each sample are given (n =3)

Table 2 Phytochemical composition of the of cornelian cherry samples

Bioactive class	Biomarker	Mean value	SD	Tukey test ($P < 0.05$)	Contribution of each biomarker
Catechins	Catechin	14.38	0.20	c	3.18
	Epicatechin	21.74	0.99	de	4.81
Benzoic acids	Ellagic acid	23.56	0.67	e	5.22
	Gallic acid	0.05	0.05	a	0.01
Flavonols	Hyperoside	1.00	0.16	ab	0.22
	Isoquercitrin	n.d.	/	/	n.d.
	Quercetin	n.d.	/	/	n.d.
	Quercitrin	0.77	0.38	ab	0.17
	Rutin	0.29	0.065	a	0.06
Cinnamic acids	Caffeic acid	0.66	0.00	a	0.14
	Chlorogenic acid	11.27	0.04	c	2.50
	Coumaric acid	3.86	0.11	ab	0.86
	Ferulic acid	2.14	0.21	ab	0.47
Tannins	Castalagin	n.d.	/	/	n.d.
	Vescalagin	4.66	0.23	b	1.03
Organic acids	Citric acid	58.24	0.87	h	12.89
	Malic acid	48.59	1.178	g	10.76
	Oxalic acid	2.11	0.13	ab	0.47
	Quinic acid	n.d.	/	/	n.d.
	Succinic acid	2.67	0.13	ab	0.59
	Tartaric acid	40.35	0.15	f	8.93
Monterpenes	Limonene	115.63	1.46	i	25.60
	Phellandrene	18.49	0.09	d	4.09
	Sabinene	n.d.	/	/	n.d.
	γ-Terpinene	18.44	1.90	d	4.08
	Terpinolene	1.42	0.31	ab	0.31
Vitamin C	Ascorbic acid	41.98	4.56	f	9.29
	Dehydroascorbic acid	19.44	2.62	d	4.30

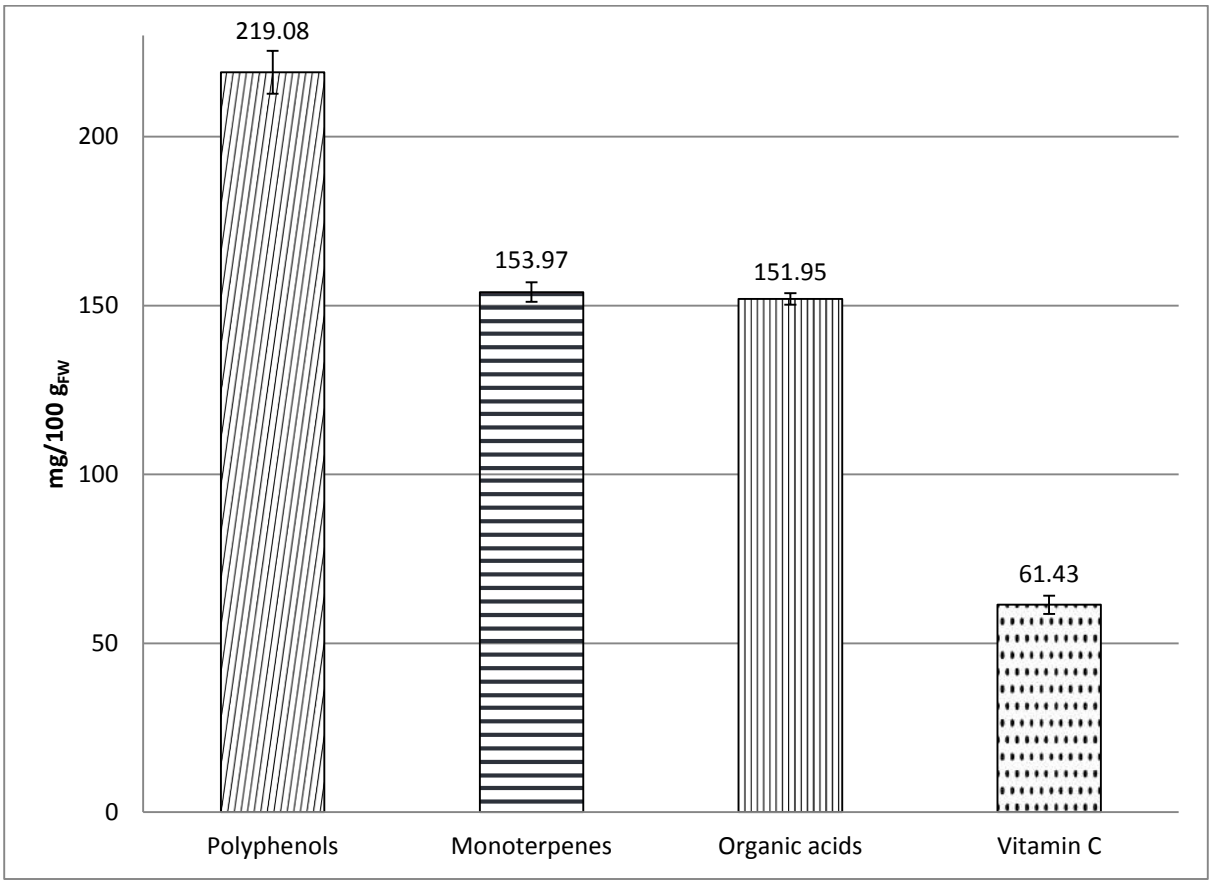
Mean value and standard deviation (SD) for each sample are given (n =3)

Results are expressed as mg/100 g_{FW}; n.d.: not detected

Different letters for each compound indicate the significant differences at $P < 0.05$

The contribution of each biomarker to the overall bioactive compound content is expressed as percentage (%).

Fig. 1 Phytocomplex representation of cornelian cherry samples



Supplementary Material

Article title: *Cornus mas* (L.) fruit as a potential source of natural health-promoting compounds: physico-chemical characterisation of bioactive components

Journal name: Plant foods for Human Nutrition

Author names: Marta De Biaggi[1], Dario Donno[1], Maria Gabriella Mellano[1], Isidoro Riondato[1], Ernest N. Rakotoniaina[2] , Gabriele L. Beccaro[1]

Affiliations:

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Largo Braccini 2, 10095 Grugliasco (TO), Italy

[2] Département de Biologie et Écologie Végétales, Faculté des Sciences. Université d'Antananarivo, BP 566, Antananarivo 101, Madagascar.

Corresponding author: marta.debiaggi@unito.it

Materials and Methods

Chemicals

Folin–Ciocalteu phenol reagent, sodium carbonate, sodium acetate, citric acid, potassium chloride, hydrochloric acid, iron(III) chloride hexahydrate, 2,4,6-tripyridyl-S-triazine, 1,2-phenylenediamine dihydrochloride (OPDA), all terpenic and polyphenolic standards, potassium dihydrogen phosphate, phosphoric acid and HPLC-grade methanol and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, acetic acid, HPLC-grade formic acid and organic acids were purchased from Fluka BioChemika, Buchs,

Switzerland. Ethylenediaminetetraacetic acid disodium salt was purchased from AMRESCO (Solon, OH, USA). Sodium fluoride was purchased from Riedel-de Haen (Seelze, Germany). Cetyltrimethylammonium bromide (cetrimide), ascorbic acid (AA) and dehydroascorbic acid (DHAA) were purchased from Extrasynthése (Genay, France). Milli-Q ultrapure water was produced by Sartorius Stedim Biotech mod. Arium (Sartorius, Göettingen, Germany).

Extraction of bioactive compounds

Polyphenolic compounds

For the extraction 10 g of fruit without kernel (three replications) were put into a 50-mL test tube and 25 mL of extraction solution (methanol : bi-distilled water, 95:5 v/v, pH adjusted with 1.5 mL of 37% HCl) and were added to the weighed samples. After 60 min in the dark, the extracts were homogenized with an Ultra-Turrax (IKA-Werkemodel T25, Staufen,Germany) for about 1 min and then centrifuged for 15 min at 3,000 rpm in a Centrifuge (ALC Centrifuge model PK 120, Cologno Monzese, Italy). This operation was carried out 3 times. All the supernatants were recovered and transferred to small glass tubes and kept frozen at -20 °C for further analysis.

Monoterpenes and organic acids

For the extraction of monoterpenes and organic acids, three replications were considered. Five grams of fruit without kernel were put into a test tube and 25 mL of 95% ethanol solution were then added. After 30 min in the dark, the extracts were homogenized with an Ultra-Turrax (T25, IKA WERKE) for about 1 min and then centrifuged for 10 min at 4,000 rpm in an ALC Centrifuge PK 120 (ALC International, Cologno Monzese, Italy). This operation was carried out 2 times. All the supernatants were recovered and transferred to small glass tubes and kept frozen at -20 °C for further analysis.

Vitamin C

A total of 10 g of fruit without kernel (three replications) was put into a 50 mL test tube and 10 mL of extraction solution (0.1 M citric acid, 2 mM EDTA disodium salt and 4 mM sodium fluoride in methanol – water, 5:95 v/v) were then added. The extracts were homogenized with an Ultra-Turrax (IKA-Werke T25) for about 1 min and then centrifuged for 10 min at 4,000 rpm at room temperature in an ALC Centrifuge PK 120. The supernatants were recovered and transferred to a 15-mL test tube through filter cloth and then acidified with 4 N HCl to decrease pH solution to a value of 2.2–2.4 pH units. Acidified samples were centrifuged for 5 min at 12,000 rpm at 4 °C with an ALC Multispeed refrigerated centrifuge PK 121R (ALC International, Cologno Monzese, Italy)[1].

Determination of quality properties

The TA (meq · L^{-1}) was determined in a mixture of 10 mL cornelian cherry juice diluted in 90 mL Milli-Q water, by titration with 0.2 M NaOH using an automatic titrator (Crison, Alella, Spain) to an end-point of pH 8.2. The pH of the fruit juice was measured directly. The TSS was measured directly in cornelian cherry juice with a digital refractometer (Tsingtao Unicom- Optics Instruments, Laixi, China), and the results were expressed as °Brix.

Spectrophotometric analysis

The TPC was determined following the Folin–Ciocalteu colorimetric method [2], and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW). Gallic acid standard solutions were prepared at 0.02–0.10 mg · mL^{-1}. The TAC in the extracts was determined using the pH-differential method [3], and expressed as milligrams of cyanidin-3-O-glucoside (C3G) per 100 grams of FW (mg_{C3G}/100 g_{FW}). The antioxidant activity was evaluated by the ferric reducing antioxidant power (FRAP) assay [4], and the

results were expressed as millimoles of ferrous iron (Fe^{2+}) equivalents per kilogram (solid food) of FW. The standard curve was obtained using $FeSO_4 \cdot 7H_2O$ at 100–1000 μmol · L^{-1}.

Chromatographic analysis

Sample preparation protocols for HPLC fingerprint

Methanolic extracts were filtered with circular pre-injection filters (0.45 μm, polytetrafluoroethylene membrane) prior to HPLC-DAD analysis. In the case of vitamin C analysis, a C_{18} cartridge for solid phase extraction (Sep-Pak® C-18,Waters, Milford, MA, USA) was used to absorb the polyphenolic fraction. Then, 250 μL of OPDA solution (18.8 mmol · L^{-1}) was added to 750 μL of each sample for DHAA derivatisation into the fluorophore 3-(1,2-dihydroxyethyl)furo(3,4-b)quinoxaline-1-one. After 37 min in the dark, these samples were analysed using HPLC-DAD [1].

Apparatus and chromatographic conditions

An Agilent 1200 High-Performance Liquid Chromatograph coupled to an Agilent UV-Vis diode array detector (AgilentTechnologies, Santa Clara, CA, USA) was used for the chromatographic analysis. Five chromatographic methods were used to separate the biomolecules on a Kinetex C18 column (4.6 × 150 mm, 5 _m, Phenomenex, Torrance, CA, USA) (Table S1). Several mobile phases were used for biomarker identification and UV spectra were recorded at different wavelengths, based on HPLC methods previously tested and validated for herbal medicines and food supplements [5].

Identification and quantification of bioactive compounds

The external standard method was used for quantitative determinations. Manual injections were performed in triplicate for each concentration level. Total bioactive compound content (TBCC) was determined as the sum of selected biomarkers having a positive role in human health (“multi-marker approach”) [6]. Five polyphenolic classes were considered: catechins

(catechin and epicatechin), benzoic acids (ellagic and gallic acids), flavonols (hyperoside, isoquercitrin, quercetin, quercitrin and rutin), cinnamic acids (caffeic, chlorogenic, coumaric and ferulic acids) and tannins (castalagin, vescalagin). Organic acids (citric, malic, oxalic, quinic, succinic and tartaric acids), monoterpenes (limonene, phellandrene, sabinene, g-terpinene, terpinolene) and vitamin C (ascorbic and dehydroascorbic acids) were considered in order to obtain a complete analytical fingerprint. All the results were expressed as mg/100 g_{FW}.

Supplementary tables:

Table S1 Chromatographic conditions of the applied methods

Method	Compound of interest	Mobile phase	Flow	Analysis time	Gradient[b]	Wavelenght
			(mL min^{-1})	(min)		(nm)
A	Cinnamon acids, flavonols	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8 B: CH_3CN	1.5	20 + 2 (CT[a])	Yes	330
B	Benzoic acids, catechins, tannins	A: $H_2O/CH_3OH/HCOOH$ (5:95:0.1 v/v/v), pH = 2.5 B: $CH_3OH/HCOOH$ (100:0.1 v/v)	0.6	23 + 2 (CT[a])	Yes	280
C	Monoterpenes	A: H_2O B: CH_3CN	1.0	17 + 3 (CT[a])	Yes	210, 220, 235, 250
D	Organic acids	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8 B: CH_3CN	0.6	13 + 2 (CT[a])	No	214
E	Vitamin C	A: 5 mM $C_{16}H_{33}N(CH_3)_3Br$/ 50 mM KH_2PO_4, pH = 2.5 B: CH_3OH	0.9	10 + 5 (CT[a])	No	261, 348

[a] CT = condition timing

[b] Evolution conditions

Method A gradient: 5%B to 21%B in 17 min + 21%B in 3 min

Method B gradient: 3%B to 85%B in 22 min + 85%B in 1 min

Method C ratio of phase and B: 95:5

Method D ratio of phase and B: 95:5

Table S2 Correlation among antioxidant activity and TPC, TAC and main bioactive compounds

Pearson correlation coefficient (R)				
	TPC	TAC	Polyphenols	Vitamin C
Antioxidant activity	0.6318	0.7190	0.7687	0.3572
Correlation	Positive Strong	Positive Strong	Positive Strong	Positive Weak

References

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