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Towards Circularity in Agriculture: A Case of Bioactive Compound Recovery from Sea Buckthorn Residual Leaves and Twigs

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Abstract: In recent years, agricultural by-products have become more valued due to the growing emphasis on sustainable agriculture and circular economies. This study concentrated on sea buckthorn (*Hippophae rhamnoides*) by utilizing its residual leaves and twigs—traditionally discarded or composted—as a source of high-value bioactive compounds. Aqueous and alcohol extraction was applied to sea buckthorn biomass using varying cycle numbers and extraction durations. The resulting extracts were analyzed for total phenolic content (TPC), proanthocyanidins (PACs), and antioxidant activity. The findings revealed that leaf extracts tended to have higher TPC, with the peak value in Leaves extract_4 (61.22 ± 0.26 mg_{GAE}/g), whose extraction was performed using 60 cycles of 10 min duration. Sea buckthorn twig water extracts demonstrated higher concentrations of PACs compared to leaf extracts, with the highest value observed in Twigs extract_5 (52.79 ± 0.21 mg_{CE}/g) whose extraction was performed using 60 cycles of 5 min duration. The antioxidant activity assessed via DPPH revealed significant functional potential, with sea buckthorn leaf extracts (aqueous and ethanolic) showing 82–90% activity, and twig extracts 77–90%. Environmental impacts of the tested extraction scenarios, assessed using a life-cycle assessment methodology, confirmed that extraction scenarios yielding higher concentrations of bioactive compounds from the same biomass were associated with lower environmental burdens.

Keywords: bioactive compounds; circular agriculture; environmental impact; sea buckthorn; solid–liquid extraction

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1. Introduction

The advancement of circular agriculture is increasingly recognized as a crucial strategy for promoting sustainability within agricultural systems. This shift from linear pro-duction models to circular ones seeks to enhance resource efficiency and minimize waste [1,2]. The transition towards circular agriculture emphasizes the need for sustainable practices that enhance agricultural productivity while minimizing waste and promoting the reuse of resources. Researchers have emphasized the importance of integrating innovative technologies and sustainable practices into agricultural systems to facilitate this transition [3]. One promising approach in this respect is the recovery of bioactive compounds from by-products of agricultural systems, particularly the leaves and twigs of sea buckthorn (*Hippophae rhamnoides*). Sea buckthorn is native to regions of

Europe and Asia and thrives in temperate climates where it has been cultivated for centuries [4]. The estimated global wild and cultivated area of sea buckthorn is approximately 3 million hectares, as it has gained attention for its ability to grow in harsh environments and its role in soil conservation and ecosystem restoration [4,5]. The wide profile of biologically active compounds in this plant gives it a high potential.

Sea buckthorn leaves contain various bioactive substances including flavonoids, phenolic compounds which are known for their antioxidant, anti-inflammatory, and antimicrobial properties [6]. Research has demonstrated that extracts from both the leaves and twigs of sea buckthorn can exhibit remarkable health benefits. For instance, Skalski et al. clarified the antiplatelet effects of phenolic extracts derived from sea buckthorn leaves and twigs, attributing these effects to their high content of phenolic compounds [7]. Further investigations revealed the anticancer and antioxidant properties associated with phenolic extracts from these plant parts, suggesting their potential as functional ingredients in food and pharmaceuticals [8]. Moreover, sea buckthorn leaves and twigs have a rich nutrient profile, particularly high in essential minerals like iron, highlighting their potential as effective dietary supplements [8].

Generally, sea buckthorn berries are valued more highly than leaves or twigs, but recent studies show that sea buckthorn leaves and twigs have a rich phytochemical profile, indicating a potential for their use in the production of nutrients and functional foods [9–11]. Janceva et al. [12] provide insights into how sea buckthorn waste biomass can be valorized into valuable bioactive compounds, thereby pointing to the capacity of specific plant materials to contribute to the circular economy through the recovery and repurposing of agricultural waste. In addition, there is scientific research that suggests that separating biologically active compounds from the twigs of the sea buckthorn accelerates their composting [13]. All of these insights can provide a double benefit when developing a closed-loop model.

Earlier studies have mainly examined fresh sea buckthorn leaves using techniques such as organic solvent extraction, ultrasound, or microwave-assisted methods to isolate or enhance phenolic content [14–18]. While these methods are effective in maximizing bioactive compound yields, they often involve the use of hazardous solvents, high energy consumption, and complex equipment, which can limit their sustainability and practical applicability on a larger scale. Consequently, the focus is now shifting toward more sustainable approaches that balance extraction efficiency with environmental and economic considerations. Aqueous extraction methods, which mimic real-life applications such as infusions or extracts, remain underexplored, despite their environmental friendliness and practical relevance. These water-based techniques use safer solvents, generally require less energy, and align well with consumer preferences for natural and “green” products. Solid–liquid extraction using green solvents offers a promising approach for recovering bioactive substances from sea buckthorn biomass. Although this method has been more commonly applied to berries and pomace [19], its adaptation to residual leaf and twig materials could significantly enhance valorization efforts. Innovative techniques that leverage pressure gradients for compound extraction, while still scarce in the context of sea buckthorn leaves or twigs, hold potential for increasing extraction efficiency without compromising compound stability [20]. Such advancements support the broader goal of transforming agricultural by-products into high-value ingredients, aligning with sustainable resource use and circular-economy principles.

Overall, the literature highlights a clear consensus on the important role of circular practices in fostering innovation, reducing environmental impact, and promoting sustainable productivity in agriculture, particularly through the valorization of agricultural by-products such as those derived from sea buckthorn. Despite increasing recognition of sea buckthorn’s phytochemical potential, much of its biomass—especially residual leaves and twigs—remains underutilized. This research addresses this gap by exploring the extraction of bioactive compounds from residual leaves and twigs, aiming to shift their fate from disposal or composting, to functional reuse. The study also included a comparison of extractions based on their environmental impact. This approach not only enhances resource efficiency, but also demonstrates a scalable strategy for integrating circularity into perennial crop systems.

2. Materials and Methods

The study was implemented in two main steps: extracting bioactive compounds from sea buckthorn leaves and twigs, and then comparing the environmental impact of the extractions.

2.1. Extraction of Bioactive Compounds from Leaves and Twigs

2.1.1. Materials

Sea buckthorn (*Hippophae rhamnoides*) leaves and twigs remaining after separating the sea buckthorn berries were obtained from the local production farm ("Amberry" farm, identification code: 1365579, Venciūnai, Lithuania). Before extraction, the leaves and twigs were crushed by Universal dry product shredder MLVS-M1000 (MLVS, China) (particle diameter 100–500 μm) and dried at room temperature (d.m. 94%). All other reagents were of analytical grade and were used as received, without further purification.

2.1.2. Proximate Composition Analysis of Sea Buckthorn Biomass

Moisture content for sea buckthorn leaves and twigs was determined according to the Association of Official Analytical Collaboration (AOAC) method 925.10-1925 [21]. Crude protein content was determined by the Kjeldahl method (N × 6.25), according to the AOAC 978.04 [21]. Total ash content was determined according to the AOAC Method 930.05 [21]. Crude fat and fatty acids content was determined according to ISO:12966-2:2017 [22]. Dietary fiber content (soluble and insoluble) was determined according to the American Association of Cereal Chemists (AACC) method 32-07.01 [23] and the AOAC method 991.43 [23], using a Total Dietary Fiber assay kit (K-TDFR-200A Megazyme Int., Wicklow, Ireland). Total carbohydrate content, sugar (glucose, fructose and saccharose) content measurements were performed using Sucrose/D-Fructose/D-Glucose Assay Kit Megazyme (K-SUFRG Megazyme Int., Wicklow, Ireland) according to the manufacturer's instructions. Microelement (calcium, potassium and magnesium) content was determined according to European Standard: EN 15621:2017[24].

2.1.3. Preparation of Sea Buckthorn Biomass Extracts

Sea buckthorn leaves or twigs (200 g) were placed in a porous bag and then placed in the extraction chamber of a Naviglio Extractor (mod. 1 L capacity, Nuova Estrazione S.a.s., Naples, Italy) [20], and 1 L of distilled water or ethanol (biomass/solvent: 1:5) was added. Sea buckthorn biomass extract was obtained by extraction for 20–60 cycles of 2–10 min each, at room temperature and 8.5 Bar pressure. In total, the extraction took 2 to 12 h (2 min in the static phase and 2 min in the dynamic phase) to obtain the extracts. The extraction conditions of sea buckthorn leaves and twigs are systematized and presented in Tables 1 and 2. The extracts were stored in a freezer at −80 °C degrees until the analysis of biologically active compounds.

Table 1. Preparation conditions of sea buckthorn biomass aqueous extracts.

Sample	Extraction Cycles	Cycle Duration, min
Leaves extract_1 Twigs extract_1	20	10
Leaves extract_2 Twigs extract_2	20	5
Leaves extract_3 Twigs extract_3	20	2
Leaves extract_4 Twigs extract_4	60	10
Leaves extract_5 Twigs extract_5	60	5
Leaves extract_6 Twigs extract_6	60	2

Table 2. Preparation conditions of sea buckthorn biomass ethanol extracts.

Sample	Extraction Cycles	Cycle Duration, min	Ethanol conc., %
Leaves extract_4-1 Twigs extract_4-1	60	10	70
Leaves extract_4-2 Twigs extract_4-2	60	10	60
Leaves extract_4-3 Twigs extract_4-3	60	10	50

2.1.4. Determination of Proanthocyanidins (PACs) by the Bate-Smith Assay

The PACs concentration was determined using the methodology proposed by Bate-Smith [25,26]. Each sea buckthorn biomass extract sample was diluted at a ratio of 1/50 (*v*/*v*) with deionized water or ethanol. A total of 4 mL of the diluted sample, 2 mL of deionized water and 6 mL of hydrochloric acid were added into two separate test tubes. One test tube (reaction tube) was placed in a water bath at 100 °C for 30 min, and the other test tube (blank tube) was left to stand in the dark for the same time. After 30 min, 1 mL of ethanol was added to each tube, and the tubes were left in the dark until the heated reaction tube has cooled. The absorbance of each test tube was measured in a spectrophotometer at 550 nm using deionized water as the blank. The absorbance difference was multiplied by the factor 19.33, and the concentration of PACs was expressed in mg (+)-catechin equivalent (CE)/g of dry weight of sea buckthorn biomass.

2.1.5. Total Phenolic Content (TPC) by Folin−Ciocalteu Assay

The TPC was determined by the Folin−Ciocalteu assay, as previously described by Singleton et al. (1999), with same modifications [27]. Sea buckthorn biomass extract solution was mixed with 5 mL Folin−Ciocalteu reagent (1:9 *v*/*v*) and after 5 min 4 mL 7.5% (*w*/*v*) sodium carbonate solution was added. The absorption was measured at 765 nm after 2 h using spectrophotometer Cary 60 (Agilent Technologies, Santa Clara, California, USA). The TPC was expressed in mg of gallic acid equivalent (GAE)/g of dry weight of sea buckthorn biomass.

2.1.6. Assessment of Antioxidant Activity

The assessment of extract antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Brand-Williams et al. (1995) [28]. Briefly, 0.5 mL of extract sample was mixed with 1.5 mL of 0.004% of DPPH solution (dissolved in ethanol) and incubated for 30 min at room (approximately 25 °C) temperature, in darkness. The DPPH radical scavenging activity was estimated using Equation (1).

$$\text{DPPH radical scavenging activity (\%)} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100 \quad (1)$$

2.1.7. Statistical Analyses

All experiments were performed at least in triplicate. Average values and standard deviations were calculated using MS Excel 2019 (Microsoft Corp, Albuquerque, NM, USA), while statistical analysis was performed using the Statgraphics Centurion 19 statistical package. One-way analysis of variation (ANOVA), followed by Tukey's honest significant difference (HSD) test was performed to determine significant differences ($p \leq 0.05$).

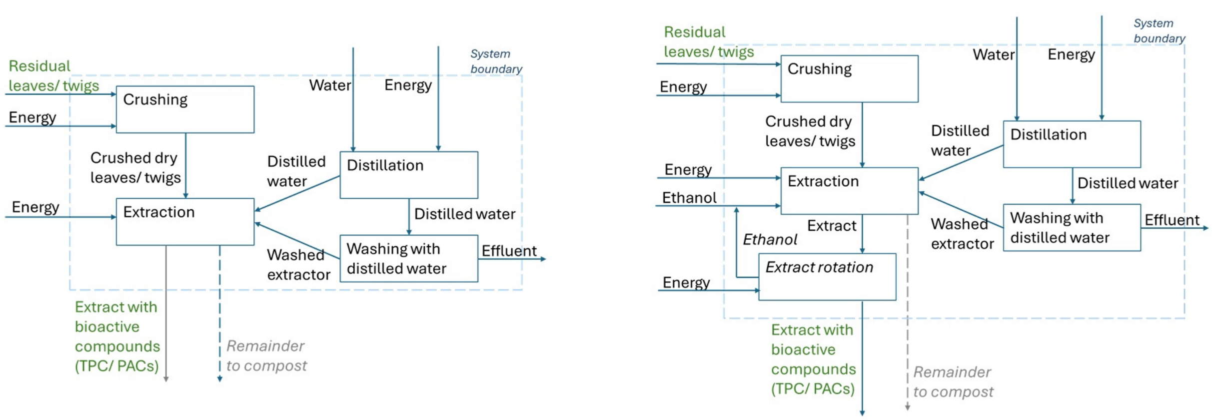
2.2. Environmental Impact Assessment of the Performed Extractions

In order to identify the extraction that causes the least environmental impact, a life-cycle assessment (LCA) methodology, described in standards ISO 14040 and ISO 14044, was used [29,30]. In accordance with the standards, the LCA analyses were performed following these steps:

- Definition of the goal and scope of the study, and identification of a functional unit and system boundaries;
- Life-cycle inventory analysis;
- Life-cycle impact assessment and interpretation.

The main goal was to determine which extraction scenario causes the least negative environmental impact, as well as to find out what, i.e., which processes, materials or reagents, contribute to the impact the most.

System boundaries are presented in Figure 1. The analyzed processes start with crushing of leaves and twigs. These are the residues from the processing of the sea buckthorn harvest, which would otherwise be sent directly to composting or even discarded. The processes leading up to the generation of these residues are not included, as they are part of the normal sea buckthorn cultivation and processing, common to all the extraction scenarios we analyzed. It is from the crushing that the new extraction-related processes of interest to us are introduced.



(**a**) (**b**)

Figure 1. Scheme of processes and flows of the assessed extractions: (**a**) aqueous, (**b**) ethanolic.

Details of the preparation of the extracts are described in Section 2.1.3, "Preparation of sea buckthorn biomass extracts", and the scenarios assessed correspond to preparation conditions of each of the samples listed in Tables 1 and 2. In the case of ethanol extraction, scenarios were assessed where the ethanol used for extraction is not rotated, and scenarios where the ethanol is rotated and the rotated amount can be used again for extraction.

The functional unit was 1 g of bioactive compound (PACs or TCP) extracted from the raw material (leaves or twigs).

Inventory data were collected from the performed laboratory scale analysis. Regarding energy, electricity generation by solar panels, mounted on slanted roof, single-Si wafer, was used, as these are the solar panels installed in the sea buckthorn farm, which was a partner in the current research. The laboratory equipment itself was not within the scope of the analysis. Two outputs are generated that lead to a potentially useful product, i.e., extracts with bioactive compounds and a remainder of leaves and twigs that is diverted to composting. Nevertheless, the assessment allocated all the impact to bioactive compounds in extracts, because the remainder is generated anyway, with or without extraction, while we are interested in the impact of the processes that are targeted on obtaining extracts.

The impact was calculated using SimaPro 9.6 software (PRé Sustainability, the Netherlands) in combination with the Ecoinvent 3.6 database. The calculation method was the ReCiPe 2016 v1.1 midpoint method, the Hierarchist version. Results are presented for all impact categories.

3. Results

3.1. Results of Extraction of Bioactive Compounds

Sea buckthorn (*Hippophae rhamnoides*) is a plant known for its valuable nutritional and medicinal properties. In recent years, growing attention has been directed toward exploring not only its berries, but also other parts of the plant, such as leaves and twigs [31].

Table 3 provides a summary of the nutritional properties and proximate composition of sea buckthorn leaves and twigs. The two samples' moisture contents were relatively similar, with the leaves having slightly lower levels (5.37 ± 0.23%) than the twigs (5.87 ± 0.21%). The crude protein content of sea buckthorn leaves was higher (15.40 ± 0.57%) than that of the twigs (13.20 ± 0.39%).

At 7.30 ± 0.36% and 3.60 ± 0.19%, respectively, the crude fat content in the leaves was more than twice that of the twigs. Polyunsaturated fatty acids were the main fatty acids in both samples; the leaves had higher levels of these fatty acids (4.45 ± 0.07%) than the twigs (2.02 ± 0.05%). The leaves had a significantly higher concentration of omega-3 fatty acids (3.37 ± 0.03%) than the twigs (0.80 ± 0.04%). On the other hand, twigs had slightly more omega-6 fatty acids (1.17 ± 0.05%) than leaves (1.08 ± 0.09%), and twigs also had more omega-9 fatty acids. Sea buckthorn leaves had a substantially higher carbohydrate content (27.85 ± 1.51%) than twigs (15.79 ± 0.74%). But, compared to leaves (2.94 ± 0.09%), twigs had a significantly higher total sugar content (5.90 ± 0.06%), with higher concentrations of each of the individual sugars (fructose, glucose, and sucrose). Sea buckthorn twigs had a significantly higher total fiber content (59.60± 0.78%) than the leaves (38.73 ± 1.05%), in terms of dietary fibers. The main cause of this variation was the higher percentage of insoluble fibers in the twigs (55.70± 1.62%), as opposed to the leaves (35.22 ± 0.98%). An analysis of the mineral content showed that leaves had a higher total mineral content (5.35 ± 0.16%) than twigs (1.94± 0.09%). Twigs had slightly higher potassium levels (75.98 mg/100 g vs. 66.29 mg/100 g), but leaves had significantly higher calcium (353.05 mg/100 g vs. 68.08 mg/100 g) and magnesium (59.04 mg/100 g vs. 20.96 mg/100 g). This comparative analysis highlights the unique and complementary nutritional profiles of sea buckthorn leaves and twigs, suggesting their potential as valuable plant-based ingredients in functional foods and nutraceutical applications.

Table 3. Nutritional, compositional, quantitative, and qualitative indicators of raw materials of sea buckthorn leaves and twigs.

Parameters	Sea Buckthorn Leaves	Sea Buckthorn Twigs
Moisture content, %	5.37 ± 0.23	5.87 ± 0.21
Crude protein content, %	15.40 ± 0.57	13.20 ± 0.39
Fat content, %,	7.30 ± 0.36	3.60 ± 0.19
of which:		
saturated fatty acids, %	2.49 ± 0.11	0.91 ± 0.06
monounsaturated fatty acids, %	0.37 ± 0.04	0.67 ± 0.05
polyunsaturated fatty acids, %	4.45 ± 0.07	2.02 ± 0.05
omega-3 fatty acids, %	3.37 ± 0.03	0.80 ± 0.04
omega-6 fatty acids, %	1.08 ± 0.09	1.17 ± 0.05
omega-9 fatty acids, %	0.30 ± 0.02	0.61 ± 0.04
Carbohydrates, %	27.85 ± 1.51	15.79 ± 0.74
Total sugar content, %	2.94 ± 0.09	5.90 ± 0.08
of which:		
fructose, %	0.34 ± 0.11	1.79 ± 0.07
glucose, %	1.28 ± 0.09	1.89 ± 0.08
sucrose, %	1.34 ± 0.07	2.22 ± 0.12
Dietary fiber content, %	38.73 ± 1.05	59.60± 0.78
of which:		
soluble	3.51 ± 0.16	3.90± 0.17
insoluble	35.22 ± 0.98	55.70± 1.62
Mineral content, %	5.35 ± 0.16	1.94± 0.09
of which:		
potassium (K), mg/100 g	66.29 ± 2.91	75.98± 2.81
magnesium (Mg), mg/100 g	59.04 ± 2.78	20.96± 1.05
calcium (Ca), mg/100 g	353.05 ± 5.45	68.08± 2.78

The increasing focus on sustainable resource use has led to increased interest in recycling underutilized plant parts such as leaves and twigs. Instead of discarding these biomass residues, which are rich in valuable nutrients, targeted extraction processes allow the extraction of high-value phytochemicals that can be used in various high-value-added sectors. Utilizing both aqueous and ethanolic extraction approaches allows for a broader recovery of target compounds, supporting the development of functional ingredients derived from post-harvest plant residues [12,32].

Different extracts of sea buckthorn leaves and twigs, collected post berry harvest, were prepared using water and ethanol as the extraction solvent. The extracts were obtained through solid–liquid extraction with varying numbers of extraction cycles and durations (see Tables 4 and 5). Biologically active compounds such as TPC and PACs and antioxidant activity were determined in the extracts.

Table 4. Concentrations of biologically active compounds and their properties in different aqueous extracts of sea buckthorn biomass.

Sample	Amount of PACs, mg_{CE}/g	Amount of TPC, mg_{GAE}/g	Antioxidant Activity, %
Leaves extract_1	12.36 ± 0.16 b	41.94 ± 0.17 f	86.08 ± 0.09 d
Twigs extract_1	10.30 ± 0.08 a	14.20 ± 0.05 a	88.98 ± 0.12 g
Leaves extract_2	17.30 ± 0.21 d	44.05 ± 0.14 g	86.57 ± 0.11 e
Twigs extract_2	36.13 ± 0.21 i	27.09 ± 0.06 d	89.70 ± 0.05 h
Leaves extract_3	22.18 ± 0.19 f	49.24 ± 0.93 i	84.20 ± 0.03 c

Twigs extract_3	14.80 ± 0.09 c	15.33 ± 0.20 b	90.37 ± 0.11 i
Leaves extract_4	27.58 ± 0.31 a	61.22 ± 0.26 j	81.97 ± 0.06 a
Twigs extract_4	31.12 ± 0.16 h	27.06 ± 0.48 d	87.71 ± 0.14 f
Leaves extract_5	19.63 ± 0.35 e	46.47 ± 0.08 h	82.95 ± 0.12 b
Twigs extract_5	52.79 ± 0.21 j	33.96 ± 0.12 e	89.74 ± 0.12 h
Leaves extract_6	21.78 ± 0.16 f	49.92 ± 0.09 i	85.97 ± 0.11 d
Twigs extract_6	25.13 ± 0.15 g	21.17 ± 0.22c	89.81 ± 0.13 h

Data values are expressed as means with the standard deviation ($n = 3$). Means in the same column with different lowercase letters differed significantly ($p < 0.05$).

Sea buckthorn twig water extracts demonstrated higher concentrations of PACs compared to leaf extracts, with the highest value observed in Twigs-extract_5 (52.79 ± 0.21 mg_{CE}/g) whose extraction was performed using 60 cycles of 5 min duration. In contrast, leaf extracts tended to have higher TPC, with the peak value in Leaves-extract_4 (61.22 ± 0.26 mg_{GAE}/g), whose extraction was performed using 60 cycles of 10 min duration. As for antioxidant activity, all extracts exhibited high values (>80%), but Twigs-extract_3, whose extraction was performed using 20 cycles of 2 min duration showed the highest antioxidant activity (90.37 ± 0.11%), surpassing all leaf-based samples. However, this was not directly correlated with either TPC or PAC content, suggesting that other bioactive compounds might also be contributing to the antioxidant capacity. These results highlight that both leaf and twig water extracts have distinct phytochemical profiles and antioxidant potential. Twig extracts, particularly extract_5, are promising sources of PACs, whereas leaf extracts offer richer phenolic content. This complementary composition suggests the potential for combined or targeted use of these extracts, depending on the intended application (e.g., antioxidant enhancement, functional food formulation, etc.). It is also noted that a longer extraction process allows for extracts containing the highest amount of biologically active compounds, compared to extracts with the shorter extraction time.

Table 5. Concentrations of biologically active compounds and their properties in different ethanolic extracts of sea buckthorn biomass.

Sample	Amount of PACs, mg_{CE}/g	Amount of TPC, mg_{GAE}/g	Antioxidant Activity, %
Leaves extract_4-1	29.88 ± 0.21 a	37.17 ± 0.24 a	85.09 ± 0.61 b
Twigs extract_4-1	54.43 ± 0.13 c	39.90 ± 0.21 b	90.31 ± 0.42 c
Leaves extract_4-2	40.85 ± 0.52 b	64.72 ± 0.87 d	89.97 ± 0.17 c
Twigs extract_4-2	62.80 ± 0.31 d	37.15 ± 0.25 a	77.46 ± 0.36 a
Leaves extract_4-3	39.19 ± 0.31 b	77.89 ± 0.81 e	85.70 ± 0.51 b
Twigs extract_4-3	64.72 ± 0.36 e	50.79 ± 0.31 c	89.56 ± 0.55 c

Data values are expressed as means with the standard deviation ($n = 3$). Means in the same column with different lowercase letters differed significantly ($p < 0.05$).

The ethanol-based sea buckthorn twig extracts exhibited superior PACs content with Twigs-extract_4-3 at 64.72 ± 0.36 mg_{CE}/g and Twigs-extract_4-2 at 62.80 ± 0.31 mg_{CE}/g. The higher bioactive compound values in twig biomass over leaf extracts demonstrate twigs as a superior source when using ethanol extraction methods. Leaves-extract_4-3 displayed the highest total phenolic content (77.89 ± 0.81 mg_{GAE}/g) among leaf extracts, while demonstrating enhanced phenolic compound retrieval efficiency when using 50% ethanol. Twigs-extract_4-1 maintained the highest antioxidant activity at 90.31 ± 0.42%, which remained above 75% for all samples, even though it registered lower values in both TPC and PACs. The antioxidant capacity is likely affected by other compounds in the extracts. The results further confirm that the profiles of sea buckthorn leaves and twigs are distinct and complementary: the PAC content is higher in twig extracts, but the use of lower ethanol concentrations results in a higher extraction of phenolic compounds from leaves.

As research on the use of solid–liquid extraction for processing sea buckthorn waste such as leaves and twigs is still lacking, this study provides new knowledge by demonstrating the effective application of these methods to underutilized biomass, enabling sustainable and value-added use of plant parts.

3.2. Comparison of Extractions Based on Their Environmental Impact Assessment

A comparison of the environmental impacts of extractions shows that the lowest impacts are from those extraction scenarios that yield the most (highest concentration and thus the largest amount of) extractable compounds.

As noted in Section 3.1, when analyzing the extraction results of bioactive compounds, a trend was observed, although not strictly, for aqueous extracts from twigs to yield higher concentrations of PACs than leaf extracts. Thus, the environmental impact of extracting 1 g of PACs will generally be lower when water-extracting twigs rather than leaves (Figure 2). In the comparison figures, an extraction scenario with the highest impact is assigned a value of 100%, while the other scenarios are indicated as a percentage of the highest impact scenario.

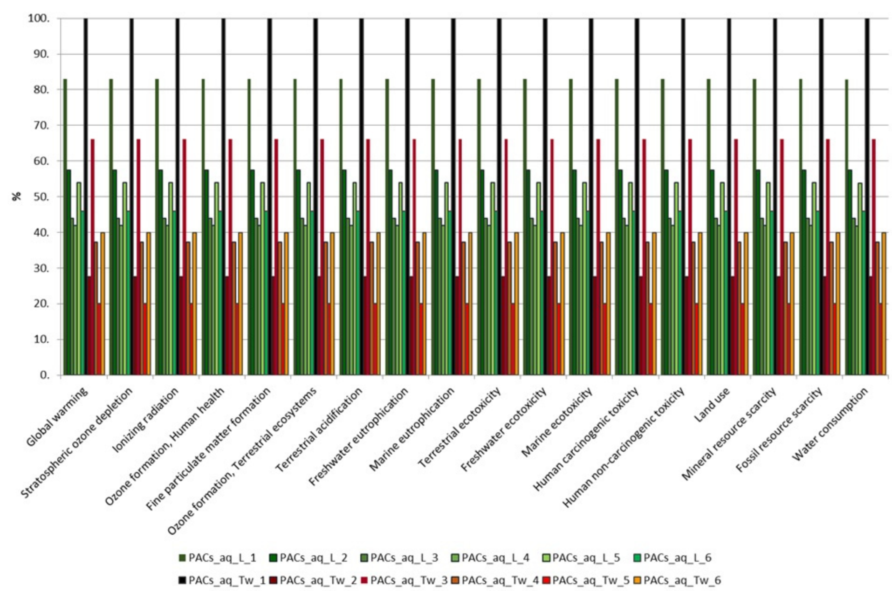


Figure 2. Comparative results of environmental impact of aqueous extractions to obtain PACs from leaves (marked with "L" in the abbreviation, green columns) and twigs (marked with "Tw" in the abbreviation, brown-red columns).

An even more pronounced trend was observed when extracting TPC by aqueous extraction: the concentration of these bioactive compounds was higher in all aqueous leaf extracts compared to twigs, and thus the environmental impact of extracting 1 g of TPC is lower for all leaf extractions (Figure 3).

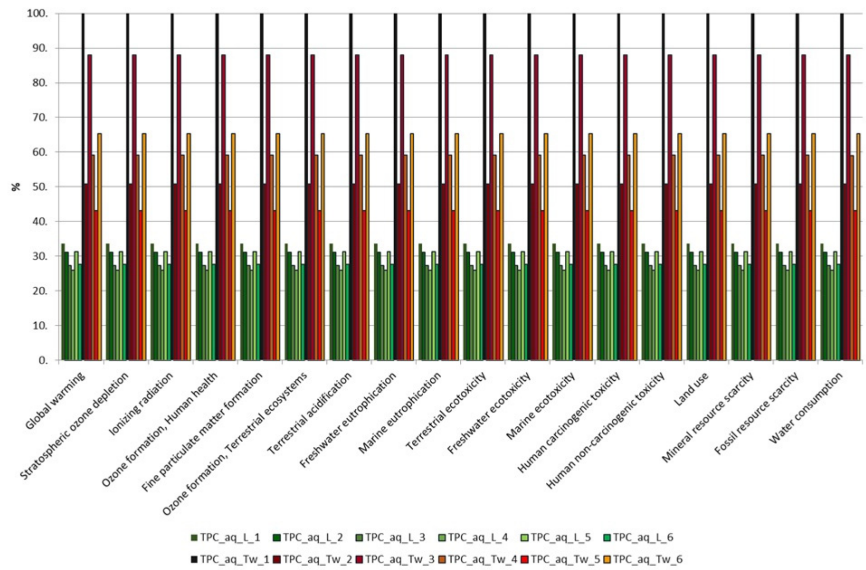


Figure 3. Comparative results of environmental impact of aqueous extractions to obtain TPC from leaves and twigs.

The same trends were observed during ethanol extraction: more PACs were extracted from twigs, and more TPC from leaves, except from one sample. The environmental impact of extracting these bioactive compounds is therefore lowest when obtaining 1 g of PACs from twigs and 1 g of TPC from leaves (Figures 4 and 5).

In the case of ethanol extraction, ethanol can be rotated from the extract and then used again for other extractions. Scenarios both with and without rotation are presented in Figure 4. Impacts of both scenarios are very similar for most impact categories. The recovery of ethanol is offset by the consumption of electricity required for rotation. However, in the category Fossil resource scarcity, the difference is larger: the impact without rotation would be 5–9% higher.

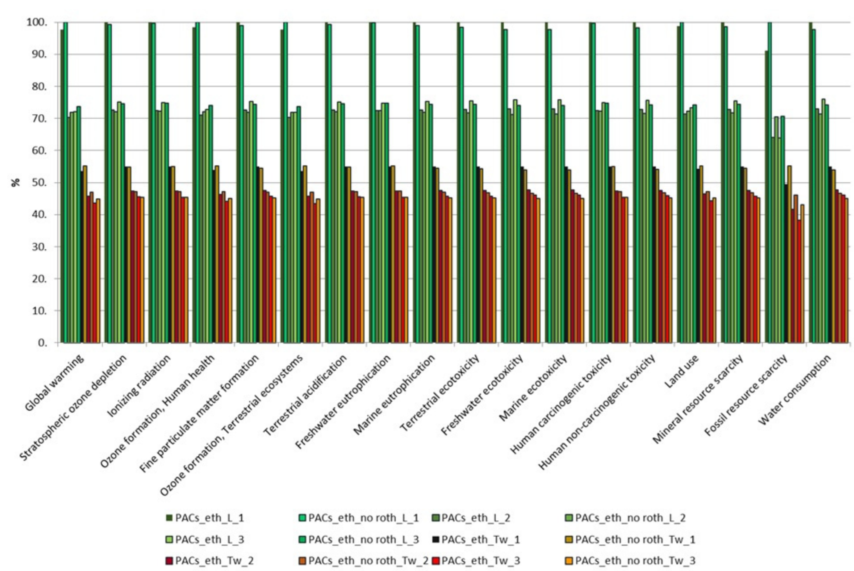


Figure 4. Comparative results of environmental impact of ethanol extractions to obtain PACs from leaves and twigs. Scenarios without ethanol rotation and its further reuse are marked with "no roth".

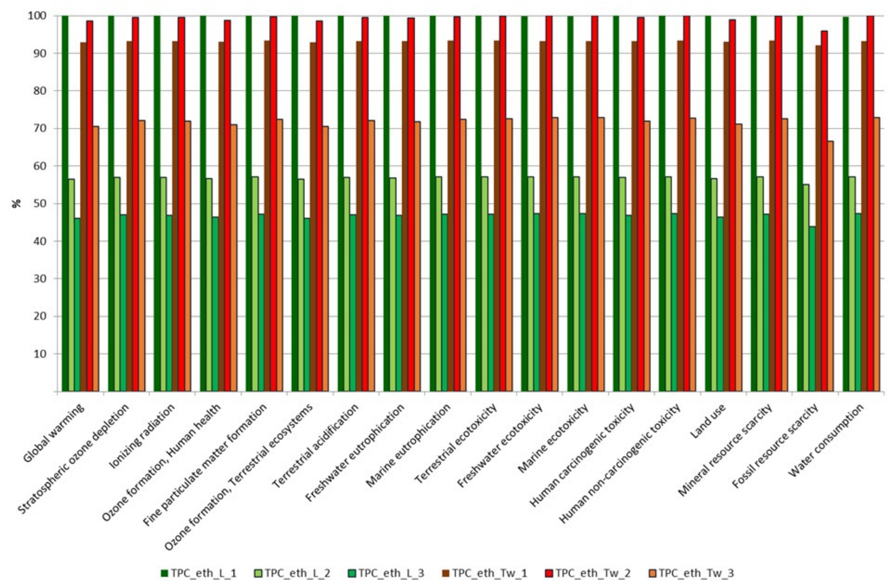


Figure 5. Comparative results of environmental impact of ethanol extractions to obtain TPC from leaves and twigs.

Normalization of the impact results using SimaPro allowed us to clarify those impact categories in which the impact is most significant. Using an example of sample "TPC in water extract_4 from leaves", Figure 6 shows the most significant impact categories. For all extractions, these are human carcinogenicity, as well as freshwater and marine ecotoxicity. The cause of all these impacts is the use of electricity. For human carcinogenicity, this is mainly related to Cr VI (from Czochralski process and steel production, needed for PV panels and wafer), while for freshwater and marine ecotoxicity this is mainly due to Cu from electric wiring in photovoltaic electric installation.

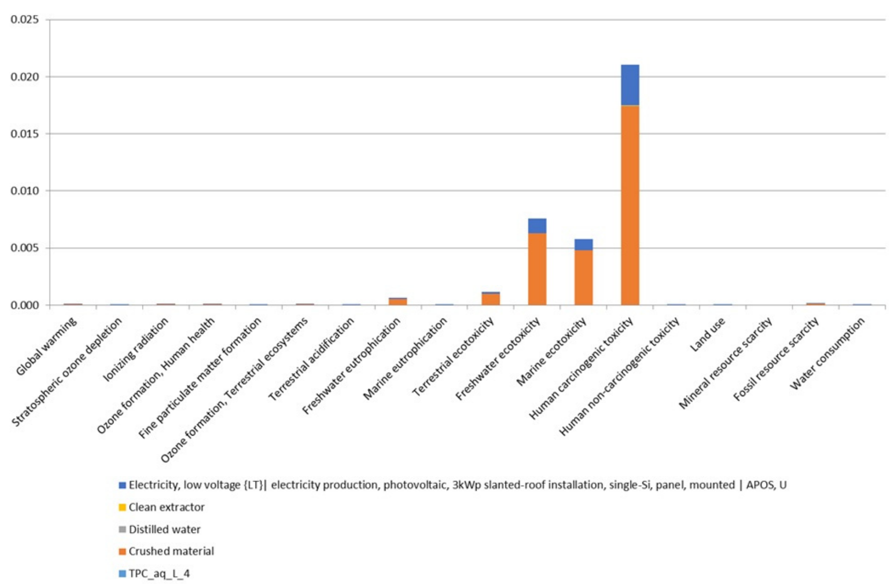


Figure 6. The most significant impact categories, exemplified by normalization of impact results for aqueous leaf extraction, TPC in sample 4.

Analysis of the processes contributing the most to the environmental impact showed that the most significant impact (82—83% of the overall impact in different impact categories) is caused by the crushing of leaves and twigs, due to high energy (electricity) demand. This process has the greatest impact in both water and ethanol extractions.

This, as well as other statements from the subsequent analysis, are illustrated by the figures presented in the Supplementary Materials.

Based on overall assessment when the process of crushing raw material is included, there is a slight trend for ethanol extraction to cause lower environmental impact than water extraction. This situation is due to the fact that ethanol extraction tends to yield more bioactive compounds, PACs and TPC, from the same amount of raw materials. Since, as already mentioned, it is the preparation of the raw material by crushing it that accounts for the largest part of the environmental impact, we see the result that in many instances the lowest environmental impact is in those extraction scenarios in which the least amount of raw material is required to obtain the same amount of bioactive compound.

However, when the crushing process is excluded from calculations, the situation becomes different. The use of electricity for the extraction itself accounts for 43–98% of the residual impact across different extraction scenarios and across different impact categories. In ethanol extraction, the influence of ethanol is also quite significant, accounting for 5–48% of the residual impact across different extraction scenarios and across different impact categories. It then turns out that the least environmental impact is caused by aqueous extraction scenarios, where the extraction takes the shortest time and uses the least energy. Of the ethanol extractions, those with the least ethanol consumption are the most favorable.

4. Discussion

In recent years, there has been increasing attention given to the utilization of agricultural waste, in order to contribute to the implementation of sustainability and circular-economy principles. Sea buckthorn (*Hippophae rhamnoides*) leaves and twigs, previously mostly considered as unused biomass, are currently being investigated as potential functional ingredients, due to their valuable nutritional and bioactive properties. The results revealed that both leaves and twigs have different, yet complementary, chemical compositional features that can be used for different purposes in the food, cosmetic or nutraceutical industries. According to nutritional analysis, sea buckthorn leaves contained more protein (15.40%), fat (7.30%), carbohydrates (27.85%), and minerals, especially calcium and magnesium, than twigs. Meanwhile, twigs had significantly higher dietary fiber (59.60%) and sugars, which may be significant for the potential use of secondary raw materials.

When solid–liquid extraction was applied to obtain bioactive compounds from sea buckthorn leaves and twigs, clear differences in the efficiency of aqueous and ethanol solvents in extracting TPC and PACs were observed. As expected, ethanol-based extracts generally yielded higher TPC, especially when 50% ethanol was used, which is consistent with previous studies [33] showing that moderate ethanol concentrations improve the solubility of polyphenols. This is attributed to the increased polarity of the solvent, which improves the solubility of both polar and moderately polar polyphenolic compounds [34]. Similarly, other researchers found that the highest TPC for different types of fruit husk was achieved using a 50% ethanol solution [31]. PACs were more concentrated in twig extracts, especially during ethanol extraction, suggesting that woody tissues may contain more condensed tannins or polar compounds bound to lignin [35]. Aqueous extracts, although less efficient for the recovery of phenols in general, still showed a notable efficiency in the extraction of polar compounds from twigs, suggesting the potential of water as a more environmentally friendly solvent for targeted extractions. Interestingly, the extraction time and number of cycles had an impact on the optimization of the process, but the differences between long and short extraction times were not always significant. Overall, the different profiles of leaves and twigs and between solvents suggest that a tailored extraction method, depending on the desired concentration of bioactive compounds, could optimize the use of sea buckthorn biomass for nutritional supplements or cosmetic applications.

The environmental impact assessment of extractions, which aimed to select and recommend the scenario with the lowest environmental impact and to find out which processes have the highest impact, showed that the critical process, which accounts for 80% or more of the impact, is the crushing of the raw material—leaves and twigs. Thus, those extraction scenarios that had the highest yield of bioactive compounds PACs and TPC from 1 g of prepared raw material have the lowest environmental impact. The authors did not identify studies that would have analyzed the environmental impact of extraction of sea buckthorn leaves, twigs, or other parts, quantitatively. However, a number of studies qualitatively identify the negative environmental impact associated with the energy use and extractants used [36,37]. Our study confirmed the significance of energy usage and extractant (ethanol). The primary goal should be to optimize the crushing process to use equipment that is as energy-efficient as possible. Based on LCA results, leaf extracts should be used to obtain TPC, and twig extracts should be used to obtain PACs. A total of 50% ethanol should be chosen for ethanol extractions. In the case of aqueous extraction, there is no straightforward answer regarding extraction parameters: the scenario with the least impact differs for different bioactive compounds, and depends on the assessment system boundaries. The not-recommended scenario, due to the higher environmental impact, is the one with 20 extraction cycles of 10 min-cycle duration.

5. Conclusions

Sea buckthorn (*Hippophae rhamnoides*) leaves and twigs, though traditionally underutilized, demonstrate valuable and complementary nutritional and phytochemical properties. Leaves are richer in protein, fat, carbohydrates, and minerals, while twigs contain higher amounts of dietary fiber and sugars. Extraction trials showed that twig extracts, especially with ethanol, yield more proanthocyanidins (PACs), whereas leaf extracts, particularly with 50% ethanol, provide higher total phenolic content (TPC). Furthermore, antioxidant activity assessments indicated strong functional potential of both leaf and twig extracts, with leaf extracts showing 82–90% DPPH radical scavenging activity and twig extracts showing 77–90%.

LCA analysis revealed that the crushing of raw material is the most energy-intensive step, contributing the majority of the impact. Extraction scenarios yielding higher concentrations of bioactive compounds from the same biomass were associated with lower environmental burdens. Thus, the environmental impact is lower when extracting PACs from leaves and TPCs from twigs.

The results underline the feasibility of integrating waste biomass recovery into sea buckthorn production systems, contributing both to waste reduction and the development of value-added products for food, cosmetic, or nutraceutical industries. The proposed approach supports the advancement of circular agriculture, by enhancing resource efficiency and creating economic value from previously underutilized biomass.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr13061884/s1, Figures S1–S7: Comparison and analysis of water and ethanol extractions.

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Abbreviations

The following abbreviations are used in this manuscript:

LCA	Life-cycle assessment
PACs	Proanthocyanidins
TPC	Total Phenolic Content

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