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Elderberry Stalks as a Source of High-Value Phytochemical: Essential Minerals and Lipophilic Compounds

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Abstract: Elderberry (*Sambucus nigra* L.) consumption has been growing in the last years, generating a large number of stalks (~10% of the berries bunch) that are still under-valorized. This study focused on the evaluation of elderberry stalks as a source of high-value phytochemicals. In this vein, the essential mineral content and lipophilic composition were analyzed for the first time. In addition, the polar fraction was evaluated regarding its total phenolic content (TPC) and antioxidant activity by both 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assays. The lipophilic fraction was mainly composed of triterpenic acids (2902.20 mg kg^{-1} of dry weight (dw)), fatty acids (711.73 mg kg^{-1} dw) and sterols (288.56 mg kg^{-1} dw). Minor amounts of long-chain aliphatic alcohols and other components were also detected. Ursolic acid (2265.83 mg kg^{-1} dw), hexadecanoic acid (219.85 mg kg^{-1} dw) and β-sitosterol (202.74 mg kg^{-1} dw) were the major lipophilic components verified. The results of this study also indicated that elderberry stalks might be used as a natural source of essential minerals, particularly calcium, iron and potassium, which are known to play important roles in various body functions. The analysis of the polar fraction also showed that elderberry stalks present TPC as high as elderberry themselves as well as considerable antioxidant activity (1.04 and 0.37 mmol TE g^{-1} of extract, against respectively ABTS and DPPH radicals). These results highlight the potential of elderberry stalks as a natural source of high-value phytochemicals that may be explored in several fields.

Keywords: *Sambucus nigra* L.; elderberry stalks; bioactive compounds; lipophilic compounds; ursolic acid; minerals; antioxidant activity

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1. Introduction

The production and consumption of fruits, mainly berries, grapes, apples and citrus, has been increasing in the last years, generating consequently a rising number of by-products. From a circular economy and a sustainable point of view, it is crucial to find ways to recycle fruit by-products, most of them considered as wastes and commonly discarded, which could be exploited in novel products or applications. Actually, a great effort has been devoted to the exploitation of agricultural and agri-industry by-products and wastes, on the one hand, valorizing them, for example, as a source of bioactive compounds for food or non-food applications, thus obtaining value from the whole biomass and, on the other hand, reducing the negative impacts of one of the major environmental problems of fruit processing industries [1,2].

In a processing plant, about one-third of the fruit will likely end up as pomace, which comprises peels, skin, pulp, seeds, leaves and stalks. In Europe alone, it is estimated that

these by-products and wastes can account for up to 1.3 billion tons per year [3,4]. These by-products have been studied as a possible source of bioactive compounds such as phenolic compounds, fatty acids, polysaccharides, and dietary fibers, among other health-promoting nutrients [1,2]. Pomaces have been reported to have, in some cases, higher amounts of these compounds of interest when compared with corresponding fruit juices themselves [1].

A growing trend in using agricultural and agri-industry by-products as a source of bioactive compounds has been observed lately [4,5], with a major interest in apple [6–10], grape [11–13], citrus [14], tomato [15–17] and olive [18,19] pomaces but also in other fruit by-products such as from almond [20], mango [21] or coffee [22] processing, just to name a few. Due to the high volume produced worldwide, grape by-products have been among one of the most studied, where stalks are also included. Grape stalks have been addressed as a promising source of phenolic compounds presenting antimicrobial activity [23]. Additionally, this lignocellulosic biomass, presenting a high content of cellulose (12–40%), hemicellulose (12–35%) and lignin (15–47%), has also been noted as a possible source to produce activated carbon [24] or fermentable sugars after hydrolysis [25–27], which could be used for bioethanol production, for instance [28]. The applications of the lignocellulosic fraction may be perfectly integrated with bioactive compounds valorization.

The consumption of healthy foods has been a trend in the past years, leading to increasing demand in the search for natural products. *Sambucus nigra* L. is a deciduous shrub whose berries and flowers have been used since ancient Rome in formulations for areas as diverse as folk medicine or food industries [29]. Elderberries, due to their high content in anthocyanins and polyphenols and their peculiar sensorial characteristics, are being used in jams, jellies, pies, syrups, beverages and also as concentrates and infusions [30,31]. Elderberries consumption has been associated with the prevention and therapy of a variety of diseases, such as cardiovascular diseases, diabetes, and obesity. Elderberries extracts have also shown promising antiviral, antibacterial, antifungal and antidepressant activities, as well as a significant impact on the immune system [32,33].

The plantation of *S. nigra* has been growing since the 1980s throughout all Europe, though an increased interest has been noticed in the last 20 years [31] and an elderberries production of 1500–2000 tons per year is estimated in Portugal alone, specifically in the Varosa Valley, located in northern Portugal [34].

Elderberries industrial processing originates by-products, with elderberries stalks the most abundant, accounting for more than 10% of the initial elderberries weight [34]. Currently, these stalks, as most food by-products [4], are disposed of, used for composting, or used for heating purposes [34]. Either way, these by-products are associated with costs for the companies, which have to pay a third-party company to get rid of these residues. Even though these by-products are not environmentally hazardous, as they are all harvested in a particular period of the year, they could pose some potential environmental problems, such as alteration of the chemical composition of the soils, if used for composting, or in the case where they are accumulated in industrial plants, apart from occupying space, they usually attract insects and rodents [24,35].

Elderberry stalks acquire a purplish color in the late stages of elderberries ripening, suggesting the presence of high levels of anthocyanins, which are also responsible for the color of ripe berries [29,36]. Characterization of the phenolic profile of elderberry stalks was performed by Silva et al. [34], who showed that their composition is quite similar to elderberries, with both presenting cinnamic acid derivatives, anthocyanins, and flavonols, but in different amounts. However, there is still a lack of information regarding the lipophilic composition of this by-product. Actually, the lipophilic fraction of elderberries showed to be composed of a variety of interesting families of compounds, such as fatty acids, long-chain aliphatic alcohols, sterols, and particularly triterpenic acids [37]. Thus, the exploitation of this fraction of elderberry stalks could be a valuable strategy for the valorization of this by-product. In addition, it becomes crucial, from a circular economy point of view, to evaluate the potential of by-products with an integrated perspective, thus integrating the valorization of this fraction with that of phenolic and lignocellulosic

fractions. In this vein, a lipophilic fraction of elderberry stalks were characterized for the first time by gas chromatography–mass spectrometry (GC–MS). In addition, the polar fraction was evaluated regarding its phenolic content and antioxidant activity, and the stalks mineral content was determined to also evaluate their possible use as a food additive or dietary complement.

2. Materials and Methods

2.1. Chemicals

Dichloromethane (99%), gallic acid (purity higher than 97.5%), Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were supplied by Sigma Chemical Co (Madrid, Spain). Methanol (>99.8%) was purchased from FlukaChemie (Madrid, Spain). N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) (99% purity), trimethylchlorosilane (≥99% purity), ursolic acid, stigmasterol, hexadecanoic acid, nonadecan-1-ol, tetracosane (≥99% purity), potassium persulfate and Trolox were supplied by Fluka Chemie or Sigma Chemical Co. (Madrid, Spain). Calcium carbonate and sulfuric acid (≥96% purity) were purchased from Merck KGaA (Darmstadt, Germany).

2.2. Raw Materials

Elderberries (*S. nigra* L.) from the 3 cultivars ('Sabugueira', 'Sabugueiro' and 'Bastardeira') mainly cultivated in the Varosa Valley (Portugal) were collected at Vila Pouca de Salzedas (41°04′11.9″ N 7°45′00.8″ W) cultivation field, during the first week of August of 2017 and transported under refrigeration (ca. 4 °C) to the laboratory. It was prepared a composite sample (ca. 10 kg) containing a mixture from the 3 cultivars, in similar proportions, simulating what happens during the collection of the berries to be delivered to suppliers, in which there is no separation of cultivars. Thus, this composite sample is representative of the stalks wastes resultant from the elderberries processing, which was stored at −20 °C until further analysis.

Before analysis, the berries were detached from stalks, which were then washed with water to remove any remaining juice. Stalks were freeze-dried using VirTis BenchTop K (SP Industries, Warminster, PA, USA) and ground in a mill (Ika A10, Staufen, Germany) prior to extraction. Freeze-drying is based on the dehydration by sublimation of a frozen product. This procedure was selected for drying stalks before analysis, as due to the absence of liquid water and the low temperatures required for the process, it preserves the materials from physico–chemical and microbiological deteriorations [38]. To determine the water content, three samples of biomass were dried at 105 °C until reaching a constant weight. The water content of elderberry stalks was 51.72 ± 4.35%.

From freeze-dried stalks, as shown in the workflow in Figure 1, the mineral content was determined, and extractions were performed to study the lipophilic profile and to evaluate the polar fraction regarding its total phenolic content through the Folin–Ciocalteu method and the antioxidant activity through ABTS and DPPH assays.

2.3. Minerals Determination

Trace element analysis was performed based on previously established methodologies in which nearly 100 mg of the freeze-dried sample was digested with 3 mL of HNO_3 at 160 °C in the Microwave Digestion System (MARS 5, CEM, Montvale, NJ, USA). Ca, Fe, K, Mg, Na and Zn, and Pb concentrations were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Horiba Jobyn Yvon, model: Activa M, Orange, NJ, USA). Calibration curves were made for mineral quantification. Analysis was performed in triplicate, and the results were provided in mg for 100 g dry weight (dw).

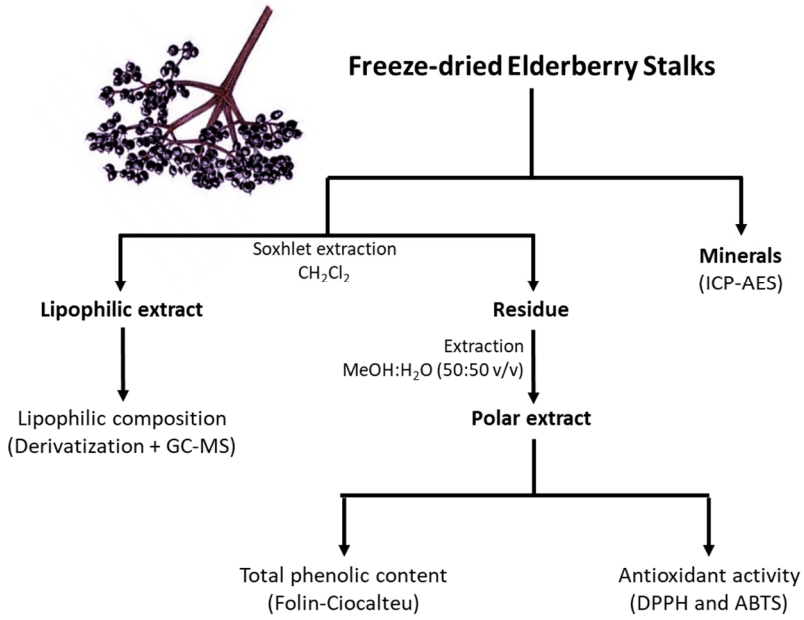

Figure 1. Scheme of the workflow followed in this work.

2.4. Lipophylic Fraction Characterization

2.4.1. Extracts Preparation

Freeze-dried elderberries stalks (ca. 2.6 g) were Soxhlet extracted using ca. 150 mL of dichloromethane for 8 h. The solvent was removed by low-pressure evaporation at 35 °C, and the lipophilic extract was weighed. Extraction was performed in triplicate, and the results were expressed as a percent of dry weight (% dw).

2.4.2. Analysis by GC–MS

Before GC–MS analysis, nearly 20 mg of extract was converted into trimethylsilyl (TMS) derivatives according to previously optimized methodology [39]. GC–MS analysis was performed using a Trace Gas Chromatograph 2000 Series equipped with a Thermo Scientific DSQ II mass spectrometer (Shimadzu, Kyoto, Japan) using helium as carrier gas (35 cm/s) equipped with a DB-1 J&W capillary column (30 m × 0.32 mm i.d., 0.25 m film thickness, Clara, CA, USA). The chromatographic conditions were as follows: initial temperature 80 °C for 5 min, temperature rate of 4 °C/min up to 260 °C, and 2 °C/min until the final temperature 285 °C, then maintained at 285 °C for 13 min, injector temperature of 250 °C; transfer-line temperature of 290 °C, split ratio: 1:50. The MS was operated in the electron impact mode with an electron impact energy of 70 eV and data collected at a rate of 1 scan/s over a range of *m/z* 33–700. The ion source was maintained at 250 °C. Compounds were identified as TMS derivatives by comparing their mass spectra with the GC–MS spectral library (Wiley-NIST Mass Spectral Library 1999), with literature MS fragmentation and also by injection of standards. Lipophilic compounds were quantified by their peak areas, being GC–MS calibrated with pure reference compounds, representative of each family, namely hexadecanoic acid for fatty acids, nonadecanol for alcohols, stigmasterol for phytosterols, and ursolic acid for triterpenic compounds, relative to tetracosane (the internal standard). The respective response factors were calculated as the average of six GC−MS runs. Three aliquots of the lipophilic extract were injected in duplicate, and the results represent the average of the concordant values obtained for the six runs (n = 6, 3 extracts, obtained from 3 independent extractions, each extract was injected in duplicate).

2.5. Polar Fraction Characterization

2.5.1. Extracts Preparation

After Soxhlet extraction, the resulting lipophilic-free residue (ca. 0.5 g) was suspended (1:100 *w/v*) in a methanol and water mixture (MeOH:H_2O, 50:50 *v/v*) for 24 h at room temperature under constant stirring, based on previous publications [40]. The suspension was then filtered, the methanol was removed by low-pressure evaporation, and the extracts were freeze-dried. The dried polar extracts were weighted, and results were expressed as % dw. Extractions were performed in triplicate.

2.5.2. Total Phenolic Content

Total phenolic content (TPC) of stalks polar fraction was determined using Folin–Ciocalteu's reagent. Briefly, the polar extract was suspended in MeOH:H_2O (50:50 *v/v*) in concentrations ranging between 125–500 μg mL^{-1}. Then, 30 μL of these extract solutions were mixed with 150 μL of Folin–Ciocalteau's reagent previously diluted with water (1:10, *v/v*). After 10 min, 120 μL of Na_2CO_3 (7.5% *w/v*) was added, and the solution was left to stand for 25 min before the absorbance was measured at 760 nm using a UV/Vis V-530 spectrophotometer (Jasco, Tokyo, Japan). Gallic acid (25 to 300 μg mL^{-1}) was used as the standard for constructing the calibration curve, and the results were expressed as g of gallic acid equivalents (GAE) per 100 g of extract. All determinations were performed in triplicate.

2.5.3. Antioxidant Activity Determination

ABTS Radical Scavenging

The ABTS assay is based on the scavenging of the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation, $ABTS^{+\bullet}$ converting it into a colorless product. In this methodology, $ABTS^{+\bullet}$ cation was previously prepared to react 10 mL of ABTS 7 mM solution with potassium persulfate 2.45 mM, following a methodology described before with minor modifications [40]. This mixture was then incubated in the dark for 16 h, at room temperature. Before usage, the $ABTS^{+\bullet}$ solution was diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Briefly, 30 μL of extracts (with concentrations ranging between 150 and 348 μg mL^{-1}) or Trolox, used as standard (50–400 μg mL^{-1}), were mixed with 3 mL of $ABTS^{+\bullet}$ solution. The absorbance was measured after 6 min at 734 nm (Shimadzu UV-1800 spectrophotometer, Kyoto, Japan), and the results were expressed as mmol TE g^{-1} dw. Triplicate measurements were performed.

DPPH Radical Scavenging

Antioxidant activity of elderberry stalks' polar fraction was assessed using the stable free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) to measure their hydrogen-donating or radical scavenging ability, following an adaption of the methodology described previously [39] for 96-well plates. Briefly, 9.6 μL of extract (with concentrations ranging between 120 and 190 μg mL^{-1}) was added to a 96-well plate containing 29 μL of DPPH radical solution (0.8 mM in methanol) and 192 μL of methanol. The mixture was shaken vigorously and left to stand for 20 min in the dark at room temperature. The absorbance was then measured at 517 nm in the microplate reader (Biotek Eon microplate spectrophotometer). Trolox calibration curve (50–800 μg mL^{-1}) was prepared in methanol. The radical scavenging results were expressed as mmol Trolox equivalents (TE) per g of dw. All determinations were performed in triplicate.

3. Results and Discussion

Elderberry stalks were comprehensively evaluated concerning their mineral content, as well as lipophilic and polar fractions composition.

3.1. Elderberry Stalks Mineral Composition

The essential mineral content of elderberry stalks, assessed by ICP-AES, is listed on Table 1. The dietary reference intake (DRI) recommended dosage per day is also presented for comparative purposes.

Table 1. Elderberry stalks' essential minerals content and their respective dietary reference intakes (DRIs).

Mineral	Concentration (mg/100 g dw)	DRI (mg) [41]
Ca	497 ± 5	800
Fe	6.5 ± 0.1	14
K	2000 ± 200	2000
Mg	110 ± 11	375
Na	14 ± 1.4	6000
Zn	2.53 ± 0.05	10

Results represent the mean ± standard deviation (n = 3).

Except for sodium, the minerals content of elderberry stalks ranged from 25 to 100% of the DRI, suggesting that elderberry stalks could be used as a natural source of these essential minerals. In fact, the ingestion of a portion of 100 g (dw) would contribute to the dietary reference intake of minerals such as calcium, iron, potassium, magnesium and zinc [41]. These minerals are associated with the body function, for instance, in hemoglobin production (iron), in keeping the blood pressure stable (calcium) and in the regulation of the osmotic balance and muscles activity (potassium). Elderberry stalks showed similar contents of Mg when compared with stalks from different grape cultivars, while the other minerals were present in lower amounts [42].

The ICP-AES analysis was also used to search for lead due to its possible toxicity [43], and the results allowed to verify the lead content on elderberry stalks was detected with a concentration of <0.1 mg 100 g^{-1} dw (limit of detection of the methodology). Although, for these samples, the lead content does not represent a concern, it is important to control this element in this type of waste as its content is related to several parameters associated with environmental issues, agricultural practices, among others.

3.2. Characterization of The Composition of the Elderberry Stalks Lipophilic Fraction

Elderberry stalks presented a dichloromethane extraction yield of 2.2% dw. The lipophilic extract of elderberry stalks was studied in detail by GC–MS analysis. Lipophilic compounds were identified as their trimethylsilyl derivatives after the derivatization of the lipophilic extract. The identification of the main lipophilic compounds and their quantification is summarized in Table 2. Nineteen compounds, particularly fatty acids, long-chain aliphatic alcohols, sterols, triterpenic compounds and minor amounts of other compounds, were identified when comparing the fragmentation profile of the TMS derivatives both with libraries and other studies as well as the injection of standards [44–48]. In general, the lipophilic extract was mainly composed of triterpenic acids, accounting for 70.4% of all identified lipophilic compounds, followed by fatty acids with 17.3%, phytosterols with 7.0%, long-chain aliphatic alcohols with 1.2% and lately others with 4.1%.

Table 2. Lipophilic compounds determined in elderberry stalk dichloromethane extract expressed in mg g^{-1} of extract and mg kg^{-1} dw.

Retention Time (min)	Compound	mg g^{-1} Extract	mg kg^{-1} dw
	FATTY ACIDS	32.50	711.73
	Saturated fatty acids		
6.3	Hexanoic acid	0.44 ± 0.12	9.57 ± 2.64
29.5	Nonanoic acid	0.43 ± 0.05	9.36 ± 1.03
30.9	Tetradecanoic acid	0.40 ± 0.03	8.72 ± 0.65
35.9	Hexadecanoic acid	10.04 ± 0.64	219.85 ± 14.00
38.2	Heptadecanoic acid	0.25 ± 0.03	5.55 ± 0.76
40.4	Octadecanoic acid	3.89 ± 0.19	85.24 ± 4.22
48.4	Docosanoic acid	0.90 ± 0.04	19.72 ± 0.92
52.1	Tetracosanoic acid	1.42 ± 0.07	31.08 ± 1.43
60.6	Octacosanoic acid	2.29 ± 0.21	50.18 ± 4.67
65.7	Triacontanoic acid	6.94 ± 0.80	151.98 ± 17.55
	Unsaturated fatty acids		
39.5	Octadeca-9,12-dienoic acid	2.76 ± 1.48	60.38 ± 32.37
39.6	Octadeca-9,12,15-trienoic acid	1.53 ± 1.03	33.60 ± 22.45
39.7	Octadeca-9-enoic acid	1.21 ± 0.72	26.50 ± 15.71
	LONG-CHAIN ALIPHATIC ALCOHOLS	2.29	50.14
58.7	Octacosanol	2.29 ± 0.12	50.14 ± 2.68
	STEROLS	13.18	288.56
60.8	Campesterol	1.85 ± 0.45	40.46 ± 9.88
61.5	Stigmasterol	2.07 ± 0.49	45.36 ± 10.72
62.9	β-sitosterol	9.26 ± 2.25	202.74 ± 49.26
	TRITERPENIC COMPOUNDS	132.52	2902.20
69.4	Oleanolic acid	29.06 ± 2.10	636.37 ± 45.98
71.1	Ursolic acid	103.46 ± 6.84	2265.83 ± 149.79
	OTHERS	7.77	170.22
14.3	Glycerol	7.12 ± 2.22	155.99 ± 48.64
30.3	Benzene—1,2-dicarboxylic acid	0.65 ± 0.20	14.23 ± 4.39

Results represent the mean ± standard deviation (n = 6).

Dichloromethane extract of elderberry stalks showed to be mainly composed of triterpenic acids (132.52 mg g^{-1} extract), accounting for 2902.20 mg kg^{-1} dw. Ursolic (2265.83 mg kg^{-1} dw) and oleanolic acids (636.37 mg kg^{-1} dw) were the only triterpenic compounds detected. A vast range of biological activities and health benefits have been addressed to these compounds. Ursolic acid is well-known for its antioxidant activity, antiviral, antidepressant, anti-inflammatory, antiarthritic and anti-ulcerous properties, apart from the demonstrated effective properties against cancer and as hepatoprotective [49]. Oleanolic acid, has also shown worthy hepatoprotective and antioxidant effects and has also been reported to have anti-cancer and anti-inflammatory activities [50].

Fatty acids, both saturated and unsaturated, were the second most abundant family of compounds detected in the lipophilic fraction of elderberry stalks. Palmitic acid (hexadecenoic acid) (219.85 mg kg^{-1} dw) was the most abundant saturated fatty acid, followed by triacontanoic (151.98 mg kg^{-1} dw) and octadecanoic acids (85.24 mg kg^{-1} dw). On the other hand, octadeca-9,12-dienoic (60.38 mg kg^{-1} dw) and octadeca-9,12,15-trienoic acids (33.60 mg kg^{-1} dw), which are omega-6 and omega-3, respectively, were the most abundant unsaturated fatty acids detected. These are associated with aiding to reduce triacylglycerol levels and have been pointed as beneficial in the prevention and treatment of cardiovascular diseases [51].

B-sitosterol (202.74 mg kg^{-1} dw) was the major sterol identified, followed by stigmasterol (45.36 mg kg^{-1} dw). These compounds have been reported as lowering agents of cholesterol levels [52,53] and β-sitosterol has also been shown to attenuate hepatotoxicity and cardiotoxicity [54].

As previously verified for the phenolic composition [34], the lipophilic profile of elderberry stalks is very similar to that of elderberries [37], and their content is within the range presented for elderberries in terms of triterpenic acids and phytosterols. Interestingly, elderberry stalks showed nearly two-fold the content of fatty acids present in elderberries. The number of fatty acids on elderberry stalks is similar to that verified previously in grape stalks [55], with hexadecanoic acid the most abundant fatty acid in both by-products. However, a higher abundance of triterpenic compounds was verified in elderberry stalks when compared to that reported for grape stalks, highlighting the potential of this by-product.

The analysis of lipophilic fraction of elderberry stalks suggests that this by-product could be therefore pointed out as a promising base material to be incorporated in cosmetics, nutraceutical, or pharmaceutical industries due to their high content on triterpenic acids, namely ursolic and oleanolic acids, which have been widely used in formulations for cosmetics and pharmaceuticals [56,57]. Similarly, phytosterols such as β-sitosterol and stigmasterol have been generally used in pharmaceuticals (therapeutic steroids manufacture), cosmetic (creams and lipsticks) and nutraceuticals (anti-cholesterol additives in functional foods) [58], as well as fatty acids, which are an important constituent of cosmetics and personal care products [59] and also have a vital rule, considering the omega-6/omega-3 ratio, in the human diet. However, such exploitation requires more detailed studies, such as evaluating their side effects and toxicity, as well as finding a sustainable and environmentally friendly extraction methodology.

3.3. Polar Extract Characterization

The methanol:water (50:50) extraction yield obtained from the lipophilic-free residue of elderberry stalks was 34.4% dw. This value is considerably higher than that previously reported for grape stalks residue (although using methanol:water (75:25) as extraction media) after dichloromethane extraction (6.4%) [35].

The TPC of elderberry stalks polar fraction was 0.83 g $\pm$ 0.21 GAE/100 g fw, or, in a dry weight basis, 1.7 $\pm$ 0.42 g GAE 100 g^{-1} dw. This content is in the range of that already obtained for elderberry stalks (0.71 GAE 100 g^{-1} fw) [34] and is also within the values known for elderberries (0.36–1.95 g GAE 100 g^{-1} fw) [60–62]. Furthermore, the TPC obtained is very similar to those verified previously in similar matrices such as grape stalks, which presented 1.6 g GAE 100 g^{-1} dw for Chardonnay and 2.0 g GAE 100 g^{-1} dw for Müller-Thurgau varieties [55].

The polar extract was also evaluated for its radical scavenging activity by both ABTS and DPPH assays. The activities, expressed as Trolox equivalents accounted, respectively for 1.04 $\pm$ 0.14 mmol TE g^{-1} extract and 0.37 $\pm$ 0.08 mmol TE g^{-1} extract, which are slightly lower values than those obtained previously for elderberries, 1.74–2.20 mmol TE g^{-1} extract and 0.62–0.89 mmol TE g^{-1} extract [63], against, respectively, ABTS and DPPH radicals. However, this is an expected difference since most of the antioxidant compounds are supposed to be stored in berries. Our elderberry stalks showed slightly higher antioxidant activity (17.31 mmol TE 100 g^{-1} fw) when compared with the value obtained by Silva et al. [34] for elderberry stalks methanol extract (10.7 mmol TE 100 g^{-1} fw).

Elderberry stalks, as elderberries or grape stalks, have a high amount of individual phenolic compounds, most predominantly anthocyanins that are commonly used as colorants in food and are well known for their antioxidant properties [31,34]. Moreover, elderberry stalks show a total phenolic content and antioxidant activities similar to elderberries and to grape stalks, which demonstrates the potential of this by-product to be valorized.

4. Conclusions

This study shows that elderberries stalks, usually known as lignocellulosic biomass, may be considered as a natural source of bioactive compounds, notably composed of phenolic (ca. 34.4% dw), lipophilic compounds (ca. 2.2% dw) and essential minerals (ca. 2.6% dw) that are actively looked for by industry investing in functional foods and health-promoting products. Elderberry stalks may be used as a natural source of essential minerals, particularly calcium, iron, and potassium, which are known to play important roles in the body function. The GC–MS analysis of the lipophilic fraction of this by-product showed that this fraction is mainly composed of triterpenic acids, particularly ursolic acid (2265.83 mg kg^{-1} dw), followed by fatty acids, in particular hexadecanoic acid (219.85 mg kg^{-1} dw) and sterols, being β-sitosterol (202.74 mg kg^{-1} dw) the major components of this family. The analysis of the polar fraction also showed that elderberry stalks present TPC as high as elderberry themselves. These results highlight the potential of this residue to be exploited in an integrated strategy, valorizing their different components in high-value areas, such as nutraceutical, cosmetic or pharmaceutical ones.

Being known the composition and potential of this residue for high-value applications, the next step must be the development of sustainable and environmentally friendly extraction procedures involving green and/or natural extraction vehicles as natural deep eutectic solvents, properly designed for this purpose.

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