



Article

Phytochemical Characterization and Bioactivity Evaluation of Autumn Olive (*Elaeagnus umbellata* Thunb.) Pseudodrupes as Potential Sources of Health-Promoting Compounds

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Abstract: Autumn olive (Elaeagnus umbellata Thunb.) is a deciduous shrub tree widely distributed in Asia and Southern Europe and grown as ornamental species. It is locally used for human consumption, as relevant medical value is attributed to the berries. Information about its composition, especially concerning the characterization of bioactive and health-promoting compounds, is limited. The aim of the present study is to evaluate the main bioactive compounds and nutraceutical proprieties of autumn olive fruits, via high-performance liquid chromatography (HPLC) fingerprint and spectrophotometric analysis, in order to strengthen the knowledge about this underrated species and promote potential applications as a food supplement. Concerning nutraceutical traits, total polyphenolic content (325.366 ± 13.019 mg of gallic acid equivalents (mg GAE)/100 of fresh weight (g FW)) and total anthocyanin content (194.992 ± 0.817 mg of cyanidin-3-O-glucoside (mg C3G)/100 g FW) recorded considerable values. The phytochemical fingerprint revealed the presence 23 bioactive compounds. Polyphenols (65.56%) were the largest class, followed by monoterpenes (27.40%) and vitamin C (7.04%). Anthocyanins were the most represented compounds among polyphenols (71.9%). The antioxidant capacity (20.031 \pm 1.214 mmol Fe²⁺/kg) was similar to that recorded for other small fruits with proven health-promoting properties. The present work underlined the potential of E. umbellata as a source of health-promoting bioactive compounds. Further studies should deepen the knowledge of nutraceutical aspects, which turned out to be interesting.

Keywords: berry fruit; antioxidant activity; anthocyanins; HPLC fingerprint; underrated species; multipurpose tree

1. Introduction

The genus *Elaeagnus* includes 98 species belonging to the Elaeagnaceae family [1]. This family includes shrubs and small trees that produce fruits with a nutritional and ornamental value. Japanese silverberry (*Elaeagnus umbellata* Thunb), also known as autumn olive or goumi, is a deciduous small tree widely used along highways, as the hedges provide a protective screen against wind, and to prevent soil erosion [2]. Due to its ornamental value, drought tolerance, adaptability to different environments, and compact structure, it is grown in urban areas as a hedge [3]. Locally, it is employed for making artefacts such as fencing, fodder, and baskets and as a fuel wood [4]. Originally from

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Southern Europe, Central and Meridional Asia, it grows wild in its native range as a shrub, where the topographic features of the area limit the cultivation of extensive crops [5]. The autumn olive produces almost spherical fruits called pseudodrupes: 7 mm long and deep-red at fully ripening stage, highly attractive to birds, and locally used for human consumption. The fruit is astringent until ripe, when it acquires a sweet-tart flavor. Pseudodrupes can be consumed fresh or processed for preserves, juices, fruit rolls, and condiments [6]. They are composed of 69.4% moisture, 14.5% total soluble solids, 8.34% total sugars, and 1.51% acids. The content of ash, expression of the total mineral content, is 1.045% [7]. The berries are rich in oil, 7.43–8.11 g/100 g and 5.84–6.11 g/100 g, respectively, in pulp and seed. The composition of these oils contains vitamin E (tocopherol) and phytosterols, which could have significant values in medicine [4].

In the native range of the species, relevant medical value is attributed to the berries. Inhabitants used to eat them, either raw or cooked, as a remedy to reduce blood pressure, against coughs, and for pulmonary infections [7]. Recent studies have shown the antibacterial activity of aqueous extracts from fruits of *E. umbellata* in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* [8]. In some areas of China, Japan, and Korea the fruits of *E. umbellata* are incorporated into the local diet, due to their putative health benefits [9].

Little information is available regarding the cultivation of *E. umbellata*. It is considered an invasive exotic species in multiple American states [3]. Until the early 1990s, there were only four recognized cultivars in the US, that mainly differed due to their adaptations to different climatic regions [10]. In the last few years, new cultivars were selected based on the color of the berries.

In Italy, this species is mainly used for ornamental purposes, as it can easily grow over 2 m, has little white flowers with intense scent, and does not require specific plant care. Indeed, autumn olive is a very rustic tree, resistant to cold temperatures and drought, and highly tolerant to pruning. Cultivation areas also include coastal zones, since autumn olive can grow at high soil salt concentrations [11]. The berries are consumed fresh, a tradition derived from tropical and temperate Asia, the native area of the species [12]. Few cultivars are available on the market, mainly selected and registered in North European regions over the last years, which underlines the marginal role of the cultivation of *E. umbellata*.

Thus far, few studies have explored the antioxidant and bioactive compounds of autumn olive. Berries are a valuable source of carotenoids such as lycopene, α -cryptoxanthin, β -cryptoxanthin, β -carotene, lutein, phytoene, and phytofluene. Regarding lycopene (15–54 mg/100 g), the concentration is rather higher than in fresh tomato fruits [13]. Scientific research has reported on the anticancer efficacy of lycopene, represented by a high singlet oxygen quenching ability, twice that of β -carotene and 10 times that of α -tocopherol [5]. The total phenolic content, ranging from 190 to 275 mg of gallic acid equivalents (mg GAE)/100 g of fresh weight (g FW), falls within the range of many other berry fruits with proven health-promoting effects [14]. Furthermore, it is a source of vitamin C, whose content ranges between 14–17 mg/100 g FW [4].

However, at the European and national level little information is yet available on *E. umbellata*, due to the limited consumption of its berries. Several aspects of its composition and potential health benefits need to be further explored, in particular regarding the polyphenol composition.

The objective of the present work is to enhance the knowledge of the autumn olive (*Elaeagnus umbellata* Thunb.), evaluating its fruits as a potential source of bioactive and antioxidant compounds. The main phytochemical compounds, selected on the basis of their health-promoting demonstrated efficacy, were measured through the use of high-performance liquid chromatography (HPLC).

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2. Materials and Methods

2.1. Plant Material, Harvesting Site, and Sample Preparation

E. umbellata fruits were collected in the germplasm collection of the Department of Agricultural, Forest and Food Sciences of the University of Turin (DISAFA), located in Chieri (45°02′26.3″ N, 7°50′16.8″ E, at 305 m a.s.l.), Piemonte (northwest Italy). Berries were harvested manually (500 g for each replication) at the beginning of November 2018 and randomized from three different trees, so that each tree represented one biological replication. The ripening evaluation was based on pericarp color and fruit development. This work is a preliminary research on the autumn olive; therefore, the investigation did not focus on a single genotype, but rather on the entire *E. umbellata* species. The climate of the area is temperate and for 2018 the average precipitation recorded a value of approximately 770 mm. The soil at the germplasm repository is loam-clay.

After the harvest, berries were immediately stored in refrigerated containers and sent to the laboratory at the DISAFA in Grugliasco (Province of Turin). Then, selection was performed in order to discard any defective fruits from analysis. Finally, the samples were separated into two equal portions. The same day of harvest, physio-chemical parameters were assessed using one portion. The other portion was stocked for one day at 4 °C and 95% relative humidity and further used to extract the bioactive compounds.

2.2. Chemicals

The solvents and chemicals used for extractions and analysis are described in more detail in the Supplementary Materials. All the references (Company, City, State Abbr. and Country) are reported.

2.3. Determination of Morphological and Quality Parameters

One portion of the samples (250 g each replication) was used for the measurement of the fruits' physio-chemical parameters. To assess width and length, a 0.01 mm sensitive digital caliper was used (Traceable Digital Caliper-6", VWR International, Milan, Italy). Weight was measured to the nearest 0.01 g (Mettler-Toledo AG, Greifensee, Switzerland). For the assessment of pH, total soluble solids (TSS) and titratable acidity (TA), samples were firstly homogenized in a blender (Ultra-Turrax model T25, Ika, Staufen, Germany) and later centrifuged at 4000 rpm for 10 min. The remaining samples were stored at 4 °C and 95% relative humidity for further analysis.

The pH was determined on the fruit juice with the support of a potentiometric pH-meter (Crison, Alella, Spain). The TSS, expressed as $^{\circ}$ Brix, was quantified with the aid of a digital refractometer (Tsingtao Unicom-Optics Instruments, Laixi, China). Finally, the TA (meq L⁻¹) was measured by titration of a mixture of autumn olive juice (10 mL) diluted in Milli-Q water (90 mL) with the addition of 0.2 M NaOH solution using an automatic titrator (Crison, Alella, Spain) to an end-point of pH 8.2.

2.4. Spectrophotometric Analysis

A single-beam UV–Vis spectrophotometer (1600-PC, VWR International, Milan, Italy) was used to determine the total polyphenolic content (TPC), total anthocyanin content (TAC), and antioxidant capacity (AC). The Folin–Ciocalteu colorimetric method was performed to assess the TPC [15]. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW). Standard solution of gallic acid was prepared at 0.02–0.10 mg mL⁻¹. The pH-differential method was used for the evaluation of TAC [16,17], and results were reported as cyanidin-3-O-glucoside (C3G) per 100 g of FW (mg C3G/100 g FW). Anthocyanins demonstrate maximum absorbance at 515 nm at pH 1.0 and at 700 nm at pH 4.5. The colored oxonium form of anthocyanin predominates at pH 1.0, and the colorless hemiketal form at pH 4.5. The pH-differential method is based on the reaction producing oxonium forms. Finally, the AC was determined through the ferric reducing antioxidant power (FRAP) assay [18], expressing results as millimoles of ferrous iron (Fe²⁺) equivalents per kilogram (solid food) of FW. The standard curve was plotted using FeSO4·7H2O at 100–1000 μmol/L.

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2.5. HPLC Fingerprint: Bioactive Compound Extraction

Polyphenolic compounds, organic acids, monoterpenes, and vitamin C were extracted following effective protocols used in previous works [19,20]. The full description of the protocols used to extract each compound class is fully explained in the Supplementary Materials.

2.6. Chromatographic Analysis

2.6.1. Sample Preparation and Chromatographic Analysis

Before the HPLC-DAD (diode array detector) analysis, circular pre-injection filters were used to filter methanolic extracts (0.45 μ m, polytetrafluoroethylene membrane, PTFE). For the absorption of the polyphenolic fraction for the vitamin C analysis, a C₁₈ cartridge was applied for solid phase extraction (Waters, Milford, MA, USA). Then, for dehydroascorbic acid (DHAA) derivatization into the fluorophore 3-(1,2-dihydroxyethyl)furo(3,4-b)quinoxaline-1-one (DFQ), 750 μ L of each sample were diluted with o-phenylenediamine (OPDA) solution (18.8 mmol L⁻¹). After 37 min in the dark, samples were ready to be analyzed using HPLC-DAD [21].

Concerning the chromatographic analysis, an Agilent 1200 High-Performance Liquid Chromatograph combined to an Agilent UV–Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA) was used. To separate the bioactive substances, a Kinetex C18 column (4.6×150 mm, 5 µm; Phenomenex, Torrance, CA, USA) was used. The samples were analyzed using five chromatographic methods and different mobile phases were used in order to separate and identify the compounds, while UV spectra were recorded at different wavelengths [22] (the mobile phases are listed in Table S1).

In order to obtain a phytochemical fingerprint with all the relevant information on the chemical composition, with a proper resolution and a reasonable analysis time, the chromatographic conditions were set. To optimize the molecule separation, different linear gradients in different slopes were used. Indeed, the same chemical class had similar compounds in term of structure. Since most of the compounds were also weakly acidic, we added formic and phosphoric acid to enhance the resolution and eliminate peak tailing. The selection of the wavelengths was essential to develop a reliable fingerprint. As a matter of fact, only the selected wavelengths achieved more specific peaks, as well as a smooth baseline after a full-scan on the chromatogram from 190 to 400 nm.

2.6.2. Identification and Quantification of Bioactive Compounds

For the quantitative determination of bioactive compounds, the external standard calibration method was used. This is an effective method already performed with success in previous studies [19,20]. A more detailed description is reported in the Supplementary Materials (Table S2).

2.7. Statistical Analysis

The results were subjected to variance analysis (ANOVA) for comparison of means, followed by Tukey's honest significant different (HSD) multiple range test (p < 0.05). The samples were prepared and analyzed in triplicate (three samples for three biological repetitions). Statistical analysis was performed with SPSS Statistics 22.0 (IBM, Armonk, NY, USA, 2013).

3. Results and Discussion

3.1. Morphological and Quality Parameters

Japanese silverberry produced almost spherical fruits (6.92 \pm 0.11 mm in width and 6.46 \pm 0.08 mm in length) with a mean weight of 0.32 \pm 0.02 g for each berry at fully ripe stage (Table 1). Results are in accordance with previous research [6,7,11], except for the weight, which turned out to be slightly higher [4].

Analysis on quality traits revealed a TSS mean value of $16.23 \pm 0.15^{\circ}$ Brix (Table 1). These findings are partially confirmed in the literature: a study on *Elaeagnus umbellata* berries cultivated in five

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different localities showed lower values of TSS [23], while the results of six autumn olive genotypes grown in Cookeville (TN, USA) recorded TSS values ranging from 10.6 to 18.4° Brix [5].

| Unit of Measurement | $Mean\ Value \pm SD$ |
|-----------------------------|--|
| (g) | 0.32 ± 0.02 |
| (mm) | 6.92 ± 0.11 |
| (mm) | 6.46 ± 0.08 |
| (°Brix) | 16.23 ± 0.15 |
| (meq/L) | 342 ± 79.37 |
| (pH units) | 3.53 ± 0.20 |
| (mgGAE/100 gFW) | 325.366 ± 13.019 |
| (mgC3G/100 gFW) | 194.992 ± 0.817 |
| (mmol Fe ²⁺ /kg) | 20.031 ± 1.214 |
| | (g) (mm) (mm) (°Brix) (meq/L) (pH units) (mgGAE/100 gFW) (mgC3G/100 gFW) |

Table 1. Morphological, quality, and antioxidant traits of autumn olive fruits.

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

Free sugars are among the principal constituents and are essential to determine the quality of fruits [24]. Within the sugar class, many studies have reported fructose and glucose as the major contributors of fruit flavor. As proof of this, fructose turned out to be 1.8 time sweeter than sucrose [25]. Many studies on oleaster fruits revealed fructose and glucose as the main sugars during ripening stage [5,26], highlighting the palatability of *Elaeagnus* fruits.

Autumn olive berries had a pH mean value of 3.53 ± 0.20 , falling within the range found in a previous study (3.30–3.90) [23], although in contrast with the study of Khattak on the same species [27]. TA ranged from 252 to 402 meq/L, with a mean value of 342 ± 79.37 meq/L (Table 1). In order to make this value comparable, it was converted in percentage of malic acid equivalent. Therefore, titratable acidity was found to be 1.64% (equal to 1.64% of malic acid/100 g), considerably lower than the findings of Hussain [23] and Khattak [27], which were 2.20-2.94% and $3.1 \pm 0.1\%$, respectively. The differences in terms of TA could be due to many factors, among which locality, weather conditions, genotype (cultivar), and agronomic techniques play a key role. Considering the non-climacteric attitude of the berries, the time of the harvest is also essential in determining the TSS:TA ratio. All of these aspects have to be taken into account when comparing fruits grown in tropical or sub-tropical areas rather than in temperate regions [28].

The TSS:TA ratio of 9.90 observed in this study further emphasized the large intraspecific variability of the *E. umbellata* fruits, partially confirming previous studies [5,23,27].

3.2. Antioxidant Potential Properties

Berry fruits have considerable amounts of health-promoting compounds, such as phenolic compounds like phenolic acids, anthocyanins, tannins, and flavonoids [28]. Natural phenolic compounds exert healthy actions by reducing oxidative stress and inhibiting macromolecular oxidations, thus counteracting the risk of degenerative diseases [29].

Autumn olive is a multipurpose plant, whose fruits have been consumed over the centuries for many uses. Among these, especially in the native range of the species, the berries have a relevant medical value. To partially support the effectiveness of these local remedies, a study on *E. umbellata* conducted in 2007 reported the capacity of the plant extracts to inhibit the growth of *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis* [8].

The TPC value is expressed in milligrams of gallic acid equivalents and recorded a considerable value (Table 1), higher than other studies on the same species [5,14]. These results make autumn olive berries comparable to other small fruits such as red raspberry (357.83 \pm 7.06 mg GAE/100 g FW), blueberry (305.38 \pm 5.09 mg GAE/100 g FW), and cherry (314.45 \pm 5.95 mg GAE/100 g FW) [28]. Regarding TAC, no values have been reported so far in this species. The clear identification of the different Elaeagnaceae species and cultivars is often difficult; moreover, it is hard to find specific

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information on the different species. However, the observed value of total anthocyanins (Table 1), expressed as milligrams of cyanidin-3-O-glucoside (C3G) per 100 g of fresh weight, was higher than those found on other berry fruits such as blackberry, strawberry, blueberry, and red currant, but lower than the values detected in black currant and black chokeberry [28,30]. The present results are in accordance with previous research on different genotypes of Russian olive (Elaeagnus angustifolia L.), where values ranged from 115.45 to 260.52 mg C3G/100 g FW and from 116.84 to 630.1 mg C3G/100 g FW [31,32]. Genetic and environmental factors contribute to determining a large intraspecific variability on these parameters, as confirmed by the results in literature.

Ferric reducing antioxidant power (FRAP), a technique based on the ability of antioxidants to reduce ferric (III) ions to ferrous (II) ions, was performed to assess the antioxidant activity. There are several techniques to estimate this parameter. Among them, FRAP methodology represents a fast, low-cost, and comparable in vitro method widely used for screening. Despite the abundance of phenolic and antioxidant compounds, only a few studies report the antioxidant capacity of Elaeagnus berries. The value obtained in this study (Table 1) is very close to the ones observed for similar berries with demonstrated health-promoting properties, such as cornelian cherry (Cornus mas L.) and jostaberry (Ribes x nidigrolaria Rud. Bauer & A. Bauer) at 20.41 mmol Fe2+/kg and 24.09 mmol Fe2+/kg, respectively [33,34], but lower than other common berries such as blackberry (Rubus ulmifolius L.) 79.17 mmol Fe2+/kg, highbush blueberry (Vaccinium corymbosum L.) 49.45 mmol Fe2+/kg, black currant (Ribes nigrum L.) 58.43 mmol Fe2+/kg, and white currant (Ribes rubrum L.) 85.97 mmol Fe2+/kg [35]. The results of nutraceutical compounds are in accordance with a previous study on Elaeagnus angustifolia (L.), member of the Elaeagnaceae family with berries similar to the E. umbellata species [31]. The FRAP value was highly correlated with TAC (r = 0.929) and flavonols content (r = 0.962), pointing out the main role of these compounds in antioxidant activities.

3.3. Organic Acids

Organic acids are located in the fleshy parts of fruits and can affect sensory properties and consumer acceptability. A study conducted on juices of six cultivars of sea buckthorn (Elaeagnus rhamnoides L.), a berry tree of the Elaeagnaceae family, found that titratable acidity had a positive correlation with the

intensity of sourness and astringency [35]. The quantity of organic acids can vary depending on many factors; for example, in a study on two blueberry species native to Turkey (Vaccinium arctostaphylos and V. myrtillus), the amount of malic, citric, and quinic acids changed significantly during different ripening stages [36]. Other factors include cultivar choice, soil, and climate conditions. Organic acids were the most represented secondary metabolites (1890 ± 182.02 mg/100 g FW) in E. umbellata berries. The compounds detected were citric, malic, oxalix, quinic, succinic, and tartaric acids (Table 2). Succinic was the primary organic acid (490.78 mg/100 g FW) accounting for about 25% of the total acid concentration, followed by quinic (468.83 mg/100 g FW) and citric (370.15 mg/100 g FW). Results are in accordance with previous studies on the autumn olive, where the main organic acids detected were malic, quinic, and citric acids, although in smaller quantities [5,6]. Among the acids, citric was the one with the higher correlation to titratable acidity (r = 0.932).

Citric Acid Malic Acid Oxalic Acid **Quinic Acid** Succinic Acid **Tartaric Acid** 117.77 Mean value 370.15 382.43 60.69 468.83 490.58 14.28 39.76 SD 15.65 6.34 18.20 22.48

Table 2. Organic acids composition of autumn olive berries.

The results are reported as mg/100 g FW (FW = fresh weight). Mean value and standard deviation are given for each sample (n = 3).

3.4. Phytochemical Composition

Phytochemicals are secondary metabolites produced by plants. These bioactive non-nutritive compounds may provide desirable health benefits to contrast the development of chronic diseases. Appl. Sci. 2020, 10, 4354 7 of 11

Indeed, the regular consumption of phytochemicals (antioxidant compounds) help to prevent or slow oxidative stress caused by free radicals [37]. There is currently little available research on the phytochemical composition of *E. umbellata* species.

Several factors are relevant in determining the composition of bioactive compounds, such as genetic and environmental factors. Among the environmental ones, soil composition, agronomic management, and weather are the most influential variables. Nevertheless, the composition can change due to oxidative reactions during processing and storage [38]. In addition, the analytical method is discriminatory.

The phytochemical fingerprint of autumn olive berries was carried out via HPLC-DAD and results on bioactive compounds are summarized in Table 3. The total bioactive compound content (TBCC), expressed as the sum of the most important biologically active molecules detected in the extracts and reported as mg/100~g of fresh weight, showed a mean value of $413.64 \pm 39.96~mg/100~g$ FW, considerably lower than commercial berries such as goji [39], but close to the value of cornelian cherry [33]. The analysis revealed the presence of 23 compounds, grouped in three different classes to evaluate each class contribution. Polyphenols, with a percentage of 65.56%, were the largest class, followed by monoterpenes (27.40%) and vitamin C (7.04%).

Table 3. Phytochemical fingerprint of autumn olive berries.

| Bioactive Class | Compound | Mean Value | SD |
|-----------------|----------------------|---------------|-------|
| Monoterpenes | limonene | 34.34 | 1.91 |
| | phellandrene | 8.33 | 0.07 |
| | sabinene | 26.16 | 0.03 |
| | γ-terpinene | 28.90 | 1.40 |
| | terpinolene | 15.59 | 0.03 |
| Vitamin C | ascorbic acid | 5.66 | 0.70 |
| | dehydroascorbic acid | 23.46 | 2.75 |
| Cinnamic acids | caffeic acid | 0.78 | 0.02 |
| | chlorogenic acid | 10.93 | 0.13 |
| | coumaric acid | 5.82 | 0.02 |
| | ferulic acid | 1.32 | 0.19 |
| Flavonols | hyperoside | 7.20 | 0.49 |
| | isoquercitrin | 0.10 | 0.03 |
| | quercetin | 8.95 | 0.72 |
| | quercitrin | 0.15 | 0.09 |
| | rutin | 0.76 | 0.08 |
| Benzoic acids | ellagic acid | 5.50 | 1.17 |
| | gallic acid | 0.16 | 0.12 |
| Catechins | catechin | 1.62 | 1.35 |
| | epicatechin | 3.38 | 0.99 |
| Tannins | castalagin | 5.00 | 1.07 |
| | vescalagin | 24.54 | 3.34 |
| Anthocyanins | - | 194.99 | 0.817 |

The results are reported as mg/100 g FW (FW = fresh weight). Mean value and standard deviation are given for each sample (n = 3).

Polyphenols are compounds derived from secondary plant metabolism, present at high levels in several edible species. They perform many essential roles in plant physiology and as health-promoting compounds. Their healthy properties in humans were first linked to their antioxidant activity. In the last few years, recent findings have challenged this view, through the investigation of more complex actions [40]. As discussed above, very little is known about the polyphenolic composition of autumn olive berries. Among polyphenols, anthocyanins represent the most important compounds (71.9%), followed by tannins (10.9%), cinnamic acids (6.9%), flavonols (6.3%), benzoic acids (2.1%),

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and catechins (1.8%). Recently, a strong interest has been paid to the anthocyanins class, which may be among the principal polyphenols responsible for health benefits [41]. Indeed, they show strong anti-oxidant activity that helps to prevent several diseases such as cardiovascular illnesses, diabetes, cancer, and inflammation [42].

Monoterpenes, a class of volatile chemicals found in many fruits, were the second class of bioactive compounds in terms of quantity detected (27.40% of TBCC). Among them, limonene was the most represented compound found in autumn olive berries (34.34 mg/100 g FW). Many studies had focused on the anticancer properties of limonene, highlighting the positive role of monoterpenes in fighting the course of breast cancer [43].

The amount of vitamin C in *E. umbellata* berries was assayed as the sum of ascorbic and dehydroascorbic acids, shown as the average value of 29.12 ± 3.46 mg/100 g FW. This was in line with the findings of a previous study [27], but rather higher than previous research on *E. umbellate* [4,7]. Considering the ascorbic acid, berries recorded significantly lower values (5.66 ± 0.70 mg/100 g FW) compared to other super fruits (strawberry 90.13 ± 2.24 mg/100 g FW, blueberry 73.21 ± 0.35 mg/100 g FW, blackberry 52.41 ± 11.31 mg/100 g FW) [28]. However, autumn olive represents a good source of vitamin C. The consumption of 100 g of berries may cover from 30% to 50% of the recommended daily intake (60-90 mg/d) [44].

4. Conclusions

The preliminary results of this work allowed to contribute to the knowledge of *E. umbellata* fruits, especially concerning their nutraceutical aspects. Little information is yet available on the properties of this underutilized fruit. In addition, several factors make it difficult to recognize the different species and genotypes of *Elaeagnus*.

Concerning the results of phytochemical traits, phenolic is the larger class, mainly represented by anthocyanins. Although no comparable values are recorded yet for this species, studies on the Russian olive ($Elaeagnus\ angustifolia\ L$.) confirm the high content of anthocyanins. The importance of anthocyanins as health-promoting compounds is also represented by the positive correlation between total anthocyanin content (TAC) and antioxidant capacity (r = 0.929). The total polyphenolic content (TPC) is higher if compared to other studies on the same species, and similar to the values recorded for other known superfruits such as red raspberry, blueberry, and cherry. The autumn olive berries also contain a high percentage of organic acids, monoterpenes, and vitamin C.

To conclude, this work contributes to highlighting the potential of autumn olive as a source of valuable bioactive compounds, both for fresh consumption and fruit-derived products. However, further studies on *E. umbellata* will be fundamental to improve the knowledge of important aspects such as phytochemical fingerprint, health benefits, and antioxidant activity.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/12/4354/s1, Table S1: Chromatographic conditions of the methods used, Table S2: Calibration curve equations; R², LOD, and LOQ of the used chromatographic methods for each calibration.

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