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Identification of Extractives from Various Poplar Species

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Abstract: Fast-growing poplar trees from plantations are the source of inexpensive wood that is intended mainly for chemical processing in the pulp and paper industry. Their potential as raw material for biofuel manufacturing is widely discussed as well. The detailed chemical composition of this raw material is therefore an important question. Five species of poplars were investigated: *Populus alba*, *P. maximowiczii*, *P. trichocarpa*, *P. nigra*, and *P. tremula*. Wood is a material consisting mainly of lignocellulose, but there are also many extractives. In temperate zone climate they typically represent up to 5% of wood mass. Their identification is difficult, due to various classes of compounds and great number of individual ones. These compounds can affect the properties of wood—its resistance to biotic agents and suitability to enzymatic treatment towards biofuels. Gas chromatography coupled with mass spectrometer (GC-MS) was used to analysis of extractives in various species of poplar wood. Compounds from several classes were identified like phenols, fatty acids, and their derivatives, sterols. Their identification was discussed along with estimation of their content in wood of investigated species.

Keywords: gas chromatography with mass spectrometry; poplar wood; wood extractives

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1. Introduction

Trees of the genus *Populus* (poplars, aspens, cottonwoods and hybrids; hereafter: poplars) arewidely distributed species across land areas of temperate zone due to their tolerance for a broad range of environmental and soil conditions. Poplar species are characterized by rapid growth to very large size and high genetic variability. Poplar has economic feasibility for industrial products. Due to poplarsvariability, its wood maybe applied in a variety of industrial products. These are factors determining the recent fast development of poplar products and its future economic and environmental potential [1]. There are three native poplar species: European aspen (*Populustremula* L.), white poplar (*Populus alba* L.), and black poplar (*Populus nigra* L.) in Poland [2]. However, non-native species are also found in plantation for industrial purposes [3]. Similar trends can be observed around the world [4,5].

One of the most important branches of industry that use wood as a raw material is energy production. While the most common way is still direct burning of wood, new Renewable Energy Directives (RED [6] and RED II [7]) promotes the use of energy from renewable sources, thus using wood as a source of liquid biofuels, like ethanol, is a topic of growing scientific interest [8–10]. The main source of bioethanol is cellulose, hydrolysed to glucose, which can be fermented by yeasts. Contemporary expectations limit hydrolysis to enzymatic processes as environmentally friendly. The main disadvantages of the use of enzymes are their cost and susceptibility to inhibitors, which are present in raw biomass or can be formed during its pretreatmeant [11–13].

Wood is a material made of lignocellulose. The main polysaccharide component is cellulose. Together with hemicelluloses, they are based on a lignin structure. Lignin is an aromatic polymer. Wood components are complemented by extractive and mineral substances [14,15]. The individual components of wood connect with each other;

these connections are highly complicated due to the nature of the combined compounds—hydrophilic polysaccharides and hydrophobic lignin [16].

Extractives are a diverse group of compounds that can be isolated from wood with the use solvents, including essential oils, fats, waxes, phenols, etc. Their content in wood ranges from 1 to 20%, depending on the species [16,17]. Wood extractives affect many aspects of wood processing. They can influence its resistance to weathering and abiotic conditions [15]. Extractives change the color of wood and influence its durability, gluability, etc. [18]. They can affect enzymatic hydrolysis both as inhibitors and process catalysts depending on the composition, as well as on the way the effect is analyzed—the extract itself may have a different effect from individual components [19–21]. The aim of this work was to develop a method of extractives composition analysis in different poplar species Obtaining these results will allow to determine the influence of individual compounds on biochemical processes (such as enzymatic hydrolysis or alcoholic fermentation).

Individual substances used as potential inhibitors of enzymatic hydrolysis may inhibit it, but not always their mixture [22,23].

The aim of this work was to develop a method of extractives analysis and determination of their composition in various poplar species. Obtaining these results will allow to determine the influence of individual compounds on biochemical processes (such as enzymatic hydrolysis or alcoholic fermentation).

2. Materials and Methods

Wood of various poplar species (the names of wood according to EN 13556:2003 [24]) was obtained for the investigation. Origin (place of harvest—location) shown in Table 1 and Figure 1.

Table 1. Origin (location) of poplar trees used for research.

Poplar Species	Age	Location *	Number of Specimen
Japanese poplar (*Populus maksimowiczi* Henry)	3,5	52°08′42″ N, 21°04′07″ E	4
Black cottonwood (*Populus trichocarpa* Torr. and A. Gray ex. Hook.)	3,5	52°08′42″ N, 21°04′07″ E	4
European aspen (*Populus tremula* L.)	~40	52°37′40″ N, 22°51′11″ E	3
White poplar (*Populus alba* L.)	~60	52°18′50″ N, 19°04′53″ E	3
Black poplar (*Populus nigra* L.)	~60	52°09′41″ N, 21°02′52″ E	2

* Origin (place of harvest) shown in the Figure 1.

Wood of various poplar species was used to characterize the extractive content and their composition. The material obtained from each specimen (3 samples of about 100 g) was crushed with Retsch SM100 laboratory mill andsieved.Chips fraction of 0.43 ÷ 1.02 mm were used for further analysis.

All the reagents used in the paper were obtained from Avantor (formerly POCh, Gliwice, Poland).

Wood chips fraction was extracted with azeotropic mixture chloroform: ethanol containing 7% of ethanol [25]. Extraction was carried out for 10 h for every 5 g of woodchips placed in the thimble. After extraction, the solvent was evaporated and the flask was dried at 105 °C. The content of extractives was determined in relation to the mass of absolute dry wood. Before chromatographic analysis samples were dissolved in 5 cm^3 of methanol p.a.

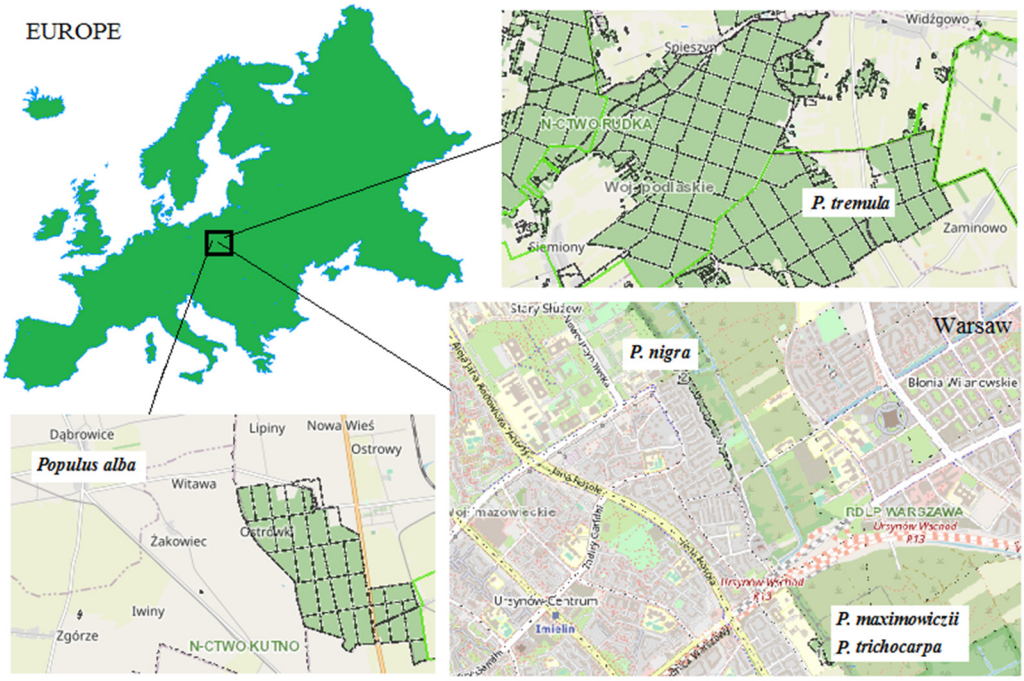

Figure 1. Origin (place of harvest) of poplar trees (https://www.bdl.lasy.gov.pl/, accessed on 1 April 2021).

The analyses were carried out on chromatograph GC-2010 Plus coupled with mass spectrometer GCMS-QP2010 (Shimadzu, Kyoto, Japan); capillary column ZB-5MS with a length of 30 m, a diameter of 0.25 mm, and a bed of 0.25µm was used. The temperature program started at 50 °C and was maintained for 7 min, then the temperature was increased by 10 °C/min to 320 °C and then maintained for 10 min. Helium 6.0 was used as the carrier gas, with the flow set at 0.8 cm^3/min. The samples were introduced directly on the column—the injection temperature was 250 °C and the detector voltage was set to 1.2 kV. Samples were introduced to the column using the AOC-20i autosampler.

Obtained chromatograms were analyzed using dedicated GCMSsolution Version 2.72 software. A single run smoothing was applied using implemented Savitzky-Golay method at 2 s smoothing width.

Individual peaks were identified by comparing the spectrum with the NIST11, NIST11b spectrum library, while spectrum processing mode was set as "Peak top spectrum" in the case of raw spectrum and "Calculated form peak: in the case of background spectrum. The most probable matches were verified on the basis of literature data on the occurrence of individual compounds in plants, including woody plants, especially different poplar species, as described in detail below.

Due to variable slope and drift of baseline, no automated integration was applied. Manual integration was applied instead, using "Link point" method in the case of fully resolved peaks or "New baseline"—allowing vertical peak splitting in the other cases. Quantitative determination was performed manually on the base of peak areas and external standard calibration. The calibration curve was obtained at the same conditions for standard solutions of vanillin, corresponding to the content at 5 to 5000 mg/kg range. No correction for detector sensitivity was applied.

3. Results and Discussion

3.1. Extractives Content

The content of extractives (Table 1) was determined in the mixture of chloroform: ethanol (POCH, Gliwice, Poland) by mass ratio of 93:7. This mixture has been applied in order to eliminate solvent containing carcinogenic benzene. The selection of the mixture was made on the base of Hansen's three-component solubility parameter system [26] and verified as comparable with standard ethanol-benzene solvent [25]. The difference

in the amount of extractives in relation to the literature data may result from the use of chloroform-ethanol mixture instead of classic solvents, as well as from different ages of some of the poplar varieties used. The smallest difference was observed for *P. maximowiczii* and the largest for *P. nigra*. The extractives content values for different poplars, with the exception of *P. trichocarpa*, reach similar values in Table 2.

Table 2. Extractives content.

Poplar Species	Extractives Content [% ± SD]	Ethanol-Benzene Extractives [%] [Ref.]
Japanese poplar (*Populus maksimowiczi* Henry)	2.4 ± 0.2	2 [15], 3 [27], 2 [28]
Black cottonwood (*Populus trichocarpa* Torr. and A. Gray ex. Hook.)	0.9 ± 0.1	3 [15], 3 [27]
European aspen (*Populus tremula* L.)	2.7 ± 0.2	3.1 [28], 2.0 [29],
White poplar (*Populus alba* L.)	2.3 ± 0.1	5 [15], 5 [27], 5.7 [28], 5.7 [30]
Black poplar (*Populus nigra* L.)	2.0 ± 0.1	5 [15], 5 [27], 2.1 [28], 1.8 [30]

Chromatograms presented in Figure 2 indicate clear differences between the species studied. The highest number of peaks, simultaneously with the highest relative intensity, were identified in the wood extract of white poplar, outside the range of retention times above 33 min. In this range, associated with the presence of mainly sterols and other terpenoids, as described below, white poplar wood extract contained the least compounds.

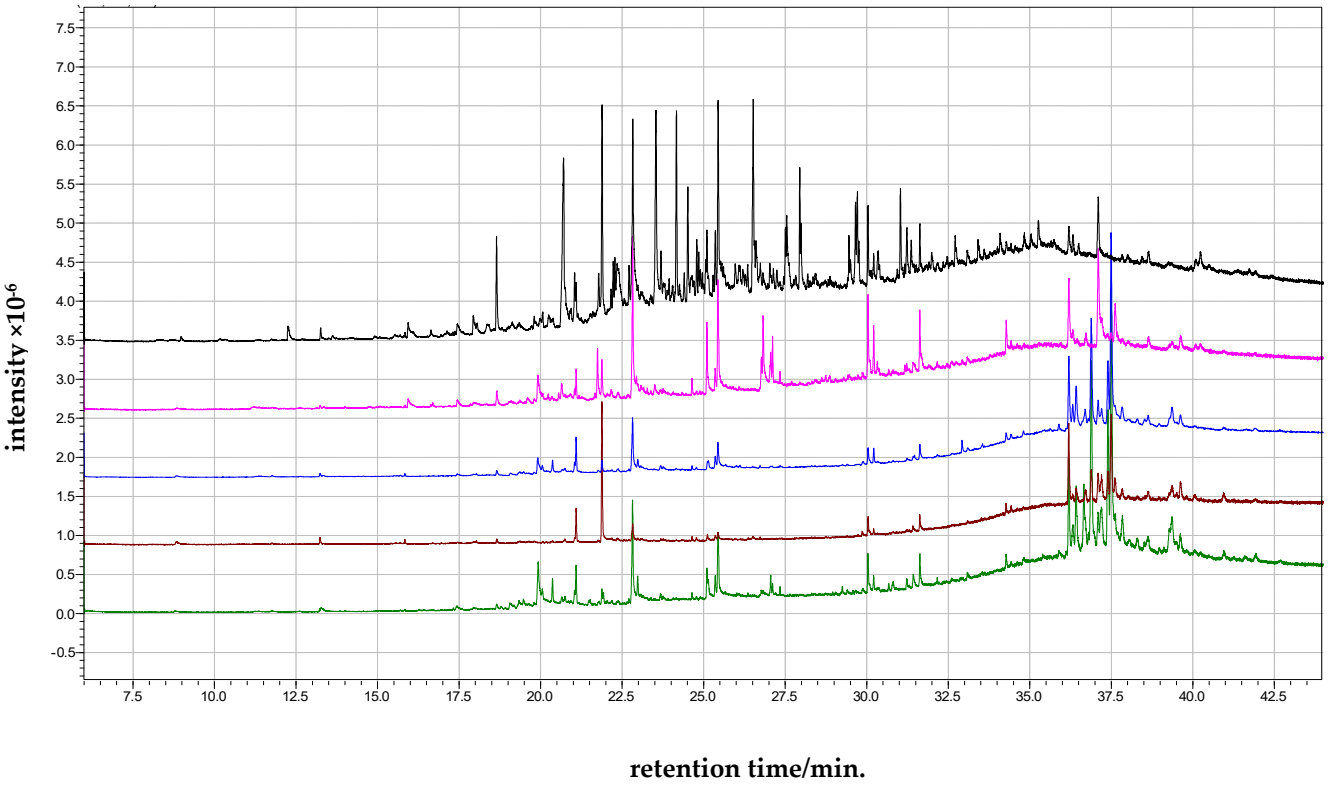

Figure 2. Raw chromatograms of individual poplar samples (black—*P. alba* L., pink—*P. tremula* L., blue—*P. nigra* L., brown—*P. trichocarpa* Torr. and A. Gray ex. Hook., green—*P. maksimowiczi* Henry.

Peaks were found to be present on all the chromatograms, as well as those found on some or even characteristic of only one extract. The separation of the substances on the chromatograms is sufficient to allow identification of the substances in the individual extracts. The compounds were best identified on European aspen chromatogram (Figure 3). The results of analyses were grouped into classes of compounds and discussed in the order of growing retention times.

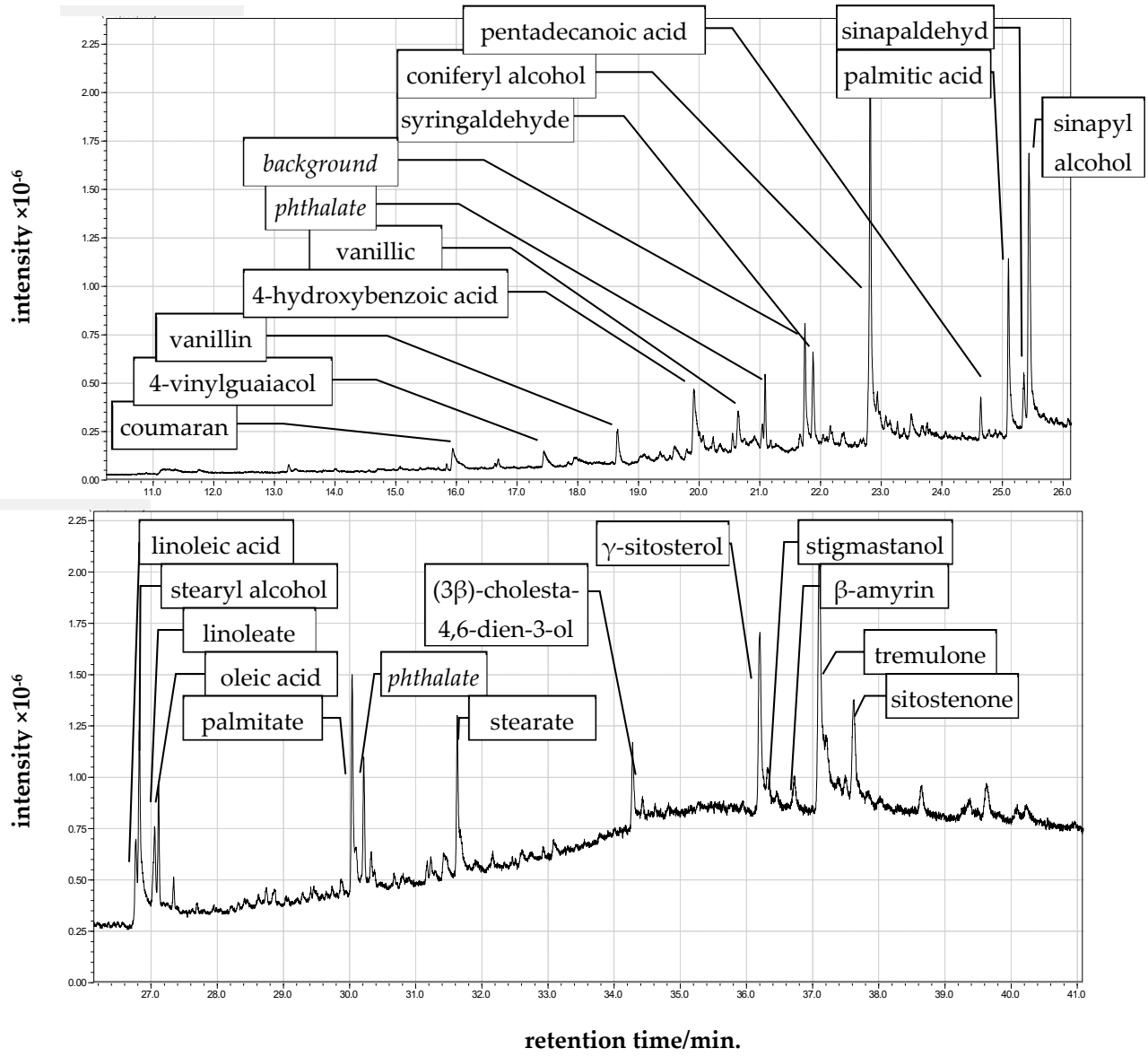

Figure 3. European aspen chromatogram (*P. tremula* L.) with peak identification.

3.2. Phenols

Table 3 presents phenolic compounds identified in wood extracts of five studied poplar species.

Table 3. Phenolic compounds identified in wood extracts of the studied poplar species.

Name	RT [min]	Content in Wood [mg/kg] (SD)				
		P. maximowiczii	*P. trichocarpa*	*P. tremula*	*P. alba*	*P. nigra*
4-vinylguaiacol	17.43	108 (10)	-	278 (26)	-	5 (1)
vanillin	18.66	47 (5)	44 (6)	328 (21)	997 (77)	80 (10)
4-hydroxybenzoic acid	19.91	622 (60)	-	922 (90)	-	322 (28)
vanillic acid	20.65	117 (17)	-	354 (39)	3 024 (195)	-
syringaldehyde	21.87	205 (19)	261 (19)	644 (56)	1 486 (91)	136 (17)
coniferyl alcohol	22.83	1 209 (109)	248 (21)	3 196 (227)	550 (48)	810 (76)
syringic acid	23.52	-	-	-	1 304 (134)	-
acetosyringone	23.96	-	-	-	190 (17)	-
ethyl vanillate	24.17	-	-	-	2 192 (148)	-
(3,5-dimethoxy-4-hydroxyphenyl)acetic acid	24.41	-	-	-	221 (17)	-
protocatechuic acid	24.51	-	-	-	741 (79)	-
sinapaldehyde	25.35	161 (15)	54 (4)	373 (35)	111 (8)	118 (8)
sinapyl alcohol	25.44	626 (40)	68 (11)	2 542 (155)	751 (69)	254 (22)
acetosyringic acid	26.51	-	-	-	1 146 (90)	-

2-methoxy-4-vinylphenol (4-vinylguaiacol) is a compound known primarily as a flavoring agent, in its natural state co-responsible for buckwheat odor. In wood, it is usually is associated with thermal modification [31] and pyrolysis processes. It was detected repeatedly in hardwood smoke [32,33]. Its presence was confirmed in bio-oil obtained by pyrolysis of genetically modified poplar varieties [34,35]. Zarzyński [36] confirms the occurrence of an isomeric compound—2-methoxy-6-vinylphenol—in the wood of, among others, European aspen (*Populustremula*) and brittle willow (*Salix fragilis*), but the author's identification may be questionable as he does not state which library he used and the compound does not appear in the most widely used NIST database.

Vanillin is one of the most commonly occurring phenolic substances in wood [37], which is confirmed by its identification in all studied poplar species, with the highest content of this compound found in the wood of white poplar (*Populus alba*). The compound is also a typical component of hydrolytic liquids formed by pretreatment of poplar wood [38,39]. Cantarella et al. [23] studied the effect of vanillin released from poplar wood by steam explosion treatment on hydrolytic enzyme activity and found that for a concentration of 0.5 g/dm3, the effect was insignificant. They also did not observe synergistic effects with compounds such as acetic acid, furfural, hydroxymethylfurfural, syringaldehyde and 4-hydroxybenzaldehyde. On the other hand, Li et al. [40] found reversible inactivation of cellulase in the presence of higher amounts of vanillin (IC50 for 30 g/dm^3).

4-hydroxybenzoic acid is the simplest of the phenolic acids, found in abundance in Japanese and black poplar wood and absent in detectable concentrations in extracts from California and white poplar. It was confirmed as a fermentation inhibitor found in poplar wood subjected to steam explosion [41]. It is also one of the primary products of lignin oxidation of various poplar clones [42]. It is a substance with relatively low toxicity—the LD50 in mice (oral) is as high as 2200 mg/kg [43].

Vanillic acid is a derivative of vanillin that occurs in abundance in white poplar wood and in smaller amounts in Japanese poplar and aspen wood. Reports of its presence in oak, cherry or acacia wood are available in the literature [44], and it isidentified as a potential fermentation inhibitor present in the wood of poplar hybrids [45]. Interestingly, it is the main phenolic substance present in argan oil [46].

Syringaldehyde is one of the most common phenolic compounds found in wood [37] and also one of the oldest recognized [47]. At the same time, it is one of the main products of wood hydrolysis during hydrothermal treatment [48], in particular steam explosion treatment of poplar wood [23], with no significant effect on hydrolytic enzyme activity found for concentrations of 0.5 g/dm^3. It has been analyzed in genetic modification studies of poplars [49,50], where its content in wood extracts is increased. The compound is considered to be an attractant for the woodworm *Scolytusmultistriatus* [51], attacking primarily elm, but also oak and aspen, among others.

Coniferyl alcohol is, apart from sinapyl and p-coumaryl alcohol, the primary precursor of lignin in the wood of both coniferous and deciduous species [52,53]. It is therefore not surprising that it is present in all the extracts examined, with only the white poplar (*P. alba*) wood extract not being one of the dominant peaks. Coniferol is also one of the primary compounds of interest in breeding poplar varieties, including genetically modified ones [50,54,55]. It is also identified as one of the phenolic inhibitors of enzymatic hydrolysis [13].

Syringic acid is a derivative of syringaldehyde that occurs as a dominant peak in white poplar extract and is undetectable in other species. The compound was identified as a potential fermentation inhibitor in poplar hybrid studies [45] andwas also detected in other types of lignocellulosic biomass—wheat straw, rice, and corn [56].

Acetosyringon is another syringaldehyde derivative that, like syringic acid, is found only in white poplar extract and is undetectable in other species. It has been linked to the plant stress response to wounding [57], which may be an alternative explanation for the presence of the compound in only one wood species.

Ethyl vanillinateis a derivative of vanillin and vanillic acid, identified only in white poplar wood. Since significant amounts of vanillic acid were also detected in this material, it is reasonable to believe that it may have been the source of the identified ester—formed during extraction with ethanol as the main solvent.

3,5-dimethoxy-4-hydroxyphenylacetic acid is an uncommon compound that is a homologue of syringic acid, with a longer alkyl fragment but shorter than in the phenylpropane unit. It is found in small amounts only in the wood of white poplar. There are reports of detection of this compound in volatiles (VOCs) present in thermally modified wood [31]. It is confirmed to be present in significant amounts in another lignocellulosic material considered as an energy source: tobacco stems [58].

Protocatechuic acid (3,4-dihydroxybenzoic acid) is one of the simplest phenolic acids that is found in abundance in numerous herbs (including rutabaga or mallow) [59]. In the extracts studied, it was detected only in white poplar. Tanase et al. [60] report the detection of significant amounts of this compound in the bark of various species of eucalyptus, as well as black spruce. It is also found in leachate obtained from hydrothermal treatment of wood [48]. Protocatechuic acid can be formed by biosynthetic processes from, among others: vanillic acid under the action of the enzyme vanillin monooxygenase (demethylase) [61] and p-hydroxybenzoic acid under the action of the enzyme 3-monooxygenase 4 hydroxybenzoate [62]. The presence of a large amount of vanillic acid in white poplar wood and the concomitant absence of a detectable amount of p-hydroxybenzoic acid points to the first pathway as a potential source of this compound. This is confirmed by the absence of protocatechuic acid in other poplar species, in which the vanillic acid content is much lower or undetectable.

Sinapaldehydeis a homologue of syringaldehyde, based on a phenylpropane skeleton, commonly found in phenolic wood [37]. It was found in a methanolic extract from the wood of quaking aspen, *P. termuloides* [63]. It was intensively studied as a marker for genetic changes in various poplar hybrids [64,65]. It was also detected alongside syringaldehyde in leachate after hydrothermal treatment of wood [48]. It occurred in every extract tested, although only in Japanese poplar in high amounts.

The sinapyl alcohol has not been identified by the library of MS spectra (Figure 4) used, which indicated 2,5-dimethoxybenzyl acetate or 3-(dimethoxyphenyl)-1-propanol as the

most likely spectra. However, the query in the database did not show the presence of the mass spectrum of sinapyl alcohol in the library. At the same time, this compound was found in all the samples tested, as one of the dominating peaks in the chromatograms. Detailed analysis of the spectrum indicated the possibility of interpreting the main spectrum peaks in decreasing m/z order (Figure 5):

210-molecular ion of sinapyl alcohol, $C_{11}H_{14}O_4^+$,
182-ethyl-syringol cation, loss of the CO fragment,
167-methylenesyringol cation, loss of CH_2CHO fragment
154-syringol cation, loss of C_3H_4O fragment.

This interpretation is also given by Takahashi [66], so the identification can be considered as confirmed. Sinapyl alcohol is one of the main monolignols, i.e., precursors of lignin [67]. It is also one of the basic compounds tested in genetically modified poplar hybrids [50]. It has cellulase inhibiting properties [68,69]. On the other hand, there are reports of its catalytic effect on lignin degradation [70].

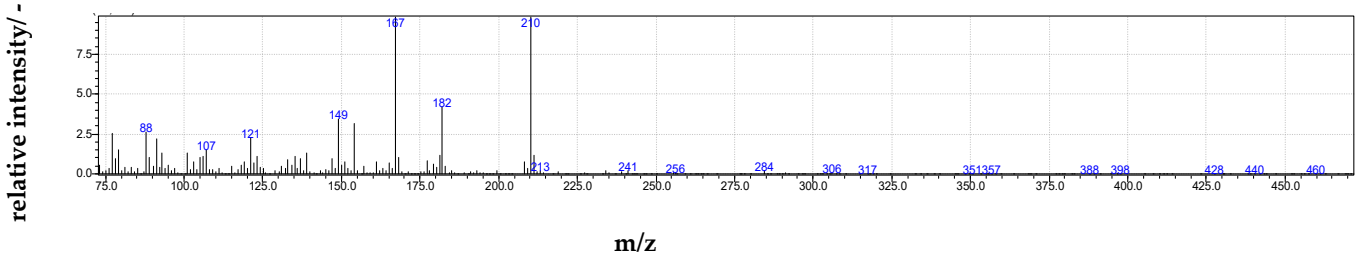

Figure 4. Mass spectrum of a compound identified as sinapyl alcohol.

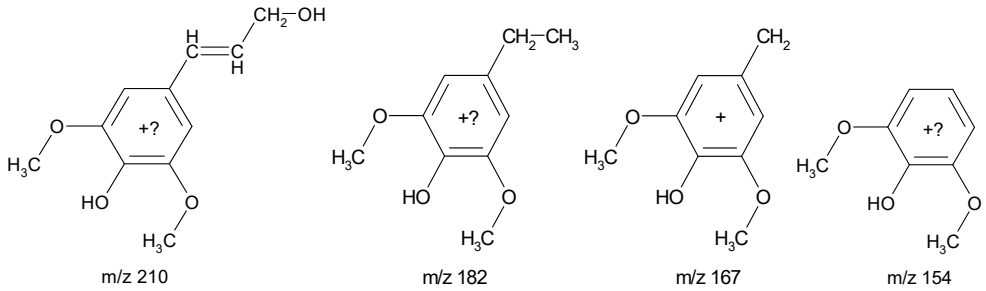

Figure 5. Presumed fragmentationof sinapyl alcohol.

The acetosyringic acid has (Figure 6) not been fully identified by the used MS spectral library. The MS spectrum shows significant similarity to syringic acid and lesser similarity to acetosyringone, but has an intense band corresponding to m/z 256. This may correspond to acetosyringic acid or be the result of fragmentation of an even larger molecule in which the hydroxyl group of syringic acid occurs as an ester. Ralph and Lu [71] reported that the acetylation of syringic compounds in position 4 may be the result of biological processes and that such groups are often found in the lignin of deciduous trees and dicotyledons. Structural units of lignin may also occur in the form of coumaric acid esters [72]. For willow and aspen wood, the hydroxyl group in position 4 often occurs in the form of p-hydroxybenzoate [71]. This type of ester linkage is also known to occur in tannins—digallicand trigallic acids are known [73].

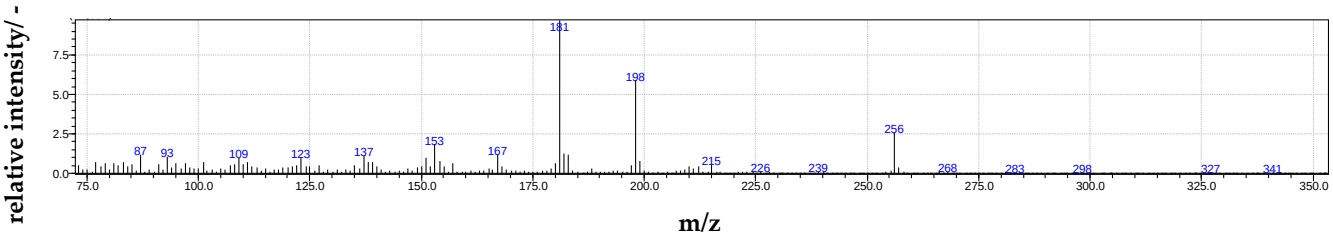

Figure 6. Mass spectrum of the compound identified as probably syringic acid acetate.

3.3. Other Compounds Corresponding to Lignin Structure

In addition to the classical phenolic compounds found in wood, three that are not obvious in terms of their presence in wood were also identified (Table 4).

Table 4. Other compounds identified in wood extracts of the poplar species tested.

Name	RT [min]	Content in Wood [mg/kg]				
		P. maximowiczii	*P. trichocarpa*	*P. tremula*	*P. alba*	*P. nigra*
coumaran	15.94	-	-	365 (33)	-	-
3,5-dimetoxyacetophenone	20.74	-	48 (4)	-	-	-
olivetol	22.98	146 (10)	-	-	-	78 (7)

The coumaran unit is considered to be a typical lignin fragment of deciduous trees [74,75]. There are also reports of identification of coumaran as a chemical compound in wood [36], but it was identified in iroko (*Chlorophoraexcelsa* Benth. and Hook). The identification of coumaran in *P. tremula* raises the suspicion that it may also be a compound not found in the MS spectrum base used, with a larger molecule containing coumaran structure. On the other hand, a short retention time is an argument in favour of volatile compound with small molecules.

3,5-dimethoxyacetophenone is based on the skeleton of phenylpropane, but unusual because of lack of hydroxyl group in position 4. It occurs only in the wood of *P.trichocarpa*. There are reports of its identification in thermal treated poplar wood [31].

Identification of olivetol is uncertain. Olivetol is a compound known to occur in different lichen species [76] but is best known as a precursor of tetrahydrocannabinol (THC), found in Cannabis sativa [77,78]. Perhaps the compound found in *P. nigra* and *P. maximowiczi* is another phenolic compound not found in NIST libraries. One of the rare connections between poplar and olivetol is the work of Gange et al. [79], which identified 12 DABB-type proteins with a sequence similar to the enzyme olivic acid cyclase.

3.4. Sterols and Terpenoids

Another group of compounds present in wood extracts of the examined poplar species are sterols, and terpenoids derived from them (Table 5). These compounds have a structure based on the triterpene skeleton, differing in the location of double bonds and the type and distribution of functional groups. More than 200 phytosterols, i.e., compounds of plant origin, are known [80]. The identification of most of them is uncertain due to the little characteristic MS spectra, typical for hydrocarbon fragmentation. These compounds are not considered as enzymatic hydrolysis or fermentation inhibitors, nor are they generally considered to be toxic [81]. There are only reports of potential adverse effects of sterols on the endocrine management of vertebrates, especially fish [82,83]. Therefore, a detailed analysis of the correctness of identification is not appropriate for the purpose of this paper. Table 4 shows compounds which identification was based only on correlation with the MS spectrum base. The highest content and diversity of these compounds was stated for the wood of Japanese poplar, and the lowest for white poplar, in the extract of which only

the presence of tremulone was detected, which was at the same time the only compound detected in all samples. The most frequently identified and present in the highest amounts were γ-sitosterol, stigmastanol, α and β-amyrin, α and β-amyrenone, and stigmastenone.

Table 5. Sterols and terpenoids identified in wood extracts from tested poplar species.

Name	RT [min]	Content in Wood [mg/kg]				
		P. maximowiczii	*P. trichocarpa*	*P. tremula*	*P. alba*	*P. nigra*
(3β)-cholesta-4,6-dien-3-ol	34.28	-	125 (10)	859 (86)	-	198 (18)
β-sitosterol	34.43	-	52 (5)	-	-	75 (5)
γ-sitosterol	36.19	1151 (111)	997 (102)	1 847 (176)	-	1 093 (74)
stigmastanol	36.32	847 (61)	126 (10)	599 (42)	-	353 (33)
β-amyrenone	36.42	801 (72)	248 (24)	-	-	739 (63)
β-amyrin	36.70	-	161 (15)	314 (30)	-	357 (19)
α-amyrenone	36.89 ()	2 001 (208)	479 (37)	-	-	1 647 (125)
cholesta-3,5-dien-7-one	37.10 ()	-	364 (28)	-	-	361 (33)
tremulone(stigmasta-3,5-dien-7-on)	37.12 ()	131 (17)	464 (38)	2 930 (277)	180 (19)	-
α-amyrin	37.19 ()	424 (32)	-	-	-	318 (17)
24-methylenecycloartanol	37.40 ()	476 (33)	362 (33)	-	-	884 (54)
sitostenone (stigmast-4-en-3-one)	37.51 ()	2 411 (230)	1 097 (76)	-	-	2 832 (274)
(5α)-stigmastano-3,6-dione	38.70 ()	866 (78)	221 (13)	-	-	160 (13)
lupan-3-ol	39.62 ()	178 (12)	302 (27)	-	-	-
9,19-cyklolanostan-3-ol	39.63 ()	-	-	-	-	149 (10)

3.5. Fatty Compounds

Numerous fatty compounds were detected on the chromatograms of the examined extracts. In the absence of standards, their identification could only be based on the degree of similarity to the MS spectra present in the base used. However, it should be stressed that the spectra of fatty acids are not very common and often the identification is based on the presence of the mother peak [84]. Moreover, fatty acids are difficult to evaporate and their quantitative analysis is carried out after derivatization [85,86]. For this reason, the exact interpretation of the compounds in this group was not carried out, especially as they are not considered to be enzymatic hydrolysis inhibitors due to their difficult solubility in water.

In the extracts studied, compounds which most probable interpretation indicated free fatty acids, methyl or ethyl esters, which may have resulted from interaction with the solvent, as well as monoglycerides, were detected. The absence of di- and triglycerides on the chromatograms is due to their very low volatility, which prevents separation by gas chromatography.

The compound most frequently identified and giving the strongest signals on chromatograms was saturated palmitic acid CH_3-$(CH_2)_{14}$-COOH, found in extracts from all wood species. Another acid is saturated stearic acid CH_3-$(CH_2)_{16}$-COOH, also present in all samples, although large quantities were detected only in *P. maksimowiczi*. The next two acids: oleic CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH and linoleic CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-$(CH_2)_7$-COOH were present in noticeable amounts only *in P. trichocapra* and *P. tremula*. Moreover, small amounts of lauric acid were detected in *P. tremula* and pentadecanoic acid in *P. alba*. In turn, the wood of *P. nigra* was probably found to contain stearyl alcohol.

3.6. Other Compounds with Difficult Identification

In addition to the identified compounds, numerous peaks were detected in the samples which could not be identified, most often due to their too low intensity, making it impossible to eliminate the background effect and obtain MS spectrum in a quality allowing for comparison with the base. In some cases identification is impossible despite the presence of large peaks, because not all compounds present on the chromatograms are in the used MS spectrum library. Another possibility is the incomplete separation of the compounds, as exemplified by the small peak with a retention time of 26.82, occurring only on the chromatogram of *P. tremula* extract. Analysis of the MS spectrum did not bring any results. The comparison of the MS spectra determined for the initial and final peak fragment showed significant differences (Figures 7 and 8). The spectrum of the initial part of the peak resembles synapyl alcohol identified slightly earlier, while the spectrum of the final part of the peak is not very typical, however, there are groups of signals, which in combination with the m/z difference of 14 (fragment -CH_2-) are typical for hydrocarbon chains. This means that it is most likely that there is a mixture of some complex phenol and a fatty compound at this point in the chromatogram. Identification of components is not possible in this case without changing the conditions of the chromatographic analysis. Considering the complexity of the chromatogram, it can be assumed that changing the temperature program, which was the only way of working, would not give satisfactory results. The use of a chromatographic column with a different stationary phase, interacting differently with compounds of different polarity, would probably be the only appropriate method. However, there is no certainty that even then separation would be possible. No further research was carried out in this direction, but the example described is given because it illustrates well the complexity of extractive substance analysis.

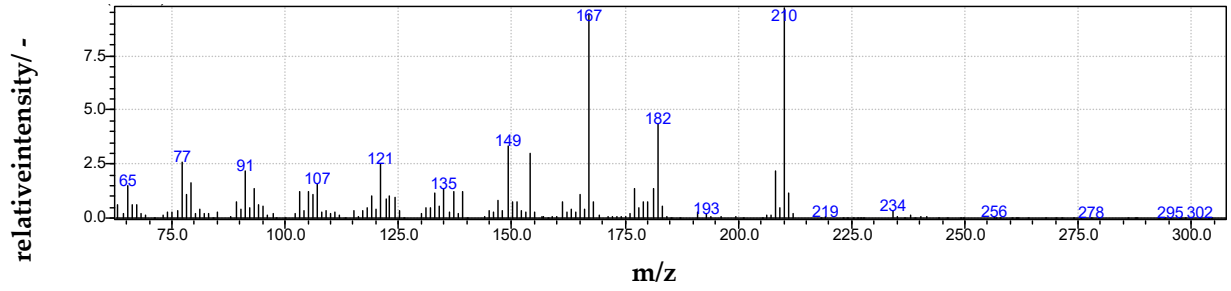

Figure 7. Mass spectrum of the initial unidentified peak fragment on the chromatogram of *P.tremula* extract with a retention time of 26.82.

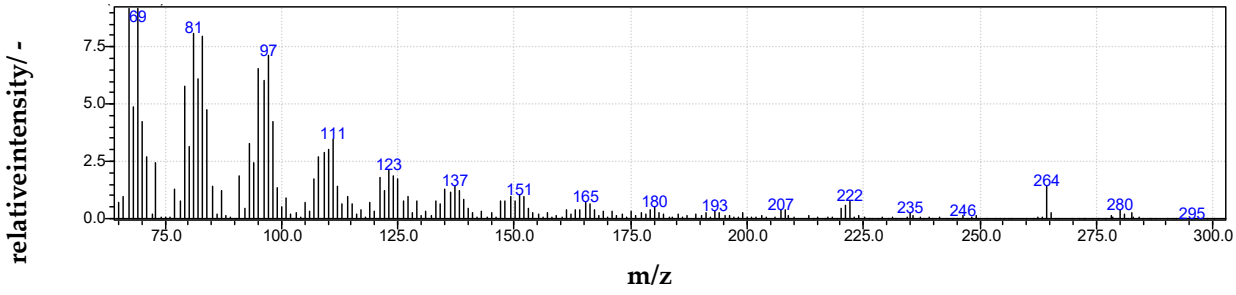

Figure 8. Mass spectrum of the final unidentified peak fragment on the chromatogram of *P.tremula* extract with a retention time of 26.82.

A separate issue is the detection of compounds identified as phthalates in some extracts. One of them was detected in samples of *P. trichocarpa* and *P. tremula*, and two more in each separately. Such compounds do not occur naturally in wood, but are commonly detected in a variety of materials, including biological origin [87]. The cause is

the contamination of samples with phthalates most often present in materials used for packaging [88,89].

4. Conclusions

The aim of the study was the qualitative and quantitative analysis of the composition of extractives in wood of various poplar species from the viewpoint of the potential impact on the rate and efficiency of enzymatic hydrolysis of polysaccharides contained in wood. It was found that individual poplar species differ significantly not only in content, but also in the composition of extractives. Only a few phenolic compounds have been detected in wood of all species—vanillin, coniferyl, and sinapyl alcohols, syringaldehyde and sinapaldehyde. 4-vinylguaiacol, 4-hydroxybenzoic acid, and vanillic acid were detected in three species, while the remaining compounds were found only in the wood of white poplar. There was no correlation between the number of compounds detected and the total content of extractives.

Three uncommon compounds corresponding to the structure of lignin were detected, of which olivetol is widely known as a component of lichen species and *Cannabis* sp. herbs.

Significant amounts of sterols and other terpenoids have been found in the wood of the studied poplars. Contrary to phenolic compounds, the least terpenoids were found in the wood of white poplar, while the highest content was found in *P. nigra*, *P. maximowiczii*, and *P. trichocarpa*. The terpenoid with the highest content was sitostenone in all cases, while it was absent in wood of *P. tremula* and *P. alba*. Other significant compounds were tremulone, γ-sitosterol, and stigmastanol, each detected in wood of four species.

The obtained results allow for the study on the effect of extractives on the activity of hydrolytic enzymes and are an attempt to correlate with the content of individual substancesidentified.

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