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# Extractable Compounds in a Birch Tree—Variations in Composition and Yield Potentials

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**Abstract:** Extractives from silver birch (*Betula pendula*) can play an important role in the future bioeconomy by delivering the feedstock, for instance, for antioxidative applications. It is, therefore, inevitable to gain knowledge of the distribution of extractive content and composition in the different tissues of the tree for estimating the potential volumes of valuable extractable compounds. This study examines the extractable compound distribution of different tree tissues such as outer and inner bark and wood, respectively, considering the original height of the stem and comparing the yields after Soxhlet and accelerated solvent extraction (ASE). Eleven parts of the model tree (seven stem discs and four branches) were separated into primary tissues and extracted with a ternary solvent system. The investigated extraction methods resulted in a comparable performance regarding yields and the composition of the extractives. The extractives were divided into single compounds such as betulin, lupeol, γ-sitosterol, and lupeone and substance groups such as carbohydrates, terpenes, aromatics, and other groups. The distribution of single substances and substance groups depends on the location and function of the examined tissues. Furthermore, the evidence for the correlation of a single substance's location and original tree height is stronger for lupeol than for betulin. Primary betulin sources of the calculated betulin output are the outer bark of the stem and the branches. By using small branches, further potential for the extraction of betulin can be utilized. A model calculation of the betulin content in the current birch tree revealed a significant potential of 23 kg of betulin available as a valuable chemical resource after by-product utilization.

**Keywords:** accelerated solvent extraction (ASE); bark; betulin; by-product utilization; extractives; lupeol; silver birch (*Betula pendula*); Soxhlet extraction; triterpenes



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## 1. Introduction

Birch is a naturally occurring commercial tree species, comprising 0.5% to 28% of the total standing volume in different European countries [1,2]. In the Northern European countries Finland, Sweden, and Norway, birch species (*B. pendula* and *B. pubescens*) make up for 16.5% [3], 13% [4], and 16% [5] of the respective standing volume and result in a combined birch growing stock of 817 million $m^3$ [1]. German forests have a harvestable timber volume (under bark) of 2901 million $m^3$ allocated to 90 billion trees, and 4% of these trees are birches, mainly silver birch (*B. pendula*) [6].

Birch wood has many possible industrial applications [7,8], and the bark as a by-product from the timber refinement processes is usually incinerated without further value creation. Bark extraction, however, could be one means to achieve a higher yield of products per invested raw material unit and a better overall raw material utilization [7,9].

The extractable components can be separated from the cell wall by extraction with organic solvents or water as typical solid–liquid extraction. Several studies were carried out with alcohols such as ethanol and methanol, other organic solvents such as chloroform,

various ethers, and hot water. In some cases, the extraction was assisted by physical methods such as microwaves, ultrasound, and other techniques [10,11]. Supercritical fluids such as $CO_2$ are an interesting alternative to typical extraction systems [12].

Extraction with ethanol of outer and inner bark parts leads to more extractable compounds from the outer parts [13]. In similarity, this applies to the extraction with ethers. Combined with alkaline substances (mostly NaOH), the yields of extracts could be increased due to the extraction of suberin, their derivatives, and the extraction of pentosanes and hemicellulose considering the extracted tissue [14,15]. Isoprenoids, phenolic compounds, and carbohydrates predominately form the substances in birch extractives. A group of isoprenoids, the triterpenoids, are primarily found in the outer bark of silver birch [16]. Pentacyclic triterpenes form the basic structure of these compounds with lupan- and oleanane-derived structures [17,18]. These components are predominately soluble in organic solvents such as alcohols, acetone, acetic esters, and others [19–21]. Within the extracts of inner bark, phenolic compounds are found in a high number. These aromatic ring structures with at least one hydroxyl group are lignans, oligolignans, flavonoids, isoflavonoids, tannins, and phenolic acids [22], which are described in detail by Hänsel et al. [23]. Carbohydrate fractions can be found in the wooden tree parts and were formed by D-glucose, D-fructose, and sucrose in the sapwood and L-arabinose in the heartwood, respectively [16]. The amounts fluctuate with the season and reach a maximum in November in preparation for the winter [24]. These components can be extracted with hot water and alkaline/water solutions [25]. Betulin and lupeol are the most abundant triterpenoids in bark extracts, followed by betulinic acid. At ethanolic alkali, hydrolysis of outer birch bark betulin and lupeol amounts were found, with 244.8 g/kg and 20.2 g/kg, respectively [14]. Other studies indicate similar average betulin amounts by extraction with ethanol of approximately 27 wt% from outer bark tissues [9]. In inner bark tissues, lower amounts of betulin (13.6–18.9 wt%) were found compared to outer bark [13,24,26]. Other extraction agents such as chloroform, heptane, and $scCO_2$ lead to betulin-enriched extracts of up to 22 wt% (chloroform), up to 30 wt% (n-heptane), and up to 25 wt% ($scCO_2$), dependent on the respective extraction parameters [12,27,28]. Yields from the extraction of wood (stem and branch) are considerably lower than from bark extraction with unconsidered extraction parameters and solvents. The typical extractive yields of wood are between 0.8 and 5 wt% [24,26,29–31]. An interesting alternative to liquid–solid extractions is the extraction with supercritical fluids (mainly $CO_2$), where betulin yields of 20 to 25 wt% were achieved dependent on the pressure and extraction temperature [9] and can be increased by utilizing polar modifiers such as ethanol to enhance the solubility of non-polar compounds. However, the yields were lower than the extraction results with a Soxhlet apparatus [32]. The pharmaceutical effects of the triterpenes, especially betulin from birch extractives, are well known and described. Symptoms of skin diseases such as neurodermatitis can be alleviated by products containing betulin or betulin-related substances [33]. Further effects of triterpenes extractives such as antibacterial, antiviral, antiparasitic, and hepatoprotective effects are described [34–37]. Additionally, an antioxidative effect of knot wood extractrives was observed [38]. A detailed description of pharmaceutical applications of extractives from bark, leaves, sap, and buds is given in Vladimirov et al. [39]. Besides the utilization of the extractives itself, portions of birch bark used as fillers can reduce the formaldehyde emissions from UF resins for plywood manufacturing [40]. Ferreira et al. postulates that birch cork is not suitable for the classic applications such as sealant, insulation, and surface materials. Based on this, a theoretical biorefinery approach is proposed for the stepwise extraction of outer bark to obtain triterpenes, long-chain fatty acids, and carbohydrates [41]. Promising applications for extracted residues may also arise here. In addition, from the perspective of sawmills, the extraction of low-quality harvested wood from selection cuttings offers a source of revenue, especially from high-priced specialties with small market sizes [42].

It must be noted that the available data are based on very specific results, often only on one tissue type from one location of the tree or random samples, related to specific ex-

traction methods and applied solvents. Therefore, the results are often difficult to compare. However, the use of triterpenoids, especially betulin, is considered very promising and relevant, but further research about the suitable techniques for extraction and isolation is recommended [39].

Therefore, this study's main objective is to describe the variations of extractive content and composition in height and different tissues of a birch (*Betula pendula* ROTH.) model tree after extraction with one ternary solvent system to evaluate the potential and theoretical extractive yields of birch by-products as a chemical resource.

Hence, we had the specific objectives to (a) study the influence of the extraction methods of Soxhlet and accelerated solvent extraction (ASE) on the extractive yield and composition; (b) investigate the influence of stem height on the extractive content and composition; and (c) investigate the extractive content and composition of different stem and branch tissues to create a first comparable data basis for future predictions of larger sample numbers based on one defined solvent system.

## 2. Materials and Methods

### *2.1. Sample Material and Preparation*

The birch tree (*Betula pendula* ROTH.) was felled in June 2020 (53°9′31.6002″ N, 10°11′35.8326″ E) at an approximate age of 80 years. The log of 19.4 m length was divided into 14 stem sections, of which 7 were selected for further analysis (SD1—SD7, Table 1). Stem discs were subtracted every 3.2 m, starting at the stem base (SD1). The center of SD1 showed severe decay, and also SD2 had some signs of decay in the wood. However, both stem discs were included in the extractive yield and composition analysis.

**Table 1.** Sample origin. The table gives the original height of the respective branch (bold, left) and stem discs (bold) on the stem axis (bold, italic), the different tissues subtracted for analysis, as well as the related sample ID (italic).

| Stem Discs Subtracted for Sampling | | | | | | HTA (m) | Branches Subtracted for Sampling | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Branches diameter > 7 cm (B > 7) | | | | | |
| Stem disc ID | | Fissured coarse bark (FB) | Outer bark (OB) | Inner Bark (IB) | Stemwood (SW) | | Branch bark (B > 7, BB) | Branch wood (B > 7, BW) | Branches of 1–7 cm (B1–7) | Twigs and foliage (TF) | | Branch ID |
| | | | | | | ***19.30*** | *11.1* | *11.2* | *15* | *19* | **B6** | |
| | **SD7** | | *7.1* | *7.2* | *7.3* | ***19.27*** | | | | | | |
| | **SD6** | | *6.1* | *6.2* | *6.3* | ***16.07*** | | | | | | |
| | | | | | | ***15.4*** | *10.1* | *10.2* | *14* | *18* | **B3** | |
| | | | | | | ***13.2*** | *9.1* | *9.2* | *13* | *17* | **B2** | |
| | **SD5** | | *5.1* | *5.2* | *5.3* | ***12.87*** | | | | | | |
| | **SD4** | | *4.1* | *4.2* | *4.3* | ***9.67*** | | | | | | |
| | | | | | | ***9.0*** | *8.1* | *8.2* | *12* | *16* | **B1** | |
| | **SD3** | | *3.1* | *3.2* | *3.3* | ***6.47*** | | | | | | |
| | **SD2** | | *2.1* | *2.2* | *2.3* | ***3.27*** | | | | | | |
| | **SD1** | *1.1* | | | *1.2* | ***0.08*** | | | | | | |

Height of tree axis (HTA (m)).

Of all seven main branches of the tree, weight and length were noted before selecting four branches for further analysis. For these four branches (B1, B2, B3, and B6), the onset on the stem was noted (Table 1). The branches were each divided into three branch categories: branch parts with a diameter over 7 cm (B > 7), branch parts between 1 and 7 cm (B1–7), and the remaining twigs and foliage (TF). The branch mass of the three different categories was measured and noted for each of the four branches. The total fresh mass of the tree, including the branches and twigs smaller than 7 cm, was weighed at 1.4 t.

### 2.2. Description of Sample Preparation

All collected samples (stem discs and branches) were further divided into different tissue (Table 1). Stem discs (SD1–SD7) and branch parts with a diameter over 7 cm (B > 7) were debarked. The wooden part of the stem discs was divided into four pieces, where two were used for further analysis (SW), and two were stored as spare.

Mature silver birch trees develop thick, fissured, coarse barks toward the base of the trunk [1] and smooth bark textures with a characteristic white color further up the stem. The phloem and the periderm are the two main tissues in the bark outside the vascular cambium. The vascular cambium is the meristem producing the xylem and wood tissue, while the phellogen, the cork cambium in the periderm, produces phelloderm toward the inside of the stem and phellem toward the outside [43]. Phloem and periderm will be referred to in this work as the inner bark (phloem) and outer bark (periderm).

The fissured coarse bark (FB) on SD1 as well as the branch parts with a diameter over 7 cm (B > 7) were used as a bark mix in further trials. For the bark of the stem discs SD2–SD7, the outer bark (OB) was separated from the inner bark (IB) before further processing and analysis.

Before initial drying (3 days, 65 °C) with circulated ventilation, wood and branch samples were prechopped. All tissues were ground afterward to 1 mm (SM 2000 Retsch) and dried again at 65 °C for at least two days. Weight before and after drying was noted for all tissues at all drying steps. Until extraction, all samples were stored in sealable containers at room temperature. Before extraction, the samples' remaining moisture content was determined by weighing a small subsample before and after drying at 103 °C. From the initial mass of the tissue and the total moisture content, the dry biomass weight fractions of the stem disc parts (wt%) were calculated (Table 1).

Table 1 gives an overview of the subtracted samples, their corresponding sample ID, and their position in the stem.

### 2.3. Extraction

The extraction was carried out with two different systems: the Soxhlet apparatus and accelerated solvent extraction (ASE). For the Soxhlet extraction, 16–18 g (accuracy: 0.0001 g) of sample material was weighed into extraction thimbles (cellulose, 33 × 118 mm). As extraction solvent, a ternary mixture of ethyl acetate (CAS-No. 141-78-6, purity: 99.5%)/ethanol (CAS-No. 64-17-5, purity: 96%)/deionized water (4.5:4.5:1 per volume) was prepared. The extraction was carried out until the solvent agent became colorless. One extraction cycle ran over 35 min, and on average, 50–55 cycles per sample were realized. The Soxhlet extraction of the 36 samples was carried out with two replicates.

In comparison to the results of Soxhlet extraction, a further extraction procedure was carried out. Here, approximately 1.6 g of sample material was weighed into the extraction cells of a Dionex ASE 350 (Thermo Fisher Scientific, 168 Third Avenue, Waltham MA 02451, USA) equipped with an autosampler. The extraction was performed at 70 °C and 100 bar. The extraction agent (ethyl acetate/ethanol/deionized water; 4.5:4.5:1 per volume) was flushed into the extraction cell with the sample, and during extraction, pressure and temperature were kept stable for 20 min. Afterward, a new extraction cycle started by automatically filling the cell with solvent. The extraction was carried out in triplicate for all 36 samples (Table 1).

After extraction with ASE and Soxhlet, the extractives–solvent mixture was transferred into weighed Petri dishes for evaporation. The aqueous part was evaporated by storing the Petri dish in a furnace at 65 °C for at least four hours. Finally, the amount of extractives was determined gravimetrically.

### 2.4. Analysis

The extracts were dissolved with a concentration of 2 mg/mL in a solution of fluoranthene (β = 202.14 µg/mL) and acetone. Afterward, the solution was filtered through a Teflon membrane with a pore size of 0.45 µm. The extractive compounds were analyzed by a GC-MS/FID (Agilent 6890 Series, MSD 5975C) system on a crosslinked methylsiloxane column (VF-5, 30 m × 0.25 mm × 0.25 µm), using He (0.7 mL/min) as carrier gas. The conditions of analysis were as follows: 150 °C (4 min), 10 K/min to 320 °C (40 min); injector: 250 °C, split injection (ratio 15:1); FID: 280 °C, $H_2$ 40 mL/min, syn air 450 mL/min, make up $N_2$ 45 mL/min; MSD transfer line 350 °C, m/z range 19–650 [44,45]. Betulin and lupeol were calibrated with two stock solutions as reference substances for quantification.

### 2.5. Statistic Analysis

A *t*-test determined possible differences between Soxhlet extraction and ASE. The Tukey–Kramer HSD (honestly significant differences, JMP Pro 14, SAS Institute Inc., Cary, NC, USA) was used to compare means on a 5% significance level. Linear regression models were used to study the influence of height on the extractive yield, betulin, and lupeol content in the samples.

### 2.6. Model Calculations

The calculations of theoretical betulin amounts are based on the weight data (Tables 2 and A1 in the Appendix A) of the complete model tree and the analytical, gas chromatographic results of extractives and, especially, the betulin amount. It was possible to assign the amount of extractives and betulin to the weighed stem sections in each case. Using these data, based on the described ASE-trials on laboratory scale, a theoretical extractable betulin content can be calculated for different tissues/parts of the model tree. These theoretical values may deviate due to technical conditions, e.g., in the context of an enlargement of scale.

**Table 2.** Dry biomass weight fractions of the stem disc parts (wt%).

| Stem Tissues | | | | | | | | Branch Tissues | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FB | OB | IB | SB | SW | HTA (m) | B > 7 | B > 7, BB | B > 7, BW | B1–7 | TF | | |
| | | | | | | | | a | | | b | c | | |
| | | | | | | | **19.30** | 39.59 | 17.75 | 82.25 | 42.59 | 17.83 | **B6** | |
| Stem disc ID | **SD7** | | 2.44 | 12.31 | 14.75 | 85.24 | **19.27** | | | | | | | Branch ID |
| | **SD6** | | 2.12 | 14.95 | 17.07 | 82.94 | **16.07** | | | | | | | |
| | | | | | | | **15.4** | 51.23 | 20.50 | 79.50 | 31.41 | 17.35 | **B3** | |
| | | | | | | | **13.2** | 57.37 | 17.14 | 82.86 | 28.50 | 14.14 | **B2** | |
| | **SD5** | | 1.20 | 12.55 | 13.75 | 86.25 | **12.87** | | | | | | | |
| | **SD4** | | 1.95 | 13.10 | 15.05 | 84.96 | **9.67** | | | | | | | |
| | | | | | | | **9.00** | 65.84 | 19.99 | 80.01 | 20.79 | 13.37 | **B1** | |
| | **SD3** | | 1.90 | 10.62 | 12.52 | 87.48 | **6.47** | | | | | | | |
| | **SD2** | | 1.82 | 8.46 | 10.28 | 89.73 | **3.27** | | | | | | | |
| | **SD1** | 14.77 | | | | 85.23 | **0.08** | | | | | | | |
| APT | | 14.77 | 1.91 ± 0.41 | 12.00 ± 2.22 | 13.90 ± 2.33 | 86.10 ± 2.33 | | 53.51 ± 11.04 | 18.85 ± 1.65 | 81.16 ± 1.65 | 30.82 ± 9.03 | 15.67 ± 2.24 | | |

Height of tree axis (HTA (m)); average per tissue type (APT); a + b + c = 100%; fissured coarse bark (FB); outer bark (OB); inner bark (IB); stem bark (OB + IB = SB); stem wood (SW); branches with > 7 cm (B > 7); branch bark (B > 7, BB); branch wood (B > 7, BW); branches of 1–7 cm (B1–7); twigs and foliage (TF).

## 3. Results and Discussion

### *3.1. Biomass Fractions*

In the sampled birch tree of this study, stem wood makes up for 86.1 ± 2.33% of the dry mass in the birch trunk. Roughly 2% of the total trunk was extractive-rich outer bark, and the total average bark content (dry mass) was 13.90 ± 2.33% (Table 2).

Bark typically represents 10–15% of the total weight of tree stems [46–48]. Our sample tree has a comparably low content of outer bark as compared to a single birch tree from the St. Petersburg area, which had an average outer bark content of 5.4% and an inner bark content of 8.6% of the dry mass [13]. Differences in the bark content could be related to geographic differences in bark thickness. Viherä-Aarnio et al. found differences in bark thickness for the Latvian and Finnish stands, where Latvian silver birch had thicker bark than the Finnish trees [49]. Branches (B > 7) have a larger bark fraction as compared to stem discs (Table 2), where the stem wood only makes up for 81.16 ± 1.65%.

### *3.2. Extractive Yields*

The extraction of different tissues from birch (*B. pendula*) resulted in a large total range in single extractive yields from 2.33 wt% to 35.44 wt%. This extensive distribution allocates to tissue-specific extractive yields (Figure 1) with ranges of 2.36 wt% (twigs and foliage, TF) and 5.33 wt% (inner bark, IB) for ASE. The outer bark (OB) had a more extensive range with 8.39 wt%. The comparison of the results from ASE and Soxhlet (Figure 1) demonstrates the comparability of the methods, which is mainly reflected in similar extractive yield ranges for the different tissues from 3.02 wt% (B1–7) to 9.13 wt% for outer bark (OB) when considering both extraction methods at once. The comparability of the methods is highlighted regarding the tissue's stem wood (SW), outer bark (OB), and inner bark, since the outliers for the respective tissues and methods all originate from one particular stem disc (OB, SD2; SW, SD1; IB, SD6).

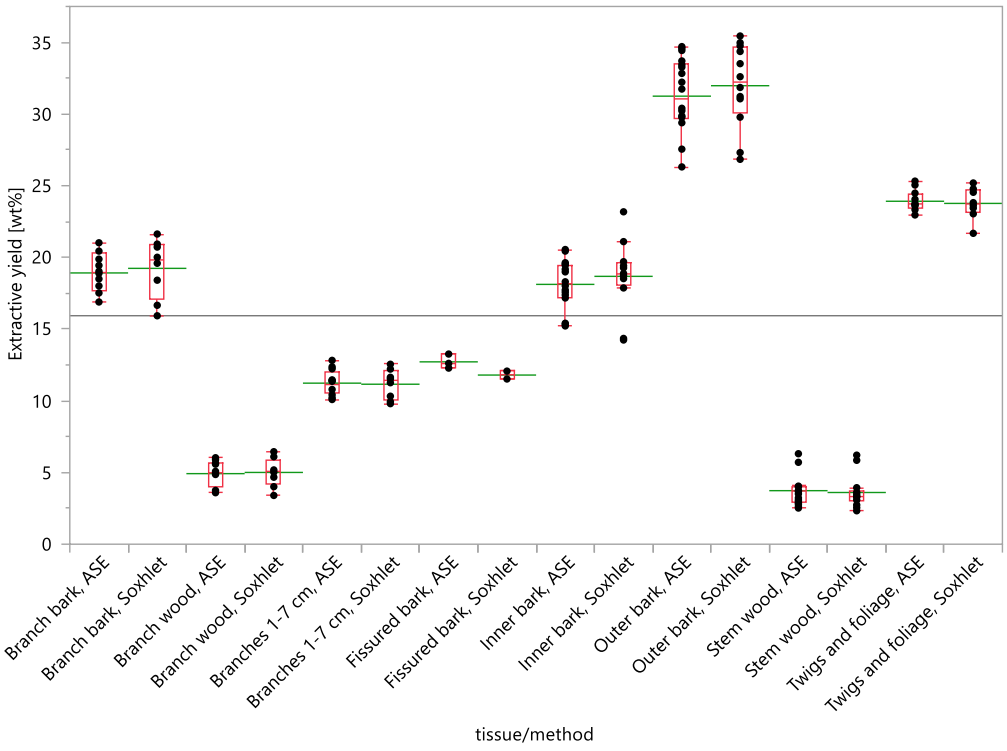


**Figure 1.** An overview of extractive yields (wt%) of the different wood tissues extracted with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with ASE and Soxhlet, and all stem discs and branches.

3.2.1. Results of Stem Discs Qualified and Quantified Contribution of Extractives, along with the Height

Outer bark tissue, independent of stem disc and original height of the stem, has the highest general extractive yield (wt%) in the study, with single sample extractive yields after ASE extraction of 26.31 wt% to 35.44 wt%. The wood fraction's stem wood and branch wood had the lowest extractive yield (wt%), with the stem wood showing generally lower yields than the adjacent branch wood sample (Tables 3 and 4). The extractive yields in the branch fraction's branch bark (B > 7, BB) and branches of 1–7 cm (B1–7) are mixtures of the outer and inner bark (B > 7, BB), or branch wood and the outer and inner bark (B1–7). These tissue mixtures are reflected in the respective extractive yields of the tissues, where the branch fraction B1–7 has single sample extractive yields after ASE extraction of 9.80–12.82 wt%, while BB has single sample extractive yields of 15.93–21.63 wt%. Within tissue materials, significant differences could be observed between stem discs and branches, respectively (Tables 3 and 4). The fraction twigs and foliage (TF) was the only fraction where no significant difference between at least some of the branches or stem discs could be observed.

A *t*-test performed on the extractive yields from the adjacent branch and stem wood samples showed that, except for the uppermost branch and stem wood combination, the branch wood had significantly higher extractive yields than the stem wood (Table 5). Significant correlations were found for the influence of branch onset in a stem (HTA, height of tree axis from the ground), extractive yield (wt%) of branch wood ($R^2$ = 0.9038), and branch bark material ($R^2$ = 0.87021). Here, the extractive yields taper off toward the top of the tree (Table 6, BB, BW). For the stem bark fraction outer bark (OB), the extractive yields increased with increasing original height of the stem (Table 6, OB, $R^2$ = 0.491282). For the stem bark fraction inner bark, only a trend of decreasing extractive yields with an increasing original height of the stem could be observed (Table 6, IB, $R^2$ = 0.307601). For the remaining branch fractions and the stem wood fraction, a correlation of stem height with extractive yield could not be found (data not shown).

Figure 1 indicates that the used ASE and Soxhlet extractions with the solvent ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) produce similar results, with comparable mean values and variations for the respective tissues. The comparability of the methods is underpinned by single direct comparisons (Table 7), which showed that the extraction yields we obtained for the different tissue with the extractive methods ASE and Soxhlet give the same extractive yields. The exceptions were the inner bark of stem disc 6 (SD6) and the fraction of twigs and foliage (TW) of branch 1 (B1), where a significant difference was found for the two preparation methods, with prob > |t| = 0.0017 and 0.0351, respectively. Additionally, comparing the betulin and lupeol yields after Soxhlet and ASE by tissue and height of the stem showed that the means were similar for all comparisons except for the betulin yields of the inner bark in SD7 (data not shown). Earlier studies on different materials have confirmed the comparability of the methods [50,51]. However, in a study on the extraction of volatile and phenolic compounds from Lamiaceae species, both techniques resulted in satisfying results, although ASE gave significantly higher yields [52]. Our results confirm ASE as a reliable extraction method for comparing the content and composition of extracts from different tissues. When also considering other factors, such as solvent consumption, extraction time, and parameter reproducibility, ASE is preferable to Soxhlet extraction.

**Table 3.** Mean extractive yields (wt%) of stem disc tissue's outer bark, inner bark, and stem wood after Soxhlet and ASE extraction. Tukey–Kramer HSD comparisons show whether significant differences exist between the different heights within branch tissues, where different letters indicate significant differences.

| Fissured Coarse Bark (FB) | | | | | | | | | Outer Bark (OB) | | | | | | | | | Inner Bark (IB) | | | | | | | | | Stem Wood (SW) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | | Soxhlet | | | ASE | | | | ID | Soxhlet | | | ASE | | | | | ID | Soxhlet | | | ASE | | | | | ID | Soxhlet | | | ASE | | | | |
| | | N | mean | range | N | mean | ±stdv | range | | N | mean | range | N | mean | ±stdv | range | TK HSD | | N | mean | range | N | mean | ±stdv | range | TK HSD | | N | mean | range | N | mean | ±stdv | range | TK HSD |
| SD1 | *1.1* | 2 | 11.80 | 0.55 | 3 | 12.72 | ±0.50 | 0.98 | | | | | | | | | | | | | | | | | | | *1.2* | 2 | 6.03 | 0.36 | 3 | 6.12 | ±0.36 | 0.64 | A |
| SD2 | | | | | | | | | *2.1* | 2 | 27.08 | 0.47 | 3 | 27.14 | ±0.72 | 1.25 | *C* | *2.2* | 2 | 22.14 | 2.08 | 3 | 20.18 | ±0.55 | 1.01 | *A* | *2.3* | 2 | 2.44 | 0.22 | 3 | 2.66 | ±0.14 | 0.27 | C |
| SD3 | | | | | | | | | *3.1* | 2 | 30.50 | 1.44 | 3 | 30.02 | ±0.34 | 0.64 | *B* | *3.2* | 2 | 18.81 | 0.15 | 3 | 17.62 | ±0.59 | 1.1 | *C* | *3.3* | 2 | 3.71 | 0.47 | 3 | 3.81 | ±0.26 | 0.39 | B |
| SD4 | | | | | | | | | *4.1* | 2 | 35.21 | 0.47 | 3 | 34.15 | ±0.62 | 1.14 | *A* | *4.2* | 2 | 19.29 | 0.82 | 3 | 19.42 | ±0.23 | 0.46 | *AB* | *4.3* | 2 | 2.91 | 0.34 | 3 | 2.83 | ±0.18 | 0.34 | C |
| SD5 | | | | | | | | | *5.1* | 2 | 31.46 | 0.79 | 3 | 30.01 | ±0.54 | 1.01 | *B* | *5.2* | 2 | 19.35 | 0.15 | 3 | 17.96 | ±0.93 | 1.82 | *BC* | *5.3* | 2 | 3.20 | 0.16 | 3 | 3.13 | ±0.08 | 0.15 | C |
| SD6 | | | | | | | | | *6.1* | 2 | 33.06 | 0.91 | 3 | 32.41 | ±0.78 | 1.53 | *A* | *6.2* | 2 | 14.30 | 0.11 | 3 | 15.32 | ±0.10 | 0.19 | *D* | *6.3* | 2 | 3.63 | 0.02 | 3 | 3.81 | ±0.16 | 0.30 | B |
| SD7 | | | | | | | | | *7.1* | 2 | 34.56 | 0.39 | 3 | 33.74 | ±0.94 | 1.87 | *A* | *7.2* | 2 | 18.20 | 0.65 | 3 | 18.20 | ±0.74 | 1.45 | *BC* | *7.3* | 2 | 3.32 | 0.04 | 3 | 3.77 | ±0.27 | 0.54 | B |

**Table 4.** Mean extractive yields (wt%) of branch tissue's branch bark (B > 7, BB), branch wood (B > 7, BW), branches of 1–7 cm (B1–7), and twigs and foliage (TF) after Soxhlet and ASE extraction. Tukey–Kramer HSD comparisons show whether significant differences exist between the different heights within branch tissues, where different letters indicate significant differences.

| Branch Bark (B > 7, BB) | | | | | | | | | | Branch Wood (B > 7, BW) | | | | | | | | | Branches of 1–7 cm (B1–7) | | | | | | | | | Twigs and Foliage (TF) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | | | Soxhlett | | | ASE | | | | ID | Soxhlett | | | ASE | | | | | ID | Soxhlett | | | ASE | | | | | ID | Soxhlett | | | ASE | | | | |
| | | | N | mean | range | N | mean | ±stdv | range | TK HSD | | N | mean | range | N | mean | ±stdv | range | TK HSD | | N | mean | range | N | mean | ±stdv | range | TK HSD | | N | mean | range | N | mean | ±stdv | range | TK HSD |
| B1 | B_a | 8.1 | 2 | 20.82 | 1.62 | 3 | 20.64 | ±0.33 | 0.58 | A | *8.2* | 2 | 6.28 | 0.35 | 3 | 5.87 | ±0.19 | 0.37 | A | *12* | 2 | 10.15 | 0.52 | 3 | 10.43 | ±0.36 | 0.71 | C | 16 | 2 | 24.99 | 0.41 | 3 | 23.58 | ±0.23 | 0.45 | A |
| B2 | B_b | 9.1 | 2 | 19.67 | 2.52 | 3 | 19.14 | ±0.70 | 1.38 | B | *9.2* | 2 | 4.94 | 0.53 | 3 | 5.17 | ±0.35 | 0.62 | B | *13* | 2 | 12.38 | 0.34 | 3 | 12.47 | ±0.31 | 0.59 | A | 17 | 2 | 23.79 | 1.50 | 3 | 24.25 | ±0.93 | 1.65 | A |
| B3 | B_c | 10.1 | 2 | 20.16 | 1.13 | 3 | 18.78 | ±0.72 | 1.43 | B | *10.2* | 2 | 5.10 | 0.16 | 3 | 4.94 | ±0.12 | 0.22 | B | *14* | 2 | 11.40 | 0.27 | 3 | 11.41 | ±0.05 | 0.10 | B | 18 | 2 | 22.75 | 2.14 | 3 | 24.38 | ±0.72 | 1.43 | A |
| B6 | B_d | 11.1 | 2 | 16.30 | 0.73 | 3 | 17.11 | ±0.36 | 0.63 | C | *11.2* | 2 | 3.71 | 0.61 | 3 | 3.71 | ±0.10 | 0.19 | C | *15* | 2 | 10.79 | 1.70 | 3 | 10.60 | ±0.34 | 0.60 | C | 19 | 2 | 23.54 | 0.19 | 3 | 23.45 | ±0.55 | 1.09 | A |

**Table 5.** Comparison of ASE extraction yields for adjacent stem and branch wood samples with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with a 2-tailed *t*-test. The values give prob > |t|, where values < 0.05 indicate significant differences.

| Stem Disc, Sample ID | Branch, Sample ID | Prob > \|t\| |
|---|---|---|
| SD7, 7.3 | B6, 11.2 | 0.7472 |
| SD6, 6.3 | B3, 10.2 | 0.0009 |
| SD5, 5.3 | B2, 9.2 | 0.0072 |
| SD4, 4.3 | B1, 8.2 | 0.0001 |

**Table 6.** Correlations of extractive yield (EY, (wt%)) after ASE extraction with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with height of tree axis from ground (HTA (m)), *p* values for intercept (prob > |t|, i, * marks significance ), slope coefficient (prob > |t|, sc, * marks significance) and $R^2$ for the respective correlations. Extractive yields for inner and outer bark yields were derived from analysis with three replicates on six different heights on the stem. Branch extractive yields BB and BW were derived from analysis with three replicates on four branches with different height onset on the stem.

| Tissue | Regression Expression | Prob> \|t\|, i | Prob> \|t\|, sc | $R^2$ | n |
|---|---|---|---|---|---|
| **BB** | EY = 23.715857 − 0.3379711 * HTA | <0.0001 * | <0.0001 * | 0.870 | 12 |
| **BW** | EY = 7.8406265 − 0.2056779 * HTA | <0.0001 * | <0.0001 * | 0.904 | 12 |
| **IB** | EY = 19.953178 − 0.1628571 * HTA | <0.0001 * | 0.0169 * | 0.308 | 18 |
| **OB** | EY = 27.616578 + 0.3219048 * HTA | <0.0001 * | 0.0012 * | 0.491 | 18 |

**Table 7.** Comparison of extraction methods ASE and Soxhlet with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with 2-tailed *t*-test. The values give prob > |t|, where values < 0.05 indicate significant differences.

| Stem Tissues Extracted | | | | | | HTA (m) | Branch Tissues Extracted | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FB | OB | IB | SW | | B > 7, BB | B > 7, BW | B1–7 | TF | | |
| | | | | | | ***19.30*** | 0.2181 | 0.9965 | 0.8631 | 0.8129 | **B6** | |
| | **SD7** | | 0.2704 | 0.9886 | 0.1016 | ***19.27*** | | | | | | |
| | **SD6** | | 0.3972 | 0.0017 | 0.1806 | ***16.07*** | | | | | | |
| | | | | | | ***15.4*** | 0.1846 | 0.2448 | 0.9299 | 0.3469 | **B3** | |
| | | | | | | ***13.2*** | 0.7465 | 0.545 | 0.7319 | 0.6668 | **B2** | |
| | **SD5** | | 0.0915 | 0.1215 | 0.5589 | ***12.87*** | | | | | | |
| | **SD4** | | 0.0908 | 0.8111 | 0.7203 | ***9.67*** | | | | | | |
| | | | | | | ***9*** | 0.864 | 0.1988 | 0.3425 | 0.0351 | **B1** | |
| | **SD3** | | 0.6222 | 0.0684 | 0.7409 | ***6.47*** | | | | | | |
| | **SD2** | | 0.9005 | 0.2903 | 0.249 | ***3.27*** | | | | | | |
| | **SD1** | 0.1117 | | | 0.757 | ***0.08*** | | | | | | |

Stem disc ID

Branch ID

Height of tree axis (HTA (m)).

Differences in the content and composition of extracts can occur due to the applied solvent. Additionally, the extracts in the wood of trees are species-specific, and differences can occur even within a species, depending on the age and location of the tree and environmental influences [9,53]. The most extractive-rich tissue, the outer bark of the stem, gave, on average, an extractive yield of 31.25 wt% in this present study. Pinto et al. found an extractive yield of 40% in the outer bark of birch after ethanol extraction [54]. Krasutsky, however, states that the average extractive content in the outer bark of *B. pendula* is equal to 27.5 wt%, showing that the presented results of this study are within the range of the expected extractive yields [9].

Vedernikov et al. investigated the chemical composition of stem bark dependent on the original height of the stem of *B. pendula*. Samples were taken from 10%, 30%, 60%, and 90% of the stem height, respectively, and extracted with ethanol in a Soxhlet apparatus. Vedernikov et al. found an increase in the extractive yield from 24.2 to 37.9%. Although our results also show increasing extractive yields from the bottom to the top of the trunk (27.14–33.74 wt%), the increase is much less pronounced [13].

The extractive yield of the inner bark with the solvent ethanol was between 13.6 and 17.3 wt% and decreased with increasing stem height [13]. Our extractions of the inner bark resulted in an average extractive yield of 18.12 wt%, with only a tendency to decrease extractive yields with the original height of the stem of the extracted sample (Table 6).

The extraction of birch stem wood resulted in extractive yields of 2.55–3.81 wt%, dependent on the height of the stem, which follows results from earlier studies reporting extractive yields between 0.8 and 5 wt% [24,26,29–31]. A Polish study showed geographic variations in extractive content in birch stem wood ranging from stand averages of 1.15–1.99 wt% [15].

Nurmi reported extractive yields of branch wood ranging from 7 to 8.2 wt%. In the present study, branch wood had higher extractive yields than stem wood, but with a total average of 4.9 wt%, the value for the wood of branches over 7 cm was lower than Nurmi reported [26].

Branch bark is younger than stem bark and hence shows less peel off through mechanical agency, contamination through the growth of algae and lichens, or simply deposition of dirt [55]. This combination could be one reason for higher extractive yields in the younger outer bark and could also be confirmed for other species, where extractive yields increased toward the top of the trunk [56]. The branches between 1 and 7 cm in thickness (B1–7) were analyzed for their extractive yield and composition as a mixture of outer branch bark, inner branch bark, and branch wood. This combination is represented in the extractive yields between 10.43 wt% and 12.47 wt%, giving a mixture of the extractive yields of branch bark and branch wood as a function of the sample-specific bark-to-wood composition.

Routa et al. summarized the extractive content of foliage of *B. pendula* from 28.8 to 33.4 wt%, and Stark et al. found latitudinal and regional influence on the extractive content composition of foliage of *B. pubescens* in Finland, where quercetin derivatives were positively correlated with latitude, and concentrations of apigenin and naringenin derivatives were negatively correlated with latitude [24,57]. The sample of twigs and foliage analyzed in the present study was a mixture of outer bark, inner bark, branch wood, and foliage, so our results (23.45–24.38 wt%) give a good representation of this mixture.

#### 3.2.2. Comparison of Extraction Methods and Statistical Tests for Conformance of Extraction Methods

The chemical composition of the different tissues shows typical distributions of single substances (betulin, lupeol, γ-sitosterol, and lupeone, Figures 2 and 3) and substance groups (carbohydrates, aromatics/phenolics, hydrocarbons, and terpenes, Figures 4 and 5). According to the statistical tests for conformance, the distribution of single substances and substance groups shows only slight variations between the extraction methods without consideration of the tissues. In contrast, major differences were observed in the occurrence of various substances or substance groups in different tissues. The outer bark section of stem discs and branches is characterized by high yields of betulin and their precursor lupeol, where the respective yields vary similarly (betulin: 300–500 µg/mg, lupeol 60–100 µg/mg, respectively, Figures 2 and 3). In addition, lupeone shows the same slight increase with the height of the tree axis. Comparing the different tissues in stem discs and branches shows that betulin is the dominant substance, with yields of up to 600 µg/mg at the outer bark. Betulin and the other detectable single substances could not be detected within the inner bark and wood tissue. Only γ-sitosterol could be detected in low amounts as a representative of plant sterols in wood.

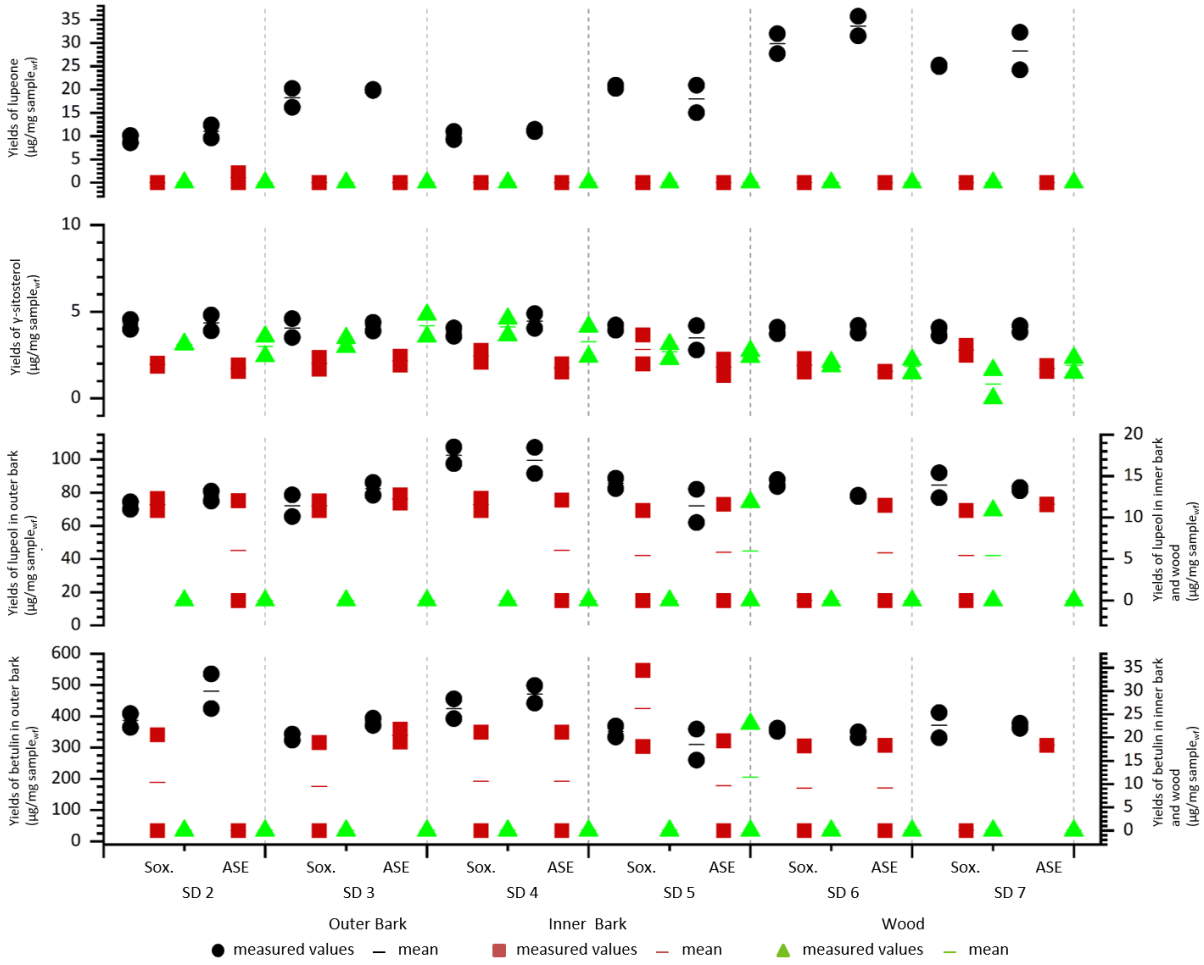


**Figure 2.** Overview of yields (µg/mg sample mf) of betulin, lupeol, γ-sitosterol, and lupeone in stem discs (SDs) of the different tissues extracted with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with ASE and Soxhlet (Sox.) (mf—moisture free).

In comparison to the outer bark tissue of stem discs, lower yields of the single substances were detectable in the corresponding branch samples. In addition, the same distribution of single substances between the outer bark and wood tissue was observed. Furthermore, the yields of betulin, lupeol, and lupeone increased at branch sample 6 (B6). The yields of extractable substances seem to increase with the height of branch onset on the stem and, indirectly, the age of the tissues [43].

Besides the single substances, several substance groups such as carbohydrates, aromatics/phenolics, terpenes, and hydrocarbons were detectable in the different tissues. However, some of these substances could not be conclusively identified during the GC-MS/FID campaigns and were therefore summarized by their basic chemical structure. While the outer bark tissue is characterized by high yields of terpenes and moderate yields of hydrocarbons and other unknown compounds, the tissues of inner bark and wood are described by higher yields of carbohydrates. Higher yields of aromatics/phenolics were only observed in inner bark tissues.

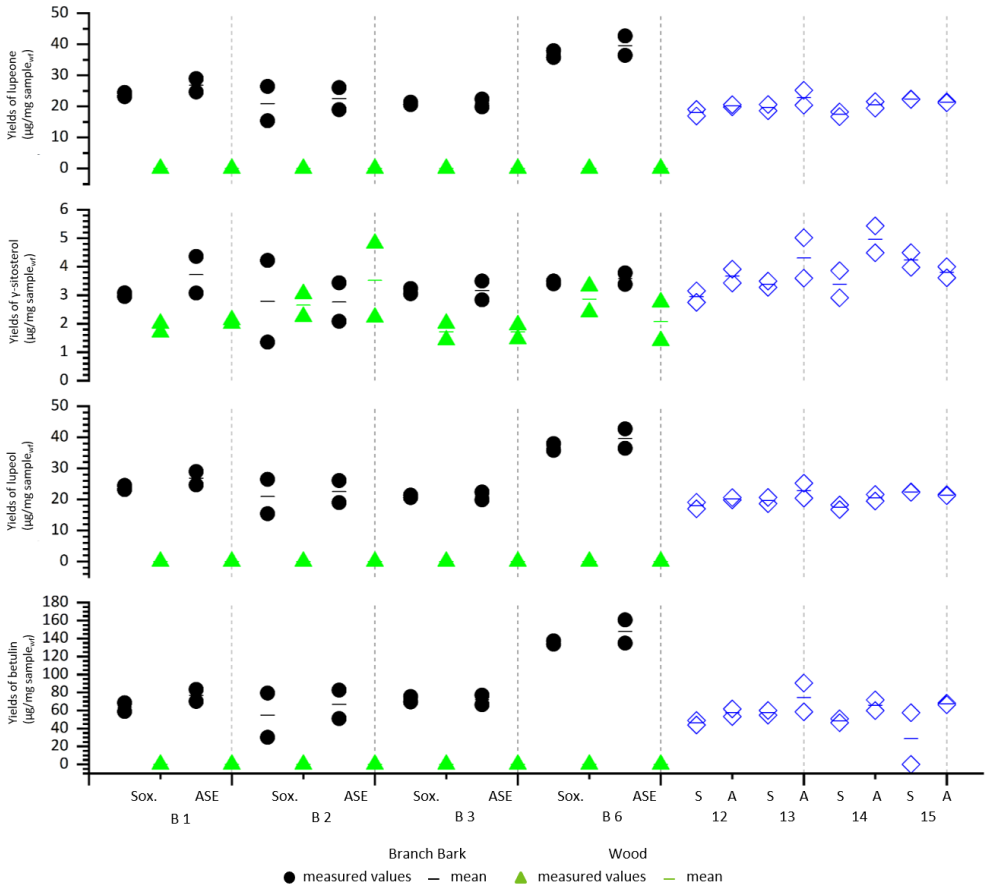


**Figure 3.** Overview of yields (µg/mg sample mf) of betulin, lupeol, γ-sitosterol, and lupeone in branches (B > 7; B1–7) of the different tissues extracted with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with ASE (A) and Soxhlet (Sox., S) (mf—moisture free).

The analysis of the outer bark extractives showed that compounds yielding 600 µg/mg and above were found. In all samples, betulin accounted for more than 60 wt% of the detectable compounds. The obtained results for Soxhlet extraction and ASE are comparable. Krasutsky describes that other triterpenes such as betulinic acid could be found besides betulin and lupeol and confirmed with the results obtained in this study [9]. According to Guo et al., the betulin and lupeol content can increase with higher temperatures and precipitation, but decrease with higher relative humidity when different birch locations are analyzed [53].

The analysis of the inner bark showed that phenolic compounds have the most significant share among the found substances and substance classes, followed by carbohydrates, betulin, and lupeol. The literature describes a high mixture of different compounds, where polyphenol-rich mixtures of procyanidin aglycones and phenolic compounds were found [58]. In most samples of the stem wood, less than 200 µg/mg was identified, and a wide variety of compounds were detected. The most significant share have carbohydrates. In contrast, the extractives of the wood sample are characterized by higher amounts of carbohydrates and average amounts of terpenes regarding the corresponding amounts in the outer and inner bark, respectively. Higher amounts of hydrocarbons also characterize the wood samples. The high amounts of unknowns are likely due to the fuzziness of the analytics than to natural distributions.

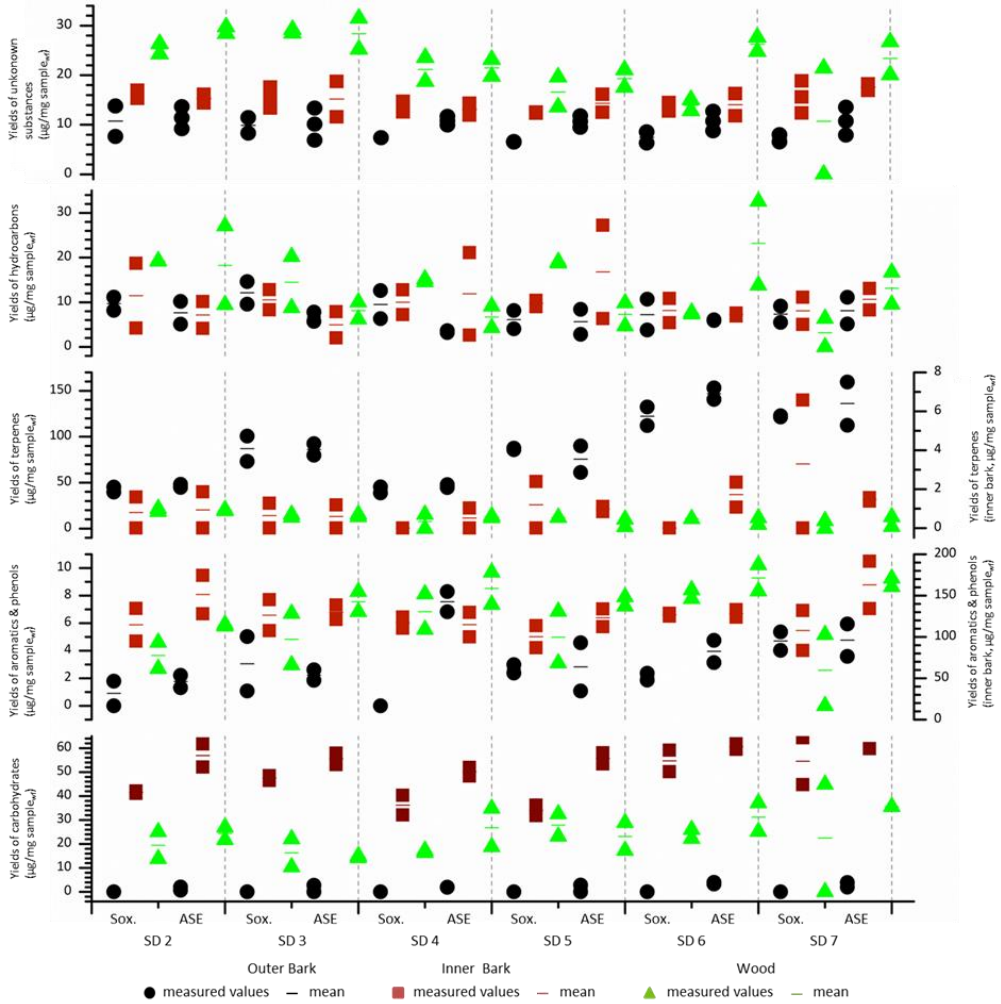


**Figure 4.** Overview of yields (µg/mg sample mf) of carbohydrates, aromatics/phenolics, terpenes, hydrocarbons, and unknown substances in stem discs of the different tissues extracted with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with ASE and Soxhlet (Sox.) (mf—moisture free).

Furthermore, except for γ-sitosterol, the named individual substances are hardly present in the wood extracts. Nor is a dependence of the amounts of the substance classes on the tree height apparent from the diagrams. Only the amounts of aromatics/phenolics show a slight increase at the branch fractions toward the stem fraction and an increase from SD 2 to SD 7.

Comparing the betulin and lupeol yields after Soxhlet and ASE by the tissue and height of the stem showed similar means for all comparisons. Only betulin yields of inner bark in SD7 were significantly different for the two extraction methods (data not shown). Linear regression models cannot prove a correlation between the height of the stem axis and the betulin content. A trend was only observed in the outer bark (OB), where the betulin content decreased with the original height of the stem, and in the branch bark (BB), where the betulin content seemed to increase with the onset of branch on the stem. For neither of these tissues, HTA was a significant variable for estimating the betulin content (Table 8). Lupeol increased with increasing height of the stem for the same tissues, branch bark, and outer bark (Table 8).

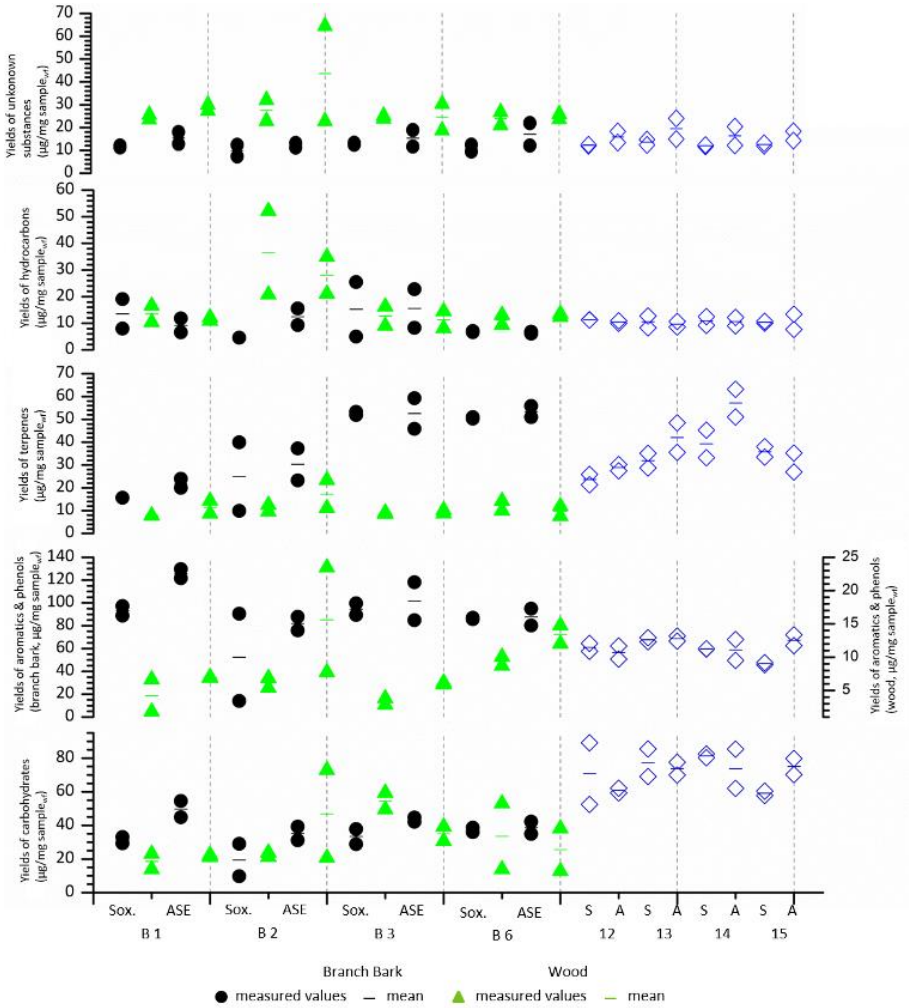


**Figure 5.** Overview of yields (µg/mg sample mf) of carbohydrates, aromatics/phenolics, terpenes, hydrocarbons, and unknown substances in branches (B > 7; B1–7) of the different tissues extracted with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with ASE (A) and Soxhlet (Sox., S) (mf—moisture free).

**Table 8.** Correlations of betulin (B, (µg/mg)) and lupeol content (L, (µg/mg)) after ASE extraction with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1) with height of tree axis (HTA (m)) from ground, *p* values for intercept (prob > |t|, i, * marks significance) and slope coefficient (prob > |t|, sc, * marks significance) and $R^2$. Outer bark betulin and lupeol yields were derived from two replicate analysis of 6 different heights on the stem. Branch bark betulin and lupeol yields were derived from two replicate analysis of 4 branches with different height onset on the stem.

| Tissue | Regression Expression | Prob > \|t\|, i | Prob > \|t\|, sc | $R^2$ | n |
|---|---|---|---|---|---|
| **OB** | B = 476.77273 – 7.5002786 * HTA | <0.0001 * | 0.0512 | 0.329 | 12 |
| **OB** | L = 6.8114721 + 1.1996753 * HTA | 0.1263 | 0.0043 * | 0.575 | 12 |
| **BB** | B = −2.851726 + 6.5923789 * HTA | 0.9463 | 0.0548 | 0.486 | 8 |
| **BB** | L = −0.268739 + 0.6458881 * HTA | 0.9347 | 0.0237 * | 0.602 | 8 |

### 3.2.3. Model Calculation

A model calculation of betulin yields (theoretical extractable) based on the described ASE trials (see Tables 2 and A1 and average botulin amounts) of different birch tissues showed a potential of approx. 23 kg of raw betulin from the model tree (Table 9). Although the outer bark only has a small share of all tissues, 40 wt% of the overall betulin yield

originates from the outer bark at stem fractions. When the bark of branches is included in the calculation, the betulin yield increases to approximately 76 wt%. However, no distinction was made between the outer and inner bark of the branches. The share of betulin from the inner bark at the whole betulin output is approximately 7 wt%, which is relatively low.

**Table 9.** Model calculation of betulin amounts (g, theoretical extractable) at different tissues, based on results of extraction trials (ASE) with ethyl acetate, ethanol, and water (v:v 4.5:4.5:1).

| Tissue | Betulin Amount (Theoretical Extractable) (g) | Standard Deviation |
|---|---|---|
| **Stem** | | |
| fissured coarse bark | 1455.0 | 0.0 |
| outer bark | 9374.6 | 420.3 |
| inner bark | 1706.6 | 76.5 |
| **Wood** | 2258.5 | 89.63 |
| **Branch (B > 7 cm)** | | |
| bark | 3087.2 | 93.84 |
| wood | 0.0 | 0.0 |
| **Branch (B < 7 cm)** | | |
| bark/wood | 5533.8 | 310.34 |
| ***Total*** | *23,415.6* | |

In contrast, the betulin yield from the branches represents at least one-third (36.8 wt%) of the whole betulin output. Thus, it is worth considering including the thinner branches in future utilization by extraction, especially if no alternative material utilization possibilities are planned or exist.

## 4. Conclusions

Separated tissues from different original heights of a single birch tree were extracted with the ternary solvent system ethyl acetate, ethanol, and water with ASE and Soxhlet extraction. The extraction methods ASE and Soxhlet resulted in comparable extraction yields, compositions, and single extractives for examined tissues and tissue mixtures. The analysis highlights that for precise and targeted analysis for specific applications, ASE emerges as the preferred method and should be strongly considered. Outer bark has the highest extractive yields; stem wood has the lowest yields; and branch wood has extractive yields above stem wood. Branches are tissue mixtures, and this mixture is reflected in attained yields. Extractive yields are correlated with the original height of the stem, where the inner bark is negatively correlated with stem height, and the outer bark is positively correlated with the original sample height of the stem. The single substances found are betulin, lupeol, γ-sitosterol, and lupeone. The yields of single components differ significantly between the different tissues. The substance classes found were carbohydrates, aromatic compounds, phenols, terpenes, and hydrocarbons. The composition of the substance classes and the distribution of single substances are tissue-dependent. Dependence on the original height of the stem is more evident for the lupeol than for the betulin concentration; increasing the original height of the stem and increasing branch onset increases the lupeol content. Primary betulin sources of the calculated betulin output are the outer bark of the stem and the branches. By using small branches, further potential for the extraction of betulin can be utilized. A model calculation of the betulin content in the current birch tree revealed a significant potential of 23 kg of betulin available as a valuable chemical resource after by-product utilization.

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**Data Availability Statement:** The data presented in this study are available in the article. Additional data material can be provided upon request.

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## Appendix A

**Table A1.** Identification of stem discs with measured parameters and subsequent extraction.

| Stem Parts Making up the Entire Trunk (SP) | Original Height of the Stem (m) | Length (m) | Diameter (cm) | Weight (kg) | X: Stem Part Selected for Extraction | Stem Disc ID for Extraction (SD) |
|---|---|---|---|---|---|---|
| SP1 | 0.08 | 0.87 | 47.00 | 118.50 | X | SD1 |
| SP2 | 3.27 | 2.47 | 40.50 | 276.40 | X | SD2 |
| SP3 | | 1.30 | 37.00 | 118.30 | X | SD2 |
| SP4 | | 1.26 | 34.50 | 110.20 | | |
| SP5 | 6.47 | 1.24 | 33.00 | 103.10 | X | SD3 |
| SP6 | | 1.01 | 34.00 | 77.10 | | |
| SP7 | | 1.23 | 34.00 | 96.40 | | |
| SP8 | 9.67 | 1.24 | 30.50 | 80.50 | X | SD4 |
| SP9 | | 1.31 | 29.50 | 80.20 | | |
| SP10 | 12.87 | 1.40 | 27.50 | 79.50 | X | SD5 |
| SP11 | | 0.98 | 25.50 | 47.90 | | |
| SP12 | | 1.27 | 24.00 | 57.50 | | |
| SP13 | 16.07 | 1.17 | 23.00 | 47.60 | X | SD6 |
| SP14 | 19.27 | 2.65 | 18.00 | 76.80 | X | SD7 |

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