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ORIGINAL ARTICLE

Extractives in Douglas firs (*Pseudotsuga menziesii* (Mirb.) Franco) from three sites in south-west Germany and potential opportunities for valorization

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Abstract
Owing to favorable wood properties and its resilience to the effects of climate change, Douglas fir (*Pseudotsuga menziesii*) is a promising tree species in Central Europe. Its wood and bark extractives could also serve as feedstock for the production of bio-based materials and platform chemicals. In this study, sapwood, heartwood, knotwood and bark extractives of Douglas firs originating from three differently aged stands in south-west Germany were investigated. Two different extraction methods with organic solvents were compared: a mixture of cyclohexane/ethanol and a successive method using petroleum ether, acetone and methanol. Extraction yields obtained with the successive method were higher, however one-step extraction was very efficient considering the number of samples that could be extracted and is therefore useful for a fast screening. At all sites, extract yields from sapwood, heartwood and bark were highest close to the tree top, while at the two older sites, an additional maximum was found at 1.3 m height. Knotwood extractives tended to decline with increasing tree height. The most abundant substance with economic importance was taxifolin, yielding up to 13% of dry weight in bark, whereas knotwood contained high amounts of resin acids and the lignan nortrachelogenin. Contrary to other studies, the present study found no evidence of a significant site effect on yield and composition of extracts. Overall, many different compounds for future bio-economic applications were found so that the preferential utilization of extractive-rich bark and crown material could foster an integral valorization of trees, supplementing with existing high grade timber production.

1 Introduction

The transition from a fossil-based economy towards a sustainable bio-economy is part of the efforts to save resources, mitigate climate change and protect the environment. In this context, by-products from the wood and timber industry are a promising feedstock for the production of bio-based materials, fuels and platform chemicals (Feng et al. 2013; Hazeena et al. 2020; Liao et al. 2020). Nowadays, the structural components cellulose, hemicelluloses and lignin are the most abundant feedstocks for biorefineries. However, accessory wood components can likewise serve as precursors for bio-based materials and economically significant chemicals. Classified as extractives, these non-structural organic compounds comprise 1–20% of the wood's dry mass, depending on tree species and tissue type. Already in the 1960s, wood and bark extractives received increased attention for their potential to generate added-value products from harvest residues (Erdtmann et al. 1968; Holmes 1961). Since then, content and composition of extractives were determined for many tree species, revealing high inter- and intraspecific variation caused by ecological factors, tree age and genetic diversity (Fries et al. 2000; Kebbi-Benkeder et al. 2015; Klasnja et al. 2003). In addition, pronounced within-tree variation along and across the stem was observed (Adamopoulos et al. 2005; Brennan et al. 2020a; Kebbi-Benkeder et al. 2017; Vek et al. 2020). Due to their enormous variability in content and chemical composition, extractives also affect the suitability of wood for technical applications. For instance, some compounds generate additional costs in paper production as they act as pulping inhibitors or cause an excessive need of bleaching to obtain the aimed degree of whiteness (Gardner and Hillis 1962). Others affect acidity and wettability of wood, which are both important factors

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regulating natural durability (Windeisen and Wegener 2003) and bonding properties of wood (Özparpucu et al. 2022; Roffael 2016).

A promising tree species to meet the current needs of the forest industry and a future bio economy is Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Due to its favorable mechanical properties (Lowell et al. 2014), it is an economically significant species in North America and might gain additional importance in Central Europe as it appears well adapted to climate change. Douglas fir could partly substitute Norway spruce (*Picea abies* (L.) Karst.), which is currently extensively cultivated in Central Europe but less resilient to increased frequency and intensity of summer droughts (Vitali et al. 2017). This raises the question how a future increased availability of Douglas fir wood and harvest residues could be exploited for innovative high-value purposes. Heartwood, knotwood and bark of Douglas fir contain economically significant polyphenols including flavonoids, lignans and stilbenes (Dellus et al. 1997; Willför et al. 2003a). These compounds consist of benzene rings with two or more hydroxyl groups and are frequently reported to exert antioxidant, antibacterial, anti-inflammatory and anti-carcinogenic activity (Miranda et al. 2021; Willför et al. 2003a). The lignan hydroxymatairesinol (HMR) was found at concentrations of up to 3% in the sapwood of Douglas fir (Mbakidi-Ngouaby et al. 2018). HMR is an excellent platform to synthesize other important lignans (Holmbom et al. 2003) and potentially has protective effects against cardiovascular diseases and hormone-dependent tumors (Saarninen et al. 2000; Soleymani et al. 2020). Taxifolin, also known as dihydroquercetin is a flavonoid usually obtained from Siberian larch (*Larix sibirica* Ledeb) and commercially used in food supplements and pharmaceuticals. In the heartwood of Douglas fir, taxifolin was present with concentrations of up to 1.5% in a study by Dellus et al. (1997). Brennan et al. (2020a) found taxifolin yields of up to 20% by weight in bark extracts of Douglas fir. Also belonging to the group of flavonoids, condensed tannins are oligomers of flavan-3-ols including catechin, epicatechin or gallocatechin. Industrially used tannins for leather tanning, drilling muds and ore flotation (Fraga-Corral et al. 2020; Oleson and Schwartz 2016) are commonly extracted from tropical *Quebracho* and *Mimosa* species, even though reasonable yields can be obtained from European conifers (Bianchi et al. 2015). In the bark of Douglas fir, condensed tannins accounted for up to 18% of dry weight in a study by Kurth (1953). In recent advanced approaches, condensed tannins were processed into industrial foams, adhesives and resins (Feng et al. 2013; García et al. 2014; Lacoste et al. 2015). Some tannin-based foams showed favorable insulating properties and fire resistance (Pizzi 2019), while tannin-based adhesives revealed low aldehyde emissions (Hemmilä et al. 2017) and are therefore suitable for indoor applications. Fatty acids, waxes, phytosterols, resin acids and other terpenoids are found in high concentrations in the bark of softwood species, but also in their sapwood and heartwood (Nisula 2018). Resin products from Douglas fir can be used to produce adhesives, printing inks and emulsifiers, while phytosterols serve as ingredients for cosmetics, food supplements and pharmaceutical steroid hormones (Nisula 2018; Oleson and Schwartz 2016).

Overall, Douglas fir wood and harvest residues are a promising feedstock for various added-value products. However, studies on extractive contents in Douglas fir grown under conditions in Central Europe are scarce and intraspecific variability as well as differences within individual trees remained mostly unregarded. The aim of this paper is therefore, to characterize extractive contents of Douglas firs grown in south-west Germany, which is assumed representative for environmental conditions in Central Europe. Special attention was paid to tree age, tissue age, tissue type and sampling position within the tree in order to provide indication, which tree compartments could be of special interest for extractives from a bio-economic point of view.

2 Materials and methods

2.1 Wood and bark sampling

Sampling trees were felled in October and November 2020 in three Douglas fir stands of different ages located in south-west Germany. Tree and site characteristics are given in Table 1. Stem discs from three trees per stand were taken within few hours after felling at 1.3 and 11.5 m tree height and at 40 and 80% length of the remaining stem length as shown in Fig. 1A. From each sampling height between 11.5 m and tree top, one disc containing knotwood (defined as the parts of branches embedded in the stemwood) was taken and another one without knotwood. In total, 7 discs per tree were sampled. The age of every stem disc was recorded along with heartwood and total diameter and bark thickness in every quadrant of a stem disc.

From the stem discs without knotwood, four samples covering an identical cambial age range were cut out from the heartwood and sapwood respectively (Fig. 1B). The range of cambial age (ring number from the pith) of each wood sample was determined. Bark samples were taken at four equally distributed positions around the disc. For the discs containing knots, a band saw and a chisel were used to separate knotwood from the surrounding stemwood. The four replicates per tissue type and disc were merged into a mixed sample and air-dried.

Table 1 Average age, height and diameter at breast height (DBH) of the three sample trees per site in addition to geographic location, elevation (m above sea level), mean annual temperature (MAT) and mean annual precipitation (MAP) of the investigated sites in Baden-Württemberg, south-west Germany

Site	Tree characteristics			Site characteristics			
	Age [a]	Height [m]	DBH [cm]	UTM Location	Elevation [m asl]	MAT [°C]	MAP [mm]
Odenwald	42	27.5	30.3	32U	280	8.2	831
		30.4	35.3	520,382.78			
		33.8	38.8	5,489,549.65			
Wildtal	89	45.5	64.3	32U	580	9.1	1200
		50.7	71.9	418,615.67			
		50.4	85.0	5,318,763.68			
Altdorfer Wald	130	46.5	74.0	32T	550	7.9	927
		48.4	83.3	549,785.15			
		49.1	85.5	5,304,870.01			

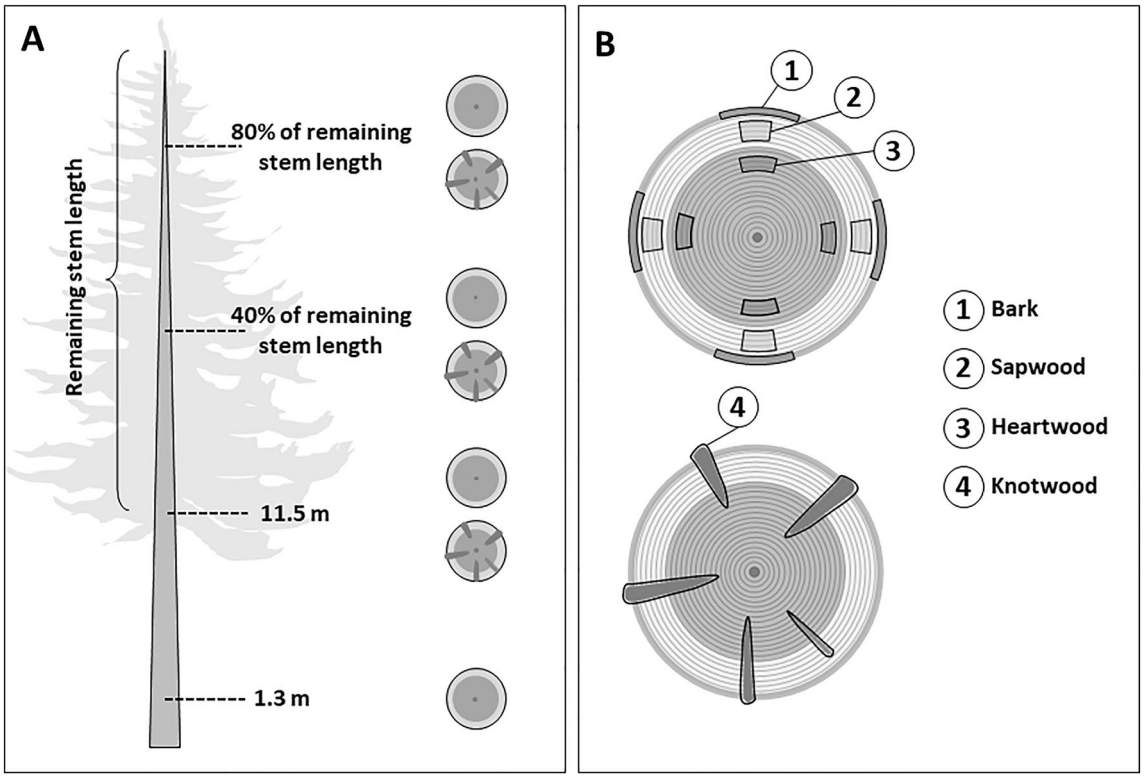

Fig. 1 Schematic overview of the sampling for stem discs at defined tree heights (**A**) and for the sampling of different tissue types originating from the stem discs (**B**)

2.2 Volumetric estimates

Total stem volume including bark and volume of stemwood and heartwood was calculated as sum of conical sections using the different sampling heights and corresponding diameters at the lower and upper height level of truncated cones according to Gominho and Pereira (2000):

$$V = \frac{h}{3}\left(s_a + s_b + \sqrt{s_a * s_b}\right) \quad (1)$$

where s_a is the area at the lower height level; s_b the area at the higher height level and h the height of the stem section. Bark volume was calculated as the difference between total tree volume and stemwood volume; sapwood volume was

calculated as the difference between stemwood and heartwood volume. Radial sapwood width relative to the stem diameter was calculated at every sampling height as a fraction of the stem disc radius. For knotwood samples, the mass of visually identified dead knots (lose knots) was calculated as a fraction of total knot mass in each sampling height.

2.3 Sample preparation and solvent extraction

Air-dried samples were ground under constant cooling with dry ice in a two-stage procedure (pre-grinding with IKA A11 basic) to a particle size of 0.75 mm (final grinding with Retsch mill SM1). All samples were extracted in a Soxhlet apparatus using 5 g of ground material and a 1:1 mixture of cyclohexane and ethanol as solvent for 6 h. Additionally, samples from one tree per site were extracted in a successive three-step procedure using solvents covering (1) petroleum ether, (2) acetone and (3) methanol (6 h extraction duration each). After extraction, the solvents were evaporated with a rotary evaporator before extract contents were determined gravimetrically relative to the dry weight of the sample.

2.4 Qualitative analysis by gas chromatography with mass spectrometer (GC/MS)

Cyclohexane/ethanol extracts from one tree per site were analyzed qualitatively. Additionally, sapwood, heartwood and bark extracts attained by successive extraction from 1.3 m sampling height and knotwood extracts from 80% remaining stem length were analyzed for one tree per site. Dried extracts were dissolved in dimethylformamide and silylated with a 99:1 mixture of N,O-bis(trimethylsilyl)acetamide (BSTFA) and trimethylchlorosilane (TMCS) before heating for 1 h at 80 °C. Heneicosanoic acid was added as an internal standard. The conditioned samples were injected into an Agilent 7890A gas chromatograph (Agilent, Santa Clara, California, USA) equipped with a mass spectrometer VL MSD 5975C (Agilent, Santa Clara, California, USA). A capillary column BPX5 with 30 m length, 0.25 mm inner diameter and a film thickness of 0.25 µm (SGE, Melbourne, Australia) was used for the separation of compounds. The temperature program started at 100 °C with a heating rate of 10 °C min^{-1} until a maximum oven temperature of 320 °C was reached. The injector temperature was set to 320 °C and a split ratio of 1:30 was applied. An internal database was used to identify the compounds.

2.5 Quantitative analysis by gas chromatography with flame ionization detector (GC/FID)

Samples for quantitative analyses were pre-treated in the same way as for the qualitative analyses. Quantification of detected compounds was conducted using heneicosanoic acid as internal standard and taxifolin and secoisolariciresinol as external standards. The gas chromatograph was equipped with a flame ionization detector FID 2010 (Shimadzu, Kyoto, Japan) and a capillary column BP5 with 30 m length, 0.25 mm inner diameter and a film thickness of 0.25 µm (SGE, Melbourne, Australia). The detector temperature was set to 330° C and a split ratio of 1:40 was used. Oven heating program and injector temperature were the same as for qualitative analyses.

2.6 Data analyses

Calculations and statistical analyses were done in R version 4.0.5 (R Core Team 2021). Dunn's test for multiple comparisons was applied to test for significant differences ($p < 0.05$) in extractive contents between sites, tissue types and sampling heights using the R package "dunn.test" (Dinno 2017). 95%-confidence intervals of medians were approximated according to McGill et al. (1978).

Multiple regression models were applied in order to analyze the relationship between captured variables and extractive contents. An approximation of the dependent variable to normal distribution was achieved through log transformation. As there was no indication of non-linearity, a linear model was chosen. Model optimization was conducted by testing all potential variables and their combinations along with subsequent comparison of R^2, and Aikaike Information Criterion (AIC). Collinearity of tested variables was evaluated by calculating variance inflation factors. Only variables with a significant effect ($p < 0.05$) were regarded in the final model.

3 Results and discussion

3.1 Tree characteristics

Extractive yield and composition differ widely between tissue types of Douglas fir (Oleson and Schwartz 2016). It is therefore important to estimate volume fractions of each tissue type in order to assess the potential for bio-economic applications. At the youngest site Odenwald, the total stem volume of Douglas firs was lowest with values ranging between 0.81 and 1.70 m^3 (Table 2). Differences between the two older stands were small with volumes of 8.16 m^3 (Wildtal) and 8.42 m^3 (Altdorfer Wald). The volumetric proportion of bark relative to the total stem volume increased with stand age from on average 11.3% at Odenwald, to 12.7% at Wildtal and to 13.1% at Altdorfer Wald. These values are in the range of observations for Douglas fir by Cardoso and Pereira (2017) and McConnon et al. (2004).

Relative radial sapwood width increased with tree height, however younger trees tended to have higher proportions of

Table 2 Total volume and volume of sapwood, heartwood and bark of field fresh Douglas firs originating from the three investigated sites

	Odenwald	Wildtal	Altdorfer Wald
Stem volume [m^3]	1.15 (0.81, 1.70)	8.16 (5.40, 9.98)	8.42 (7.09, 9.48)
Sapwood [m^3]	0.41 (0.32, 0.90)	3.28 (1.73, 3.39)	2.69 (2.57, 3.48)
Heartwood volume [m^3]	0.61 (0.38, 0.62)	3.83 (2.94, 5.13)	4.65 (3.42, 4.74)
Bark volume [m^3]	0.13 (0.12, 0.19)	1.04 (0.73, 1.46)	1.10 (0.98, 1.35)

Median values are given with respective minima and maxima in parentheses

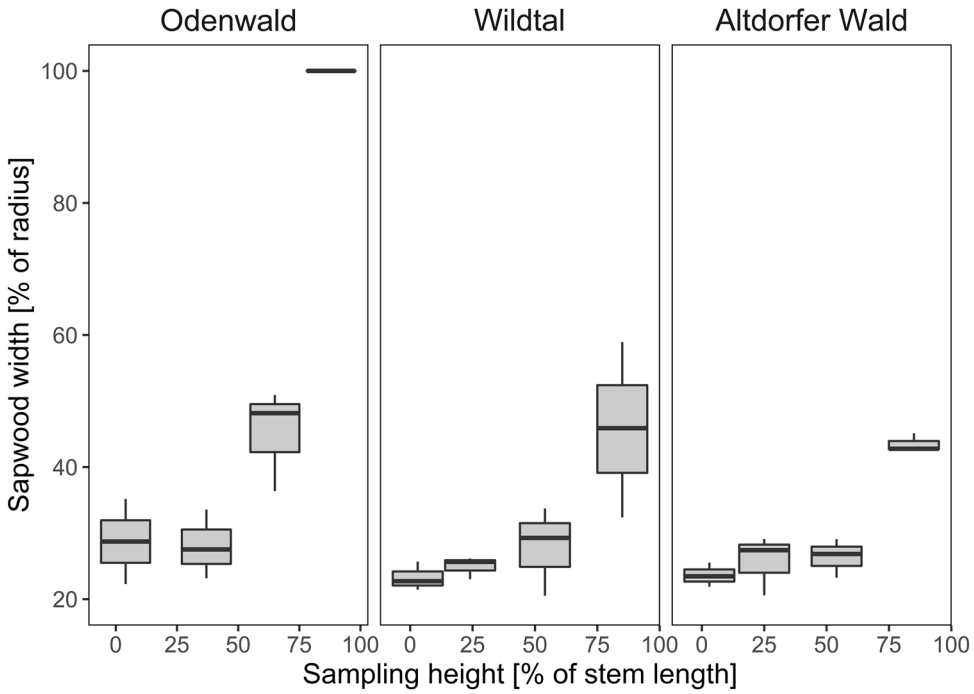

Fig. 2 Boxplots of the radial proportion of sapwood width as a percentage of total disc radius for different sampling heights of Douglas firs originating from three different sites

sapwood than older trees (Fig. 2). At the highest sampling positions of Douglas firs originating from the site Odenwald (age of the stem discs between 6 and 9 years), heartwood formation had not yet started.

Dead knots were exclusively found in samples from the two older sites Wildtal and Altdorfer Wald. Mass fractions relative to the total knotwood ranged between 10 and 100% (Fig. 3). A significant correlation between dead knot mass fraction and stem disc age was found ($R^2 = 0.55$, $p = 0.01$). The youngest stem disc containing dead knotwood was 46 years old.

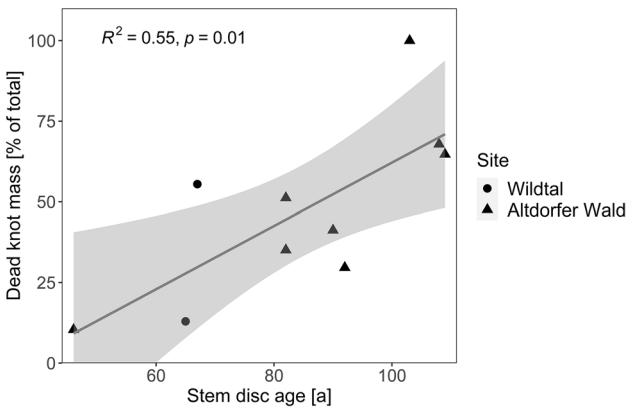

Fig. 3 Mass of dead knots relative to total knot mass as a function of stem disc age. The shaded area represents the 95% confidence interval of the linear regression line

3.2 Total extractive yields

The experimental design of the present study including different sites with several trees per site, four sampling heights and the most important tissue types is unique for studies of Douglas fir extractives. Owing to this comprehensive experimental setup, an adaption of chemical analyses was required, resulting in a single-step extraction method for the greater part of the samples, whereas three-step extraction was only performed for one tree per site. Figure 4 shows extraction yields obtained with the successive extraction method compared to the single-step extraction. Yields of the successive

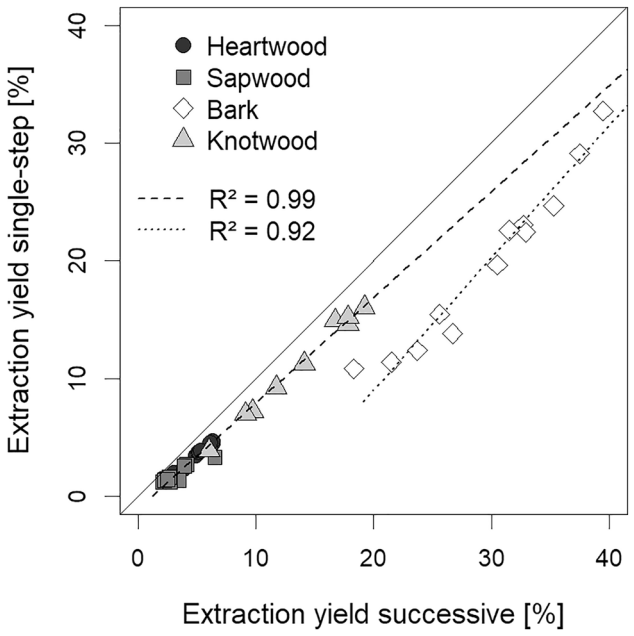

Fig. 4 Comparison of extractive contents yielded with a single-step extraction procedure with a 1:1 mixture of cyclohexane and ethanol and a successive extraction procedure with petroleum ether, acetone, methanol

extraction were in all cases higher compared to the single-step extraction due to longer extraction duration and the slightly wider polarity range of the three solvents; differences were most pronounced for the bark samples, where extraction yield was on average 11.6% lower, followed by knotwood, heartwood and sapwood with 2.8, 1.3 and 1.3%, lower yields, respectively. For an initial and comparative overview of extractives, these results are satisfactory given that a three times greater output of analyzed samples during the same time was possible. Yet, bark extraction with the cyclohexane/ethanol mixture did not recover fatty acids and other lipophilic compounds. Neither extraction method recovered suberin, which is a major cell wall component of Douglas fir bark (Cardoso et al. 2018), or building blocks of it. For analysis of suberin, a depolimerization reaction would have been necessary. Depending on the target compounds, it might therefore be necessary to optimize laboratory methods and to use more specific solvents corresponding to the polarity of the substances of interest. For instance, the recovery of tannins is best accomplished by extraction with hot water (Bianchi et al. 2015), a mixture of water and ethanol (Pan et al. 2012) or water with sodium-hydroxide, sodium sulfite and sodium bisulfite (Chupin et al. 2013).

At all investigated sites, total extractive yields (% by dry weight) obtained with the single-step method were highest in the bark with values of up to 25.2%, followed by up to 16.2% in the knotwood, 5.0% in the heartwood and 3.0% in the sapwood. Higher extractive contents in the heartwood compared to the sapwood are commonly attributed to the synthesis of organic compounds during the conversion of sapwood to heartwood (Taylor et al. 2007), providing resistance to fungal decay, microbes and insect infestation (Schultz and Nicholas 2000). In knotwood, elevated extractive contents likewise provide protection against pathogens, especially because dead or broken branches constitute a preferential entrance for microorganisms (Mbakidi-Ngouaby et al. 2018; Välimaa et al. 2007). High extractive contents in the bark result from the formation and translocation of photosynthates through the inner bark (phloem) in addition to the protective function of outer bark against pathogens by high contents of polyphenolic substances that act as biocides and radical scavengers (Pietarinen et al. 2006).

Extractive yields presented here are in agreement with other studies investigating different tissue types in Douglas fir (Ferreira et al. 2015; Kebbi-Benkeder et al. 2015; Mbakidi-Ngouaby et al. 2018; Välimaa et al. 2007). Nevertheless, one should bear in mind that cross-study comparisons are difficult due to the diversity of extraction procedures and analytical methods. Furthermore, seasonal variation of extractive contents can occur due to changes in metabolic activity (Mbakidi-Ngouaby et al. 2018), additionally complicating inter-study comparison when sampling date or season are not specified. In the present study however, seasonal effects can be excluded, since sampling of the three stands was performed at the same time of the year.

Extractive contents of all tissue types displayed a more or less pronounced dependence on sampling height, which was best described by a second-degree polynomial function (Fig. 5). Extractive yield from heartwood and sapwood was mostly higher close to the stem base and to the treetop compared to the intermediate stem parts. Knotwood extractive yield tended to decline with sampling height, even though the relationship was only significant for the site Altdorfer Wald ($R^2 = 0.65$, $p = 0.04$). This is in agreement with Kebbi-Benkeder et al. (2017) who found – starting from the crown base—decreasing extractive contents in the knotwood of *Abies alba*. For Douglas fir, a comparable trend was evident in a study by Brennan et al. (2021), however a transition zone was identified at 45–55% of the tree height, below which lower extraction yields were found than above. This pattern resembles the development of extractive yields with sampling height at the site Wildtal (Fig. 5). For bark, there was a positive trend between sampling height and bark extractive contents in the youngest stand Odenwald (42 years). Such height-dependency was likewise found in extracts of 43- to 57-year-old silver fir (*Abies alba* Mill.) bark (Brennan et al. 2020a, b) and in the bark of 53-year-old Douglas firs (Brennan et al. 2020a, b). These observations are well explicable by an increasing ratio of inner bark to outer bark with decreasing bark maturity in greater sampling heights and the fact that inner bark contains more extractives than

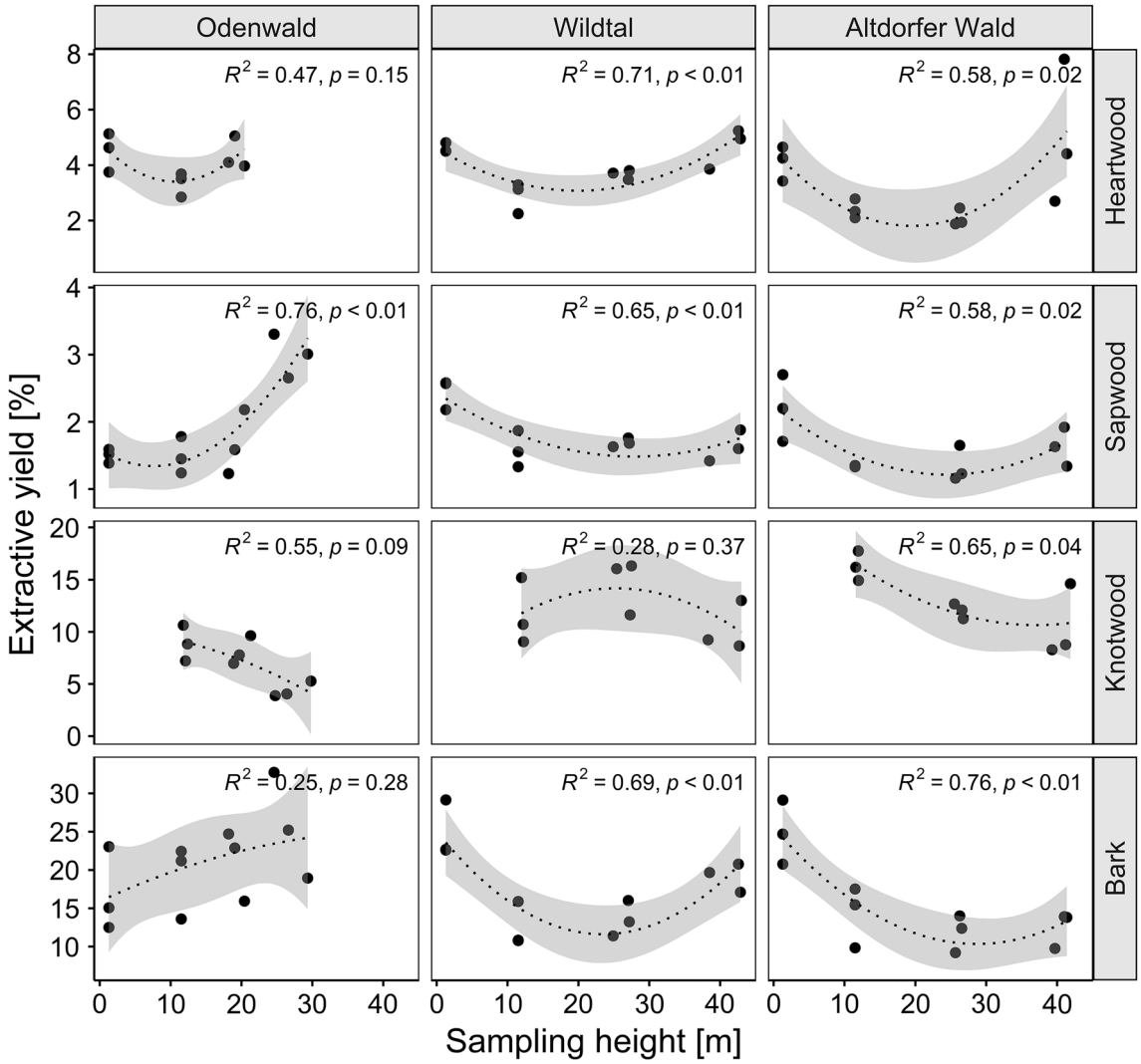

Fig. 5 Total extractive yields at the three sites in all tissue types obtained by single-step extraction with a 1:1 mixture of cyclohexane and ethanol as a function of sampling height. The shaded area indicates the 95% confidence interval of the second-degree polynomial fit (dashed lines)

outer bark (Eberhardt 2013). Moreover, formation and translocation of metabolites in the crown may explain elevated extractive contents in greater heights (Cardoso et al. 2018). At the two older sites Wildtal (89 years) and Altdorfer Wald (130 years), extractive yields had an additional maximum close to the stem base (Fig. 5). This is in line with findings at two sites in Portugal by Miranda et al. (2021) and may be explained by the formation of cork in the mature bark of Douglas fir. Extractives can make up about 50% by weight of the cork-rich outer bark (Cardoso et al. 2018), which is found in greatest amounts at the lower (older) stem parts, while cork has not yet formed in upper stem regions.

Overall, besides tissue type, the vertical position within the tree is the most obvious and straightforward factor that must be considered when an optimized valorization of extractives is aimed at. Nevertheless, there are several other variables that could explain extractive contents, possibly in more detail. For instance, the effect of tissue age could be masked when only sampling height is regarded, owing to the inherent relationship between both variables. This can be visualized for sapwood and heartwood when extractive yield is plotted against cambial age of the sample as shown in Fig. 6. Moreover, radial position in the stem (Gierlinger and Wimmer 2004; Vek et al. 2020), growth ring width (Taylor et al. 2003) or tree age (Morais and Pereira 2011) are possible factors affecting wood extractives. Thus, in order to identify further possible variables with significant impacts on total extractive yield, a multivariate approach was pursued.

Tested variables to explain *extractive yield* were *site*, *tree age*, *sampling height*, *average vertical growth rate*,

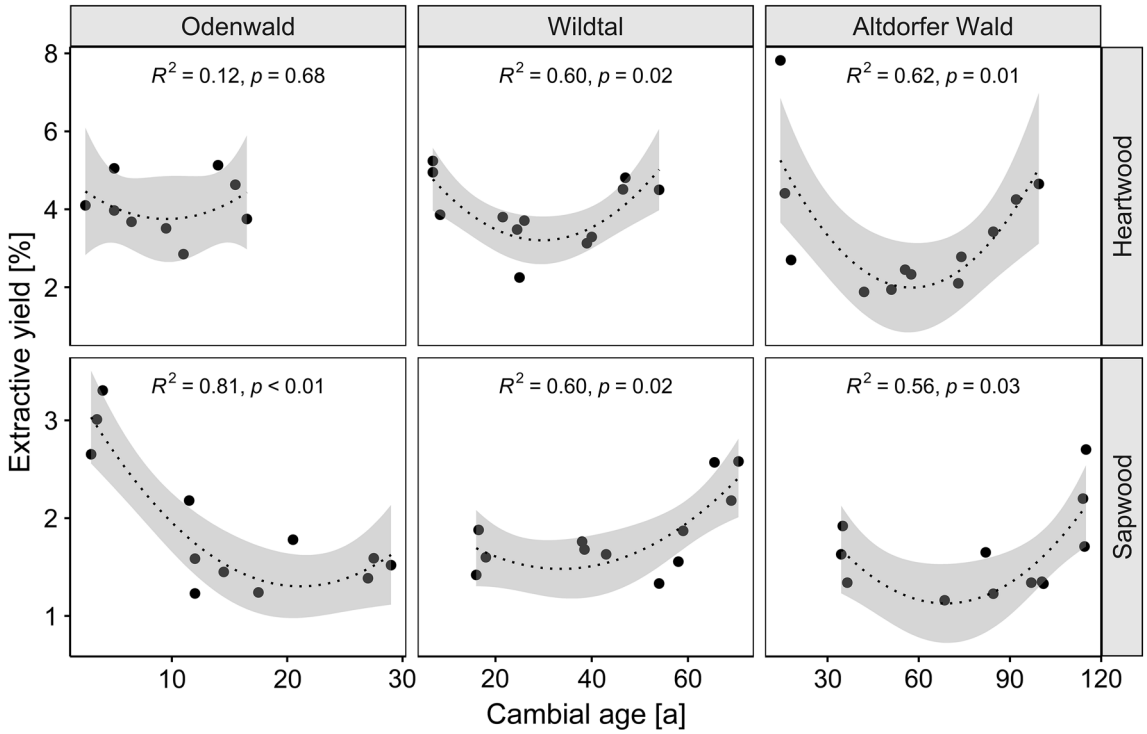

Fig. 6 Total extractive yields at the three sites in heartwood and sapwood obtained by single-step extraction with a 1:1 mixture of cyclohexane and ethanol as a function of cambial age of the sample. The shaded area indicates the 95% confidence interval of the second-degree polynomial fit (dashed lines)

average secondary growth rate, *tissue type*, *cambial age of the sample* (only for sapwood and heartwood), *radial sapwood width* and *age of the stem disc*. Initial attempts with mixed effects models were discarded, as the introduction of a random effect (e.g. a tree identification number) did not improve the model's AIC and/or R^2. The best model in terms of R^2 and AIC was obtained using the interaction between the two continuous variables *sampling height* and *age of the stem disc* along with the categorical variable *tissue type* as independent variables. As cambial age of the sample was available only for heartwood and sapwood, the *age of the stem disc* was used instead, in order to account for age effects within all tissue types. By this means, a variable was available, which was assignable to every single sampling height within each individual tree. A summary of the final model is given in Table 3. The variable *site* was not significant when introduced into the model, neither was *tree age*.

The absence of a significant effect of the variable *site* on extractive yields does not necessarily indicate that site-specific characteristics (climatic conditions, available resources etc.) are negligible. It is likely that the interaction between *sampling height* and *age of the stem disc* indirectly comprises site-specific traits, which affect tree growth and thus extractives. Specifically, the age of the stem disc of a rapidly grown tree at a defined sampling height is lower than the age of a slowly grown tree at the same height; accordingly, the interaction between those variables can be interpreted as related to growth rate. This assumption was supported by an

Table 3 Coefficients of the final linear model with indication of degrees of freedom (Df), adjusted R^2 and overall model p-value

	Estimate	Standard error	p-value	Df	Adj. R^2	p-value
Intercept	2.192	0.1127	< 0.001	125	0.89	< 0.001
Age of the stem disc	– 0.0003	0.0014	< 0.01			
Sampling height	0.0010	0.0042	< 0.05			
Tissue sapwood	-1.805	0.0764	< 0.001			
Tissue bark	0.485	0.0764	< 0.001			
Tissue heartwood	– 1.062	0.0838	< 0.001			
Age of the stem disc × sampling height	– 0.0003	0.0001	< 0.001			

additional model, where instead of the interaction term, the average vertical growth rate was used as a predictor. However, the previously described model was chosen due to a lower AIC (80.4 compared to 86.5) and a slightly higher proportion of explained variance ($R^2 = 0.89$ compared to 0.88). It is possible that site-specific traits like frequency of storms, drought, frost, snow etc. result in a physiological adaption of trees, which also reflects in extractive concentrations via growth rate (Kebbi-Benkeder et al. 2017; Taylor et al. 2003). By implication this means, that at least for a confined area like south-west Germany, the geographical origin of trees seems less suitable to predict extractive yields than more obvious tree parameters and growth rates.

3.3 Chemical composition of extracts and opportunities for valorization

Successive extraction utilizing petroleum ether, acetone and methanol with subsequent GC analyses revealed 39 substances, from which 17 and 13 were identified and quantified, respectively. Analysis of the cyclohexane/ethanol extracts yielded 33 substances, from which 11 and 9 were identified and quantified, respectively. Table 4 gives an overview of all recognized constituents, also of those which were not quantified. Tetrocosanol, β-sitosterol, lignoceric acid and behenic acid were exclusively found in the petroleum ether extract, while 15-hydroxydehydroabietic acid and sandaracopimaric acid were solely detected in the cyclohexane/ethanol extract. The one-stage extraction did not capture quinic acid, inositol and D-pinitol, which were found in the acetone and methanol extracts in not quantitatively determinable amounts.

3.3.1 Sapwood extractives

The cyclohexane/ethanol extracts of sapwood comprised low quantities of dehydroabietic, isopimaric and palustric acid (Fig. 7). None of these resin acids were found in concentrations higher than 0.1% of dry weight. Taxifolin, a secondary extractive, which is normally not found in sapwood, was nevertheless detected in a sample at 11.5 m height at the site Odenwald. Successive extraction of sapwood yielded in 7-oxodehydroabietic acid at all sites (Table 4), but did not capture isopimaric and palustric acid, as did one-step extraction. In total, at maximum 19.4% of the sapwood extracts were quantitatively characterized. It is likely that a large part of the extract consisted of compounds involved in metabolism that were not captured with the applied methodology relying on GC/FID.

The identified resin acids in the sapwood and their derivatives are potentially of interest for medical and pharmaceutical but also for material applications. Strong inhibition of biofilm formation by dehydroabietic acid was observed by Fallarero et al. (2013), while isopimaric acid demonstrated activity against multidrug-resistant strains of *Staphylococcus aureus*, which become increasingly resistant to antibiotics (Smith et al. 2005). Furthermore, an environment-friendly polymer material was produced by replacing the toxic plasticizer phthalate ester in polyvinyl chloride (PVC) by modified dehydroabietic acid (Jia et al. 2019). Nowadays, modified

Table 4 Substance name, substance class and occurrence of compounds identified in the extracts of Douglas fir wood and bark with indication of the organic solvent (PE = petroleum ether, AC = acetone, MeOH = methanol, CH/EtOH = 1:1 mixture of cyclohexane/ethanol)

Substance name	Substance class	Solvent	Occurrence in tissue types
Lignoceric acid	Fatty acids	PE	Bark
Behenic acid		PE	Bark
Abietic acid	Resin acids	PE, CH/EtOH	Knotwood
Dehydroabietic acid		PE, CH/EtOH	Sapwood, heartwood, bark, knotwood
7-Oxodehydroabietic acid		PE, CH/EtOH	Sapwood
15-Hydroxydehydroabietic acid		CH/EtOH	Sapwood, heartwood
Sandaracopimaric acid		CH/EtOH	Knotwood
Isopimaric acid		PE, CH/EtOH	Heartwood, bark, knotwood
β-Sitosterol	Phytosterol	PE	Bark
Tetracosanol	Wax alcohol	PE	Bark
Nortrachelogenin	Lignan	CH/EtOH, AC, MeOH	Knotwood
Taxifolin	Flavonoids	CH/EtOH, AC, MeOH	Heartwood, bark, knotwood
Dihydrokaempferol		CH/EtOH, AC, MeOH	Heartwood
Pinocembrin		CH/EtOH, AC	Heartwood
Inositol	Sugar alcohols	MeOH	Sapwood
Pinitol		AC, MeOH	Sapwood
Quinic acid	Carboxylic acid	MeOH	Sapwood

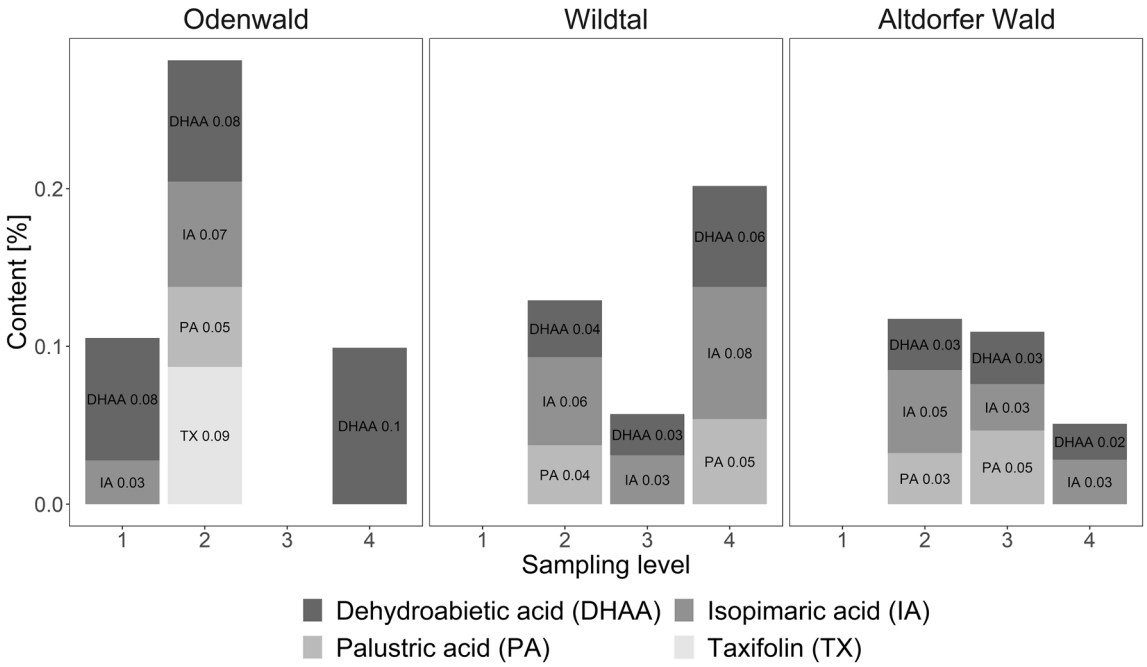

Fig. 7 Identified compounds in the cyclohexane/ethanol extracts of sapwood at the three investigated sites. Contents are given as percentage relative to the dry weight of the sample material. Missing bars indicate that the limit of determination was not achieved for any compound. Sampling levels 1 and 2 correspond to 1.3 m and 11.5 m stem length at each site. Level 3 corresponds to 18.2 m (Odenwald), 24.9 m (Wildtal) and 26.8 m (Altdorfer Wald). Level 4 corresponds to 24.8 m (Odenwald), 38.3 m (Wildtal) and 41.4 m (Altdorfer Wald)

resin acids are commercially used as binders in printing ink (Robert 2015).

Resin acids are commonly separated from tall oil, which arises as a by-product during Kraft-pulping. Since Douglas fir sapwood is more favorable for papermaking than heartwood, owing to the absence of discoloring extractives that complicate bleaching of pulp (Dellus et al. 1997), thinning material with low or no heartwood quantities could be used as a feedstock for pulp and resin acid production. Nevertheless, the identified resin acids are available to much greater extents in other tree compartments as will be discussed below.

3.3.2 Heartwood extractives

Heartwood extracted with cyclohexane/ethanol contained taxifolin as the dominant compound at all sites, comprising 1.6–2.4% by dry weight at the site Odenwald (Fig. 8). Douglas firs from the site Wildtal had lowest taxifolin yields (0.5 to 1.2%) and did not comprise any further compounds in quantifiable amounts, whereas trees from the other sites additionally contained low quantities of dihydrokaempferol (0.13–0.5%), pinocembrin (0.07–0.20%) and resin acids (only Altdorfer Wald). The quantifiable proportion of the cyclohexane/ethanol extracts ranged between 18 and 63%. Successive extraction revealed no additional compound species, however dehydroabietic and isopimaric acid were found in the extracts of each site.

The natural durability of wood is directly linked to the presence of specific extractives (Kirker et al. 2013). In case of Douglas fir, taxifolin causes moderate durability of the heartwood against fungal decomposition (Kennedy 1955), making it suitable for outdoor construction. Differences in taxifolin contents between sites and sampling heights in the present study are statistically not significant due to limited numbers of specimen. Yet, a trend towards increased taxifolin contents close to the stem base and to the crown was evident at all sites (Fig. 8). Despite the considerable quantities in Douglas fir and its wide application range in existing pharmaceutical preparations, food additives and health care products (Trivelato et al. 2016; Weidmann 2012), taxifolin is nowadays mostly extracted from the heartwood of species of the genus *Larix*, even though yields reported in the literature are not exceptionally high ranging between less than 1% (Neverova et al. 2013) over 1.8% (Kuznetsov et al. 2018) up to 2.5% (Pâques et al. 2012) and 4.1% (Windeisen and Wegener 2003) of dry weight. Thus, Douglas fir heartwood might constitute a worthwhile alternative source for this valuable compound. Moreover, with pinocembrin, a flavonoid with antibacterial, anti-inflammatory and anti-oxidant properties (Lan et al. 2016; Shen et al. 2019) was detected, that could generate additional benefit when isolated from heartwood.

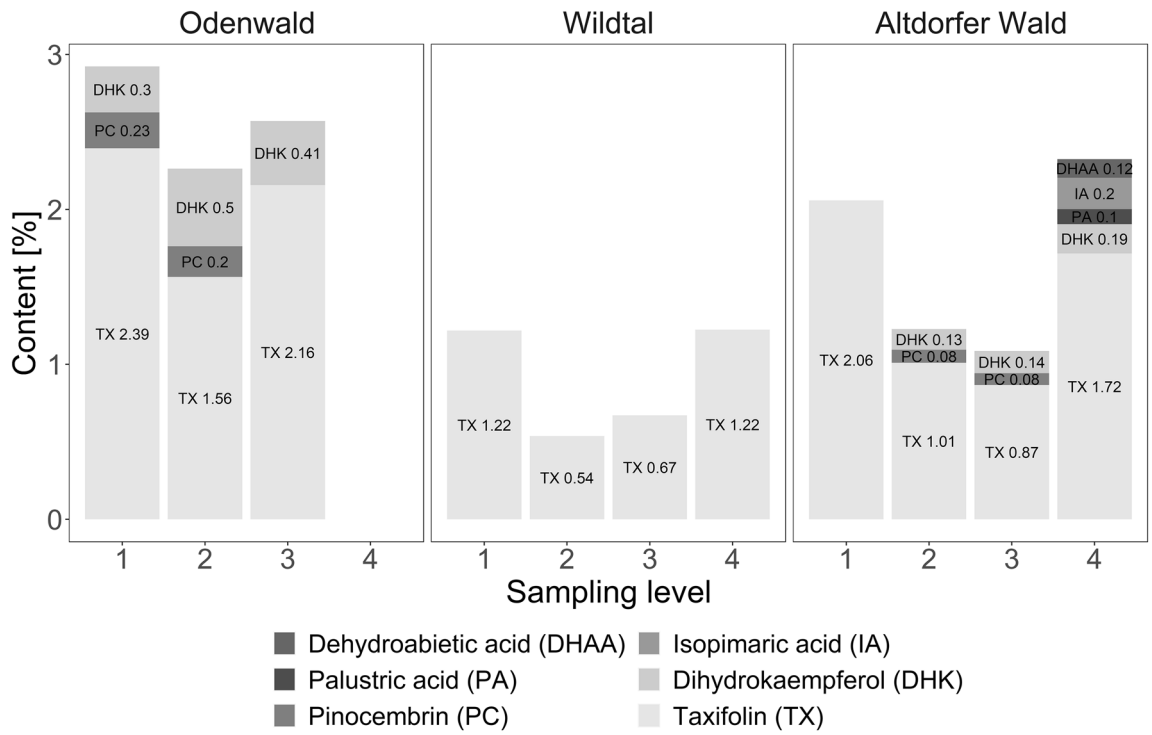

Fig. 8 Identified compounds in the cyclohexane/ethanol extracts of heartwood at the three investigated sites. Contents are given as percentage relative to the dry weight of the sample material. Sampling levels 1 and 2 correspond to 1.3 m and 11.5 m stem length at each site. Level 3 corresponds to 18.2 m (Odenwald), 24.9 m (Wildtal) and 26.8 m (Altdorfer Wald). Level 4 corresponds to 24.8 m (Odenwald), 38.3 m (Wildtal) and 41.4 m (Altdorfer Wald)

To avoid competition with high quality sawn timber production, utilization of low quality stem sections classified as pulpwood or fuelwood appears most suitable for extraction. This kind of wood will likely be found in the upper tree parts, which contained still substantial amounts of heartwood (Fig. 2) and showed even higher extraction yields than the intermediate stem parts (Fig. 5). As mentioned before, heartwood of Douglas fir is problematic in papermaking due to high contents of undesired wood components. For that reason, extraction of wood chips as a pretreatment in the production of thermomechanical pulp is a possibility to increase pulp quality and whiteness (Zimmer and Wegener 1995). As a positive side effect, recovery of extractives for added-value generation could be easily integrated into an established industrial process chain.

3.3.3 Bark extractives

In the cyclohexane/ethanol extract of bark, taxifolin and dehydroabietic acid were the only quantifiable compounds, yet they did in no case occur concurrently (Fig. 9). While taxifolin was only present in the bark of stem discs older than 36 years, dehydroabietic acid was exclusively found in stem discs younger than 21 years where it accounted for 2.7% of the bark's dry weight. Major parts of the cyclohexane/ethanol extracts remained unidentified, yet tannins that were not recovered with the applied methodology likely make up a substantial fraction (Pietarinen et al. 2006). Successive extraction yielded lignoceric acid (2.30 and 2.10% of dry weight at the sites Wildtal and Altdorfer Wald, respectively), behenic acid (1.04% at the site Wildtal), tetracosanol (0.98 and 1.73% at the sites Wildtal and Altdorfer Wald, respectively) and β-sitosterol (0.95 and 2.93% at the sites Odenwald and Wildtal, respectively).

Elevated extractive contents at the stem base of older trees as described above can in parts be explained by high taxifolin contents, which accounted for up to 12.7% of the bark's dry weight (Fig. 9). The observed rapid decrease of taxifolin contents with increasing stem height matches well with observations by Brennan et al. (2020a, b) and can be explained by the preferential storage and/or production of taxifolin in the cork-rich mature bark at the stem base (Trivelato et al. 2016). According to this, bark thickness (as a rough estimate of cork content) in the present study was strongly correlated with taxifolin content ($R^2 = 0.85$, $p < 0.01$; data not shown).

Already today, β-sitosterol, which was found in the petroleum ether extract, has economic relevance. Used as food additive, it is claimed to reduce cholesterol levels in blood (Rudkowska et al. 2008). Furthermore, β-sitosterol is used

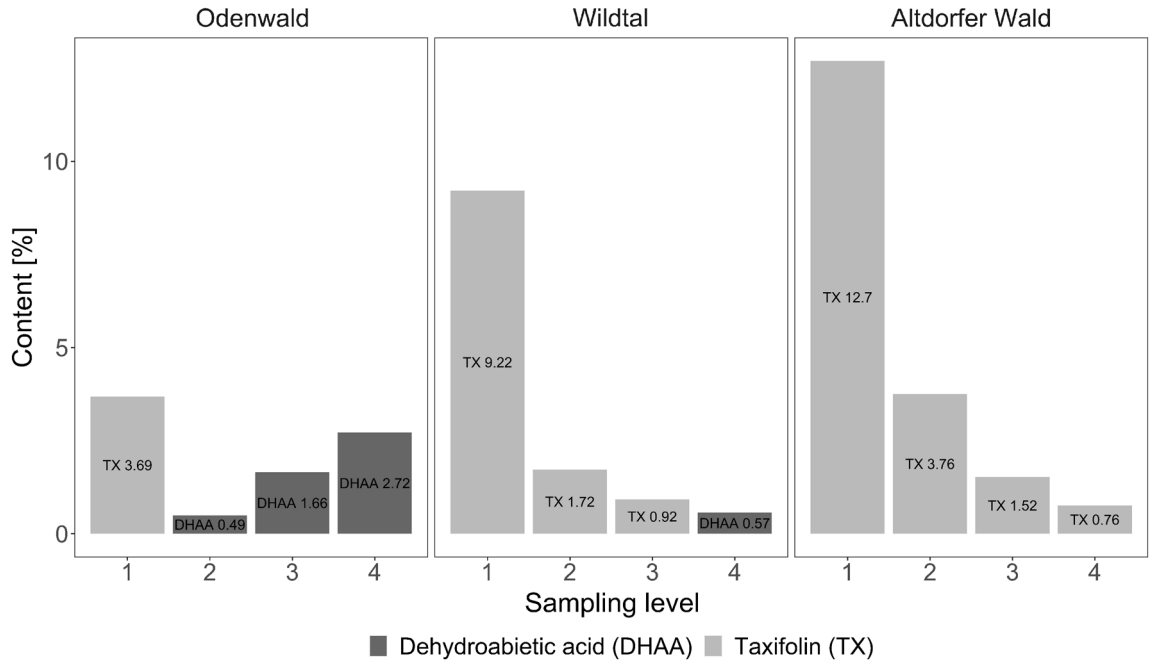

Fig. 9 Identified compounds in the cyclohexane/ethanol extracts of bark at the three investigated sites. Contents are given as percentage relative to the dry weight of the sample material. Sampling levels 1 and 2 correspond to 1.3 m and 11.5 m stem length at each site. Level 3 corresponds to 18.2 m (Odenwald), 24.9 m (Wildtal) and 26.8 m (Altdorfer Wald). Level 4 corresponds to 24.8 m (Odenwald), 38.3 m (Wildtal) and 41.4 m (Altdorfer Wald)

to treat benign prostatic hyperplasia (Wilt et al. 2000) and as a starting material for microbiological production of synthetic hormones (Johansson 1982). Nevertheless, when it comes to valorization of Douglas fir bark, taxifolin seems the most promising substance as it can be extracted in large quantities from older trees. Assuming a scenario for the site Altdorfer Wald, where (1) bark makes up around 1.1 m^3 of the stem volume, (2) average taxifolin yield along the stem is 4.7% and (3) bark density is estimated 440 kg m^{-3} (Miles and Smith 2009), around 228 g of taxifolin per tree could be produced. Given the high market price of 800–1000 $ per gram pure taxifolin (Trivelato et al. 2016), there seems great unused potential in bark extraction. A more detailed chemical analysis of bark extracts and the use of more polar solvents could reveal additional valuable components like tannins or their building blocks (Mbakidi-Ngouaby et al. 2018) with potential for production of bio-based adhesives and foams (García et al. 2014; Pizzi 2019).

3.3.4 Knotwood extractives

Extracts obtained from knotwood with cyclohexane/ethanol were more diverse than extracts from all other compartments. Taxifolin was present in the majority of samples with highest contents of up to 1.3% by dry weight at the site Odenwald (Fig. 10). Furthermore, the lignan nortrachelogenin was found in nearly all samples in concentrations of up to 0.5%. Resin acids that were identified in the sapwood were also found in the knotwood originating from the sites Wildtal and Altdorfer Wald, yet in much higher concentrations than in sapwood. Samples from the site Odenwald contained no resin acids at all. When resin acids were present, isopimaric acid was dominant, yielding up to 1.3% of dry weight. With sandaracopimaric acid, one additional resin acid was recovered in a sample from the site Altdorfer Wald. Between 9 and 29% of the cyclohexane/ethanol extract were quantified and successive extraction did not recover any further substances.

Lignans are a typical substance class in knotwood extractives (Willför et al. 2003a, b). The only lignan identified in the present study was nortrachelogenin, which was previously identified in European larch (*Larix decidua* Mill.), in Scots pine (*Pinus sylvestris* L.) (Holmbom et al. 2008; Kebbi-Benkeder et al. 2015), but also in the knotwood of Douglas fir (Brennan et al. 2021). Owing to its antitumor effects (Peuhu et al. 2013) and anti-inflammatory properties (Laavola et al. 2017), nortrachelogenin is an interesting candidate for pharmaceutical applications.

The presence of dead knots in the samples originating from the two older stands (Fig. 3) could explain high contents of resin acids as a result of physical damage. Mechanical bending stresses induced by wind or snow load increase with tree and branch size, leading to wounds in and around knots and subsequent formation of traumatic resin pockets

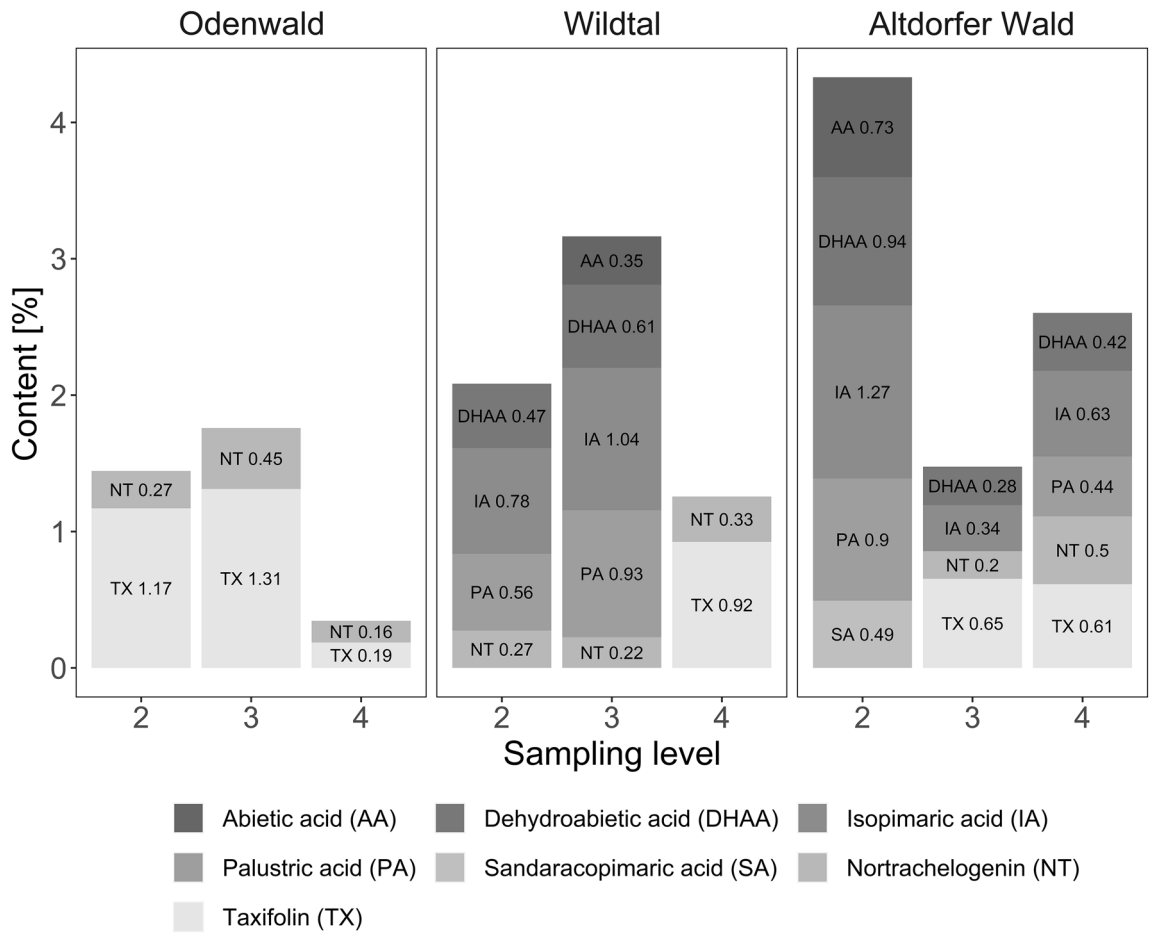

Fig. 10 Identified compounds in the cyclohexane/ethanol extracts of knotwood at the three investigated sites. Contents are given as percentage relative to the dry weight of the sample material. Sampling level 2 corresponds to around 11.5 m stem length at each site. Level 3 corresponds to 18.9 m (Odenwald), 25.4 m (Wildtal) and 26.8 m (Altdorfer Wald). Level 4 corresponds to 24.8 m (Odenwald), 38.3 m (Wildtal) and 41.9 m (Altdorfer Wald)

(Watt et al. 2009). Moreover, Willför et al. (2003b) found elevated contents of resin acids in dead knots. When utilization of resin acids is aimed for value generation, it might therefore be favorable to select larger trees with supposedly high contents of dead or wounded knots. Furthermore, pruning and tree spacing in the stand can affect crown development and branch size (Hein et al. 2008) and thus knotwood quantity in the stem. The chemical composition of Douglas fir knots, however, appeared unaffected by silvicultural treatments in a study by Brennan et al. (2021).

One critical aspect about knotwood valorization is that knots are firmly attached to the surrounding stemwood (with exception of dead knots), causing additional costs for knotwood separation. On the other hand, a technique exists for papermills that separates knotwood from wood chips in order to increase pulp quality (Eckerman and Holmbolm 2004). Integrating this method as a standard into the papermaking process, would at the same time increase pulp quality and generate a high-yield feedstock for extraction.

4 Conclusion

In this study, extractives in Douglas firs from three sites in south-west Germany with potential for material, pharmaceutical and dietary utilization were identified and especially taxifolin was found in promising amounts. At all investigated sites, the pattern of extractive contents along the stem followed a non-linear function, likely indicating zones of elevated metabolic activity and/or increased need for defense mechanisms triggered by extractives. Among the studied stands, there was no evidence for a significant site effect on total extractive yields. Differences between trees originating from different stands could rather be explained by age-related variables and the longitudinal sampling position within the tree. While this might be true for a spatially restricted area like south-west Germany, it can only be assumed that for larger areas with stronger gradients in ecological factors, differences would be more distinct, due to physiological adaption of trees.

In order to realize maximum resource efficiency, it is advisable to include wood and bark extraction into existing industrial processes, allowing for a cascading utilization of resources. In terms of carbon sequestration, long-lived wood products like construction timber should always be preferred. However, bark and crown material with no or limited use in traditional wood utilization seem particularly suitable as feedstock for a future bio-economy, also due to elevated extractive contents in these parts of the tree. For wood and bark initially assigned to combustion to produce heat and energy, prior extraction might be an option to increase value generation and resource efficiency.

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Author contributions Conceptualization: HW and FB; Methodology: HW and EW-H; Formal analysis and investigation: HW; Writing-original draft preparation: HW; Writing-review and editing: FB, ElW-H, US, KR; Funding acquisition: FB, US.

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Availability of data and materials The data presented in the study that support the findings of this research are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval Not applicable.

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