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Diversity of Essential Oils and the Respective Hydrolates Obtained from Three *Pinus cembra* Populations in the Austrian Alps

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Abstract: *Pinus cembra*, stone pine, is an Alpine coniferous tree rich in aromatic compounds. Twigs with needles are used commonly to produce essential oils for various purposes in pharmacy, food industry, and cosmetics. Hydrolates as byproducts of hydrodistillation encounter increasing interest owing to their aromatic properties. The variabilities in the compositions of essential oil and the related hydrolates are studied in samples from individual trees growing on three sites in the Austrian high mountain range. The essential oils have been obtained by steam distillation. All essential oils contained monoterpene hydrocarbons as main compounds, especially α -pinene (36–37%), β -phellandrene (27–30%), and β -pinene (7–9%). In contrast, the volatiles in the corresponding hydrolates were dominated by oxidized compounds as α -terpineol (28–34%), terpinen-4-ol (6–8%), and verbenone (6–7%). The pinene rich essential oils might be used in pharmacy as pinene containing oils from other *Pinus* species, while the hydrolates are of interest for cosmetics and other well-being promoting products.

Keywords: hydrolate; essential oil; *Pinus cembra* L.; α -pinene; α -terpineol; monoterpenes

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1. Introduction

Pinus cembra L., Pinaceae, Swiss stone pine, or arrola pine, is an alpine evergreen coniferous tree distributed in Europe in the Alps and the Carpathian Mountains. It grows in pure or mixed stands at high altitudes, 1500–2200 m, where it is well adapted to the alpine climate. Growing slowly, it can reach a height up to 25 m and an age of 1000 years [1]. However, due to natural competition and human activities such as alpine farming and timber extraction, the distribution of *P. cembra* has been seriously compromised, which has led to a displacement to marginal habitats [2–4].

Regionally, *P. cembra* has obtained economic significance. Commonly, the wood is used as building timber, for furniture production, and for artwork. Additionally, *P. cembra* is grown in parks and gardens as an ornamental tree.

Similar to other *Pinus* species, *P. cembra* is rich in aromatic volatile compounds and resins. These compounds are of interest for non-timber uses of the tree, and in some regions efforts are undertaken to promote this sector [5]. Essential oils represent the plant volatile fraction obtained by hydrodistillation or steam distillation; in the case of stone pine they are usually obtained from whole twigs with needles. In steam distillation, which is the widely used technique, a hot steam passing through the plant material extracts the volatile plant compounds. After condensation, the essential oils can be separated from the water phase [6]. Therefore, hydrolates or hydrosols are byproducts of the distillation, and contain low amounts of aromatic compounds soluble in water. Another increasingly applied technique uses supercritical CO₂ as an extractant to isolate the volatile fraction from plants. By adding modifiers, the selectivity of the extraction can be regulated [7].

However, these techniques are more sophisticated and need more intricate equipment than the classical distillation process, and are therefore costly.

P. cembra essential oils have various applications in medicine and aromatherapy, in local liquor production, and in the fragrance industry. Similarly, due to their aromatic properties, hydrolates are of interest for cosmetics. Stone pine products are sold to promote well-being. The aromatic compounds of the wood are said to have positive effects on indoor climate although this claim is not supported by scientific evidence.

Several authors reported on the *P. cembra* essential oil composition. α -Pinene, limonene, β -phellandrene, and β -pinene in varying proportions were the main compounds [8–11]. The plant material investigated for these reports came mainly from Eastern Europe or from botanical gardens. Volatiles emitted by *P. cembra* cones of different geographic origins in the French Alps include α -pinene (67–70%), β -pinene (18–20%), and limonene/ β -phellandrene (8–11%) [12]. Only a recent study dealt with plants from a location in the Austrian Alps [13]. This study reports, in twig tips with needles, 44–48% α -pinene, 13–17% β -phellandrene, 7–9% β -pinene, 4–6% limonene, and 5–7% germacrene D. As the evergreen tree produces large amounts of branches and greenery, this plant's raw material appears to be suitable for biosourcing interesting fractions or molecules. Products from other *Pinus* species are of medical interest or, as in the case of turpentine oils, have industrial applications [14]. As the biological activities may change with the composition of the essential oil, a more detailed knowledge of the oil composition becomes necessary.

As the variability of the volatiles in this species is not yet well documented and there are no reports on volatiles in *P. cembra* hydrolates, the focus of the present research was:

- (1) To investigate the variability in essential oil composition from three different collection sites;
- (2) To take sample from individual trees to get an insight in the chemical variation within populations;
- (3) To obtain data on the volatiles in the hydrolates and to learn more about the correlation to their corresponding essential oils.

To obtain the essential oils and hydrolates, we used a simple steam distillation unit which can be easily installed on the collecting sites of the plants.

2. Materials and Methods

2.1. Plant Material

The plant material was collected during September 2015 on three sites in the Austrian Alps: Kemetgebirge, Stoderalm (KEM, Styria, 47°27'56" N, 13°49'37" E and 47°28'02" N, 13°49'15" E, 1660–1701 m); Defereggental, Oberhauser Alm (DEF, East Tyrol, 46°56'40" N, 12°13'12" E, 1660–1701 m); and Sirnitz, Hochrindl (SIR, 46°51'57" N, 13°59'09" E, 1498–1588 m). The Kemetgebirge site is located north of the central Alpine ridge, while the Sirnitz and Defereggental sites are located on the south side of the Alpine chain. On each site, 10 individual trees growing within a radius of 75–225 m were samples. The diameters of the trees' trunk ranged from 68 to 250 cm. Approximately 20 kg of apparently healthy branches, with a diameter of up to 1.5 cm, including the shoot tips and the needles, were taken from each tree within a height range of 1.5 to 10 m. Voucher specimens from each site were deposited in the Herbarium of the University of Vienna (WU, <https://www.jacq.org/#database>, accessed on 18 June 2021) with the numbers WU0122822, WU0122823, and WU0122824 for SIR, KEM, and DEF, respectively. From each tree, a portion of one hundred grams of the shredded material was transported in an airtight plastic bag to the laboratory, where dry matter was determined at 125 °C for 1.5 h in a drying chamber.

2.2. Steam Distillation

The essential oils and hydrolates were obtained in a medium scale copper alembic type steam distillation unit, consisting of an 80 liter boiler fired with wood filled with 30 L water to produce the steam. About 12 +/- 0.2 kg freshly chopped branches with

needles were set atop the boiler in a 40 L column. A condenser connected to the column was constantly supplied with 5 °C cold water so the condensed essential oil and hydrolate could be caught and separated in a 1000 mL separating funnels. While the hydrolate was drained into a 10 L canister, the essential oil remained in the funnel and was collected and its amount measured at the end of the distillation process. The distillation was stopped after approximately 1 h +/− 15 min when 7.7 L hydrolate were drained.

Prior to GC/MS the essential oils were diluted 1:100 with hexane. The hydrolates (100 mL) were extracted with 10 mL hexane in a separatory funnel. The hexane layer was separated from the water, dried over sodium sulfate, and kept at −18 °C until further GC/MS analysis.

2.3. GC/MS

The GC/MS system consisted of 6890 (Agilent Technologies, Santa Clara, CA, USA) equipped with a 59772 MSD Mass selective detector and a 30 m × 25 mm fused silica column coated with 0.25 µm HP-5MS-column. The injector temperature was set at 250 °C and the injection volume was 1 µL under a split relation of 1:10. Helium was the carrier gas at a velocity of 1.4 mL/min in the constant flow mode. The oven temperature raised from 60 °C to 320 °C at a rate of 6 °C/min. The MSD recorded the mass fragments in the range m/z 40 to 500. The spectral libraries Wiley 275 and NIST 08 were used to identify the mass spectra. Additionally, retention indices were calculated using a mix of C8–C30 n-alkanes analyzed under the same conditions [15,16]. The peak areas from the total ion current without any correction were taken to calculate the percent composition of the essential oils and hydrolates.

2.4. Statistical Analysis

The statistical analyses were completed with the package SPSS for Windows, version 26.0 (IBM Corporation, Armonk, NY, USA). A one-way analysis of variance (ANOVA) with Tukey post hoc test evaluated the differences between the population for the individual essential oil compounds. For essential oils and hydrolate volatiles, the relation between major essential oil compounds was studied by a principal component analysis (PCA) using the samples as cases and the essential oil compounds as variables. Data was Z-transformed before calculating the loadings of the variables and scores of the samples.

3. Results and Discussion

3.1. Essential Oil Yield

Branches with needles were harvested from ten trees on each of the investigated sites. The essential oil yield calculated on a dry matter basis was lowest on the site KEM (1.29% v/w) and comparable for the sites DEF and SIR (1.60% and 1.66% v/w , respectively). The content of volatiles varies widely within the *P. cembra* tree. While unripe cones are rich in essential oils (2.4–3.0%) the wood is very low in these compounds [10,13]. For needles and branches, essential oil contents range from 0.46 to 0.66% in plants growing in Poland [10], to 1.1 to 2.5% from Austrian plants [13], and 2.45% in trees from Romania [8].

3.2. Essential Oil Composition

The composition of the essential oils is presented in Table 1. Monoterpene hydrocarbons were predominant, accounting for 76–80% of the essential oil, with α -pinene (approximately 37%) and β -phellandrene (27.5–30.0%) as the two main compounds in all samples. α -Pinene is widely occurring in essential oils. *Pinus* essential oils with α -pinene as a major compound are pharmaceutically used in inhalation products, unctions, or as bath additives [17]. Essential oils from *Pinus peuce*, having comparable pinene and β -phellandrene contents to the present *P. cembra* essential oil, showed promising anti-cancer and anti-inflammatory activities [18]. The analytical column used did not separate limonene from β -phellandrene. Previous experiences report that *P. cembra* essential oil contains more β -phellandrene than limonene [9,13]. β -Pinene (7.0–9.0%) was a further

remarkable monoterpene hydrocarbon, while the percentages of camphene and myrcene remained below 2–3%. Oxidized monoterpenes represented, on average, 1.9–2.6% of the essential oil, with the main compounds of thymol methylether (0.5–0.8%), bornyl acetate (0.4–0.7%), and cryptone (0.3–0.5%). Other compounds of this class mostly had percentages of 0.1% or less. Amongst the sesquiterpene hydrocarbons, germacrene D and δ -cadinene came to 4.3–7.4% and 2.1–2.8% of the essential oil, respectively. Other compounds from this class were below 1% each. Finally, six oxidized sesquiterpenes could be identified; they occur at low levels, together representing less than 1.4% of the oil, each usually below 0.4%. Diterpenoids were not present in the actual essential oils, in contrast to essential oils obtained in a laboratory scale Clevenger type hydrodistillation device, which after three hours contained small amounts of diterpenes [11,13].

Table 1. Composition of the essential oil (%) from *Pinus cembra*.

RI	Compound	KEM			DEF			SIR		
		Mean	SD	Max	Mean	SD	Max	Mean	SD	Max
	Oil yield (% v/w)	1.29	0.27		1.6	0.24		1.66	0.42	
Monoterpene Hydrocarbons										
922	Tricyclene	0.1	<0.05	0.2	0.2	<0.05	0.2	0.2	0.1	0.2
931	α -Thujene	<0.05	<0.05	<0.05	<0.05	<0.05	0.1	<0.05	<0.05	0.1
938	α -Pinene	36.8	6.2	44.8	37.2	2.6	42.2	36.8	5.0	43.9
950	Camphene	1.6	0.8	3.1	1.7	0.8	3.5	1.8	0.9	3.1
978	β -Pinene	7.0	2.4	10.3	8.7	1.6	10.6	9.0	2.0	11.9
993	Myrcene *	1.2 a	0.2	1.6	1.4 ab	0.1	1.6	1.4 b	0.2	1.6
1003	α -Phellandrene	0.1	<0.05	0.2	0.1	<0.05	0.2	0.2	0.1	0.2
1009	δ -3-Carene *	1.2 ab	0.6	2.7	0.5 a	0.5	1.6	1.2 b	0.4	1.8
1018	α -Terpinene	<0.05	<0.05	0.1	<0.05	<0.05	<0.05	<0.05	<0.05	0.1
1027	p-Cymene	0.1	0.05	0.2	0.1	0.05	0.1	0.1	0.1	0.2
1032	β -Phellandrene **	27.5	5.4	37.8	29.8	3.0	33.5	30.0	4.8	37.2
1062	γ -Terpinene	0.1	<0.05	0.1	0.1	<0.05	0.1	0.1	<0.05	0.1
1089	Terpinolene	0.5	0.3	1.2	0.4	0.2	0.9	0.6	0.3	1.0
	Sum	76.3			80.3			81.3		
Oxidised Monoterpenes										
1097	α -Pinene-epoxide	0.1	<0.05	0.1	0.1	<0.05	0.1	<0.05	<0.05	0.1
1123	trans-Pinene-hydrate				<0.05	<0.05	0.1	<0.05	<0.05	0.1
1129	α -Campholenal				<0.05	<0.05	<0.05	<0.05	<0.05	0.1
1141	trans-Pinocarveol	<0.0	0.1	0.1	0.1	<0.05	0.1	0.1	0.1	0.2
1147	Camphor				<0.05	<0.05	<0.05	<0.05	<0.05	0.1
1148	trans-Verbenol	<0.05	<0.05	0.1	0.1	<0.05	0.1	<0.05	0.1	0.2
1165	Pinocavone	0.1	<0.05	0.1	0.1	<0.05	0.1	<0.05	<0.05	0.1
1168	Borneol	<0.05	<0.05	0.1	<0.05	<0.05	0.1	0.1	0.1	0.2
1176	Isopinocampheol				<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
1180	Terpinen-4-ol	<0.05	<0.05	0.1	0.1	<0.05	0.1	0.1	<0.05	0.1
1188	Cryptone	0.4	0.4	1.1	0.5	0.2	0.9	0.3	0.5	1.7
1192	α -Terpineol	0.2	0.1	0.3	0.2	0.1	0.3	0.2	0.1	0.3
1197	Myrtenal	0.1	<0.05	0.1	0.1	<0.05	0.1	0.1	0.1	0.2
1211	Verbenone	<0.05	<0.05	0.1	0.1	<0.05	0.1	<0.05	0.1	0.2
1238	Thymol-methylether	0.5	0.2	0.9	0.6	0.2	0.8	0.8	0.3	1.2
1244	Cuminic aldehyde	0.1	0.1	0.2	0.1	<0.05	0.2	<0.05	0.1	0.3
1288	Bornyl acetate	0.5	0.3	1.2	0.6	0.4	1.6	0.7	0.5	1.6
	Sum	1.9			2.6			2.6		

Table 1. Cont.

		KEM			DEF			SIR		
RI	Compound	Mean	SD	Max	Mean	SD	Max	Mean	SD	Max
Sesquiterpene Hydrocarbons										
1354	α -Cubebene	0.2	0.2	0.5	0.1	0.1	0.5	0.1	0.1	0.3
1380	α -Copaene	0.2	<0.05	0.2	0.1	<0.05	0.2	0.1	<0.05	0.2
1391	β -Bourbonene	<0.05	<0.05	<0.05	<0.05	<0.05	0.1	<0.05	<0.05	0.1
1394	β -Cubebene	0.2	0.1	0.4	<0.05	0.1	0.2	0.1	0.1	0.2
1425	β -Caryophyllene	0.9	0.5	2.3	1.0	0.4	1.5	1.0	0.4	1.6
1446	Aromadendrene	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4
1460	α -Humulene	0.7	0.5	1.2	0.8	0.4	1.5	0.9	0.5	1.7
1470	<i>epi</i> -Bicyclo-sesquiphellandrene	0.1	<0.05	0.2	0.1	<0.05	0.1	0.1	<0.05	0.2
1483	γ -Muurolene *	0.9 b	0.3	1.4	0.3 a	0.4	1.1	0.5 ab	0.2	0.8
1488	Germacrene D *	7.4 b	2.2	10.4	4.5 a	2.7	9.9	4.3 a	2.3	7.4
1502	Bicyclgermacrene	1.8	0.6	2.7	1.5	0.3	2.1	1.3	0.4	1.8
1505	α -Muurolene *	0.7 b	0.2	1.2	0.6 ab	0.1	0.6	0.5 a	0.1	0.7
1513	β -Bisabolene	0.3	0.3	0.9	0.6	0.2	1.0	0.7	0.3	1.2
1520	γ -Cadinene *	1.4 a	0.3	1.7	1.2 ab	0.2	1.5	1.0 b	0.3	1.4
1530	δ -Cadinene	2.8	0.5	3.7	2.2	0.3	2.7	2.1	0.6	2.8
1539	<i>trans</i> -Cadina-1,4-diene	0.1	<0.05	0.2	0.1	<0.05	0.1	0.1	<0.05	0.1
1545	α -Cadinene	0.2	0.1	0.3	0.2	<0.05	0.2	0.1	<0.05	0.2
1548	<i>cis</i> - α -Bisabolene	0.1	0.1	0.2	<0.05	0.1	0.2	0.1	0.1	0.3
	Sum	18.3			13.6			13.4		
Oxidised Sesquiterpenes										
1583	Germacrene D-4-ol	0.4	0.2	0.8	0.4	0.2	0.9	0.2	0.1	0.3
1585	Spathulenol	0.1	0.1	0.3	0.1	0.1	0.2	<0.05	0.1	0.2
1591	Caryophyllene oxide	0.2	0.1	0.4	0.2	0.1	0.3	0.1	0.1	0.2
1650	τ -Cadinol *	0.4 b	0.2	0.7	0.4 ab	0.1	0.6	0.2 a	0.1	0.3
1663	α -Cadinol	0.3	0.1	0.6	0.2	0.1	0.3	0.2	0.1	0.3
1692	α -Bisabolol	0.1	0.1	0.3	0.1	<0.05	0.2	0.1	0.1	0.3
	Sum	1.4			1.3			0.9		
Other										
1199	Estragol	0.2	0.2	0.5	0.1	0.1	0.2	0.2	0.1	0.3
	Total Sum	98.0			98.0			98.3		

* For these compounds means followed by the same letter are not significantly different according to a one-way ANOVA with Tukey post hoc test; ** also contains some limonene; SD: standard deviation ($n = 10$).

The main compounds, α -pinene, β -phellandrene, β -pinene, and germacrene D were found in all samples. This is in good agreement with the published literature, where these compounds were reported to occur in varying proportions. α -Pinene ranged from 21.1% in trees from a Scotch arboretum [19] to 69.1% in samples from Romania [8]. To produce essential oils, branches with needles are usually the parts of the trees which are processed. Former research demonstrated that essential oil composition clearly differed between the needles and the twigs and branches. Needles had more α -pinene and germacrene D than the twig tips, but less limonene, β -phellandrene, and β -pinene [10,13]. Therefore, the composition of a commercially produced essential oil is strongly dependent on the ratio of twigs to needles in the distilled raw material.

A one-way analysis of variance testing the influence of sampling site on the individual essential oil compounds found significant differences between the sites only for germacrene D, and the minor compounds myrcene, δ -3-carene, γ -cadinene, α -muurolene, γ -muurolene, and τ -muurolol (Table 1). For germacrene D, the Tukey post hoc test indicated that this compound was higher in samples from KEM, in comparison to that of both other sites. Nevertheless, the main compounds, α -pinene, β -phellandrene, and β -pinene had variation coefficient from 6.9 to 10%, 11 to 16% and 17 to 32% in population DEF, SIR and KEM, respectively, suggesting that there is some variability within the populations.

To get further insight into the variability, a principal component analysis was calculated with 30 essential oil compounds as variables and the samples as cases, and resulted in eight components with an Eigenvalue greater than one, accounting for 86.7% of the variability. The loadings of the compounds on the first two components, representing together 48.2% of the variability, are displayed in Figure 1. β -Phellandrene and β -pinene, as well as myrcene, had a high positive component 1 loading, while α -pinene and germacrene D showed negative loadings on this component. All oxidized monoterpenes as minor essential oil constituents loaded positively on both components. The scores of the samples in Figure 2 show wide overlapping of the samples from the three sites, with most KEM samples scoring with a negative component 1 value. Samples from the DEF site having a lower within population variability were rather clustered in the center. Outliers S4, S8, and K7 in the lower right quadrant had the highest β -phellandrene percentages. Outlier S2 had relatively higher amounts of oxidized monoterpenes. Samples K3 and K4, with the lowest component 1 scorings, had relatively high amounts of the minor essential oil compounds δ -cadinene, bicyclogermacrene, and γ -cadinene.

Figure 1. Essential oil compounds loading on the first two axes of a principal component analysis of *P. cembra* essential oils.

Figure 2. PCA scoring plot showing the essential oil samples from the individual *P. cembra* trees.

ABisab: α -bisabolol, Ahum: α -humulene, Amuu: α -muurolene, Apin: α -pinene, Bcar: β -caryophyllene, BiGer: bicyclogermacrene, Bisab: β bisabolene, Bor: borneol, BorAc: bornyl acetate, Bphel: β -phellandrene, Bpin: β -pinene, CadOl: α -cadinol, Cam: camphene, Cryp: cryptone, D3c: δ -3-carene, Dcad: δ -cadinene, Gcad: γ -cadinene, GerD: germacrene D, GerD4ol: germacrene D-4-ol, Gmuu: γ -muurolene, Myr: myrcene, MyrAl: myrtenal, Picar: trans-pinocarveol, Spath: spathulenol, T4Ol: terpinen-4-ol, Ter: terpinolene, TerOl: α -terpineol, Tether: thymolmethylther, Tmuu: τ -muurolol, and Verb: verbenone

A great variability of essential oils within a given population has also been reported in other conifers. In *Pinus sylvestris*, individuals rich in α -pinene or δ -3-carene occurred together within a stand [20]. High diversity of terpenes might be important to increase the resistance against herbivory [21].

3.3. Composition of the Volatile Fractions from the Hydrolates

The mean composition of the volatiles isolated from the hydrolates for the three sites is given in Table 2. Oxidized monoterpenes constituted the major fraction of hydrolate volatiles. Thirty compounds were identified, of which α -terpineol was the dominant compound. This compound had, on average, 27.7–37.6% in the hydrolate volatiles, while in the distilled oil it reached only around 0.2%. Applications of α -terpineol, with its pleasant odor similar to lilac, are in various perfumes, cosmetics, soaps, and antiseptic products [22]. Terpinen-4-ol was the second major compound (6.3–7.8%) from hydrolate volatiles, and this compound was very low in essential oil, at 0.1% or less. Further remarkable volatiles were verbenone (6.1–7.0%), cis-p-Menth-2-en-1-ol (3.7–4.7%), myrtenal (3.5–4.1%), cryptone (3.2–4.0%), and borneol (3.1–3.6%). Oxidized sesquiterpenes reached 6.5–9.9% of the hydrolate volatiles, mainly with α -cadinol (3.1–5.4%) and τ -muurolol (1.8–2.9%). Terpene hydrocarbons were low in the hydrolate volatiles; on average α -pinene (1.0–2.2%), β -phellandrene (0.9–1.9%), germacrene D (1.3–5.6%), and δ -cadinene (1.0–3.1%).

An ANOVA studying the influence of the population detected significant differences for α -terpineol, α -cadinol, τ -muurolol, germacrene D, δ -cadinene, α -pinene, and β -phellandrene. The post hoc test showed that, in all cases, the samples from KEM differed from at least one of the other sites. It appears that KEM hydrolate volatiles had more terpene hydrocarbons and less oxidized monoterpenes than those from DEF and SIR.

A principal component analysis, with the hydrolate volatile compounds as variables and the samples as cases, has been calculated. Six components with an Eigenvalue greater than 1 were extracted from 23 variables. Figure 3 presents the loadings of the volatile compounds on the first two components. The main volatile constituent, α -terpineol, as well as myrtenal, had a strong negative loading on component 1; further oxidized sesquiterpenes (terpinen-4-ol, piperitone, cis-p-menth-2-en-1-ol, and trans-p-menth-2-en-1-ol) showed a high component 2 loading, while others (cryptone, borneol, and camphor) had weak negative component 1 and component 2 loadings. The terpene hydrocarbons, in contrast, were all highly component 1 loading. The plotting of the first two components for the samples, as displayed in Figure 4, presents samples from SIR mainly with negative factor 1 scores, while those from KEM were mostly positively factor 1 scoring. Therefore, most KEM hydrolate samples appear to differ from those of both other sites. Samples D5, D9, and S7 had higher terpinen-4-ol, piperitone, and trans-p-menth-2-en-1-ol in their volatile fractions. Outlier K5 had the highest germacrene D and δ -cadinene contents.

ABisab: α -Bisabolol, Apin: α -pinene, Bcar: β -caryophyllene, Bor: borneol, BorAc: bornylacetate, Bphel: β -phellandrene, CadOl: α -cadinol, Camp: camphor, CMol: cis-p-menth-2-en-1-ol, Cryp: cryptone, Dcad: δ -cadinene, Gcad: γ -cadinene, GerD: germacreneD, Lin: linalool, MeOl: mentha-1,5-dien-8-ol, MyrAl: myrtenal, Picar: trans-Pinocarveol, Piper: piperitone, Spath: spathulenol, T4Ol: terpinen-4-ol, Tcar: trans-carveol, TerOl: α -terpineol, Tether: thymolmethylether, TMol: trans-p-menth-2-en-1-ol, Tmuu: τ -muurolol, and Verb: verbenone.

Table 2. Composition of the hydrolate volatiles (%) from *Pinus cembra*.

RI		KEM			DEF			SIR		
		Mean	SD	Max	Mean	SD	Max	Mean	SD	Max
Monoterpene Hydrocarbons										
933	α-Pinene *	2.2 b	0.7	3.2	1.4 a	0.7	2.9	1.0 a	0.4	1.6
978	β-Pinene	0.2	0.2	0.6						
1032	β-Phellandrene *,**	1.9 b	0.9	3.8	1.2 ab	0.5	2.2	0.9 a	0.5	1.9
	Sum	4.4			2.6			1.9		
Oxidized Monoterpenes										
1034	1,8-Cineol	0.5	0.2	0.9	0.4	0.3	0.8	0.7	0.5	1.3
1088	Fenchone	0.2	0.1	0.5	0.2	0.1	0.3	0.3	0.2	0.6
1100	Linalool	0.6	1.1	3.7	0.2	0.4	1.3	0.2	0.4	1.2
1112	exo-Fenchol	0.8	0.2	1.1	0.9	0.1	1.1	0.9	0.1	1.0
1120	cis-p-Menth-2-en-1-ol	3.7	1.6	5.5	4.7	1.6	7.6	4.2	1.4	6.3
1126	α-Campholenal	0.3	0.1	0.6	0.3	0.2	0.6	0.3	0.2	0.5
1137	trans-Pinocarveol	2.4	1.0	4.5	3.6	3.4	10.7	2.9	2.0	7.2
1139	tr.-p-Menth-2-en-1-ol	0.7	0.4	1.4	1.5	1.2	3.3	1.4	0.7	2.2
1143	Camphor	0.7	0.8	2.3	0.6	0.4	1.5	0.8	0.6	2.2
1145	trans-Verbenol	1.0	0.5	1.6	0.9	0.2	1.3	0.9	0.4	1.4
1147	Camphene Hydrate	0.6	0.4	1.3	0.6	0.4	1.3	0.4	0.2	0.8
1160	trans-Pinocamphone	0.4	0.3	0.9	0.6	0.5	1.6	0.3	0.1	0.5
1162	Pinocarvone	0.3	0.1	0.5	0.2	0.3	0.8	0.2	0.1	0.4
1165	Borneol	3.1	1.6	6.1	3.5	1.1	5.5	3.6	1.5	6.8
1167	Mentha-1,5-dien-8-ol	1.5	0.4	2.1	1.8	0.8	3.3	2.3	0.9	3.5
1173	cis-Pinocamphone	0.6	0.2	0.9	0.6	0.3	1.1	0.6	0.2	1.1
1177	Terpinen-4-ol	6.3	1.2	7.7	7.0	2.1	10.6	7.8	1.2	10.3
1183	p-Cymen-8-ol	0.4	0.2	0.7				0.4	0.3	1.2
1186	Cryptone	4.0	1.8	6.1	3.2	1.6	5.0	3.0	1.7	5.1
1190	α-Terpineol *	27.7 a	5.4	34.6	33.1 ab	4.7	39.3	34.1 b	4.1	40.5
1196	Myrtenal	3.5	1.3	5.3	3.8	0.9	5.1	4.1	0.8	5.6
1200	trans-Piperol	0.2	0.2	0.7	0.3	0.2	0.7	0.2	0.1	0.4
1209	Verbenone	6.1	1.6	8.5	6.3	2.1	9.5	7.0	1.1	8.5
1220	trans-Carveol	1.2	0.3	1.7	1.1	0.4	1.5	1.4	0.5	2.2
1230	cis-p-Mentha-1(7),8-dien-2-ol	0.8	0.5	1.8	0.6	0.4	1.4	0.4	0.2	1.0
1236	Thymol-Methylether	0.2	0.1	0.5	0.2	0.1	0.3	0.2	0.2	0.6
1246	Carvotanacetone	0.5	0.2	0.7	0.4	0.3	0.9	0.5	0.3	0.8
1256	Piperitone	1.7	0.9	3.6	3.7	2.6	8.2	2.7	1.4	5.9
1261	trans-Myrtanol	0.3	0.3	0.9	0.2	0.1	0.4	0.3	0.3	0.9
1287	Bornyl Acetate	1.5	1.1	4.0	1.9	1.7	6.0	2.2	1.4	5.2
	Sum	71.5			82.3			84.4		
Sesquiterpene Hydrocarbons										
1423	β-Caryophyllene	0.3	0.5	1.3	0.2	0.2	0.5			
1485	Germacrene-D *	5.6 b	4.9	16.5	1.4 a	1.9	6.3	1.3 a	1.6	4.6
1504	α-Muurolene	0.3	0.2	0.6						
1519	γ-Cadinene	0.9	1.0	3.6	0.4	0.5	1.4	0.3	0.3	0.8
1527	δ-Cadinene	3.1	2.7	10.3	1.5	1.2	3.9	1.0	1.0	2.9
	Sum	10.2			3.4			2.5		
Oxidized Sesquiterpenes										
1584	Spathulenol	0.4	0.2	0.7	0.3	0.2	0.5	0.2	0.2	0.6
1634	cis-Cadin-4-en-7-ol	0.3	0.1	0.5	0.1	0.1	0.3	0.2	0.1	0.4
1648	τ-Muurolol *	2.9 b	0.8	4.2	2.4 ab	0.9	4.2	1.8 a	0.8	2.9
1652	Torreyol	0.6	0.3	1.3	0.5	0.2	1.0	0.5	0.2	0.9
1661	α-Cadinol *	5.4 b	2.1	8.3	4.5 ab	1.7	8.2	3. a1	1.2	4.7
1690	α-Bisabolol	0.3	0.5	1.4	0.6	0.3	1.3	0.8	0.6	1.7
	Sum	9.9			8.5			6.5		
	Total Sum	96.0			96.8			95.4		

* For these compounds, means followed by the same letter are not significantly different according to a one-way ANOVA with Tukey post hoc test; ** also contains some limonene; SD: standard deviation ($n = 10$).

Figure 3. Hydrolate volatile compounds loading on the first two axes of a principal component analysis of *P. cembra* hydrolates.

Figure 4. PCA scoring plot showing the hydrolate volatile samples from the individual *P. cembra* trees.

3.4. Comparison of the Essential Oils with the Hydrolate Volatiles

The comparison of Tables 1 and 2 shows that essential oils and the hydrolate volatiles differed significantly. Hydrocarbon terpenes as camphene, myrcene, δ -3-carene, terpinolene, α -humulene, and bicyclogermacrene could only be found in the distilled oils. β -Pinene was present in all essential oils, but could only be recovered in the hydrolate volatiles from KEM site. On the other hand, a range of oxidized monoterpenes occurred only in hydrolates; these were linalool, cis-p-menth-2-en-1-ol, trans-p-menth-2-en-1-ol, mentha-1,5-dien-8-ol, *trans*-carveol, and piperitone. Camphor, which could be detected in all hydrolate volatiles, was present only in a few essential oil samples at very low levels. The ratio of volatile percentages in hydrolate to distilled oil has been calculated for the compounds that occur in both sample types (Figure 5). Thus, terpene hydrocarbons having a ratio below one occurred mainly in the distilled oils, while oxidized compounds were more prominent in the respective hydrolate volatiles. In comparison to the essential oil, the hydrolate volatiles had a 158-, 186-, or 303-times higher percentage for the oxidized monoterpenes terpinen-4-ol, α -terpineol, and verbenone, respectively.

Figure 5. Ratio of volatile percentage in hydrolates to essential oils.

The fact that hydrolate volatiles are low in hydrocarbons and higher in oxygenized compounds has been reported for hydrolates from various plants. The essential oils from *Abies alba* and *A. koreana* cones and seeds is constituted by 82.4–93.4% monoterpene hydrocarbons, mainly α -pinene and limonene, and in contrast the volatiles in the respective hydrolates were composed of 83.1–94.4% of oxidized monoterpenes [23]. While *Picea glauca* essential oil contained 20% β -pinene and 12% α -pinene, these constituents were barely present in the hydrolates [24,25]. A *Solidago puberula* essential oil had 37.2% α -pinene and the respective hydrolate was devoid of it [25]. Similarly, myrcene and β -phellandrene, accounting together for 72–74% of the essential from *Anthriscus sylvestris*, occurred from traces to 1.6% in the hydrolate volatiles [26]. This distribution is, to a large extent, due to the low solubility of hydrocarbons in water; for monoterpene hydrocarbons such as α -pinene, β -pinene, camphene, myrcene, and α -phellandrene, the solubility is below 10 mg/L at 25 °C. The water solubility of α -terpineol and erpinene-4-ol is reported to be 7100 and 387 mg/L, respectively [27].

Additionally, the enrichment of oxidized compounds in the hydrolates is documented. The distilled oils from *Myrica gale* contained none to 0.2% α -terpineol, and none to 0.4% terpinen-4-ol, while the hydrolate volatiles accumulated these compounds to 11.3–15.6% and 13.4–14.3%, respectively [28]. In the case of *Teucrium kabylicum*, a percentage of 0.4% terpinen-4-ol was found in the essential oil, and of 23.2% in the hydrolate volatile fraction [29].

The partition of a compound between the essential oil and the water phase of the hydrolate also depends on the solubility of the oil compound in the essential oil itself. *P. cembra* essential oil is composed of more than 90% terpene hydrocarbons, a very apolar matrix. This is a less favorable environment for oxygenated compounds, supporting their transfer into the water phase. Therefore, we obtained higher ratios (Figure 5) between hydrolate and essential oil for these compounds than those reported in the literature. For example, in the present study, camphor occurred up to 2.7% in the hydrolate volatiles, while in distilled oil it could only be found in traces (<0.05%). In contrast, an enrichment of camphor at 65.5% occurred in the volatile fraction of a *Picea glauca* hydrosol where the corresponding essential oil had 20.2% of this compound [25].

A further factor influencing the composition of the hydrolate volatile fraction might be the distillation process itself, considering the dimensions of the distillation unit, the

amount of water consumed, and the experimental duration. However, in this study, these factors could not be further investigated using a medium scale device operated under field conditions.

4. Conclusions

The hydrodistillation of *Pinus cembra* branches with needles gives an essential oil rich in monoterpene hydrocarbons, mainly α -pinene and β -phellandrene. The hydrolates, as byproducts of the distillation process, had a different volatile fraction, where mainly oxygenated compounds were present, including even such compounds that are below the detection limit in the essential oil.

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