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Analysis of quantitative and qualitative extractive components extracted from hornbeam (*Carpinus betulus L.*) and yakal (*Shorea astylosa* Foxw.) wood

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Abstract: The aim of the study was analysis of quantitative and qualitative extractive components extracted from hornbeam *(Carpinus betulus L.)* and yakal *(Shorea astylosa Foxw.)* wood. Two wood species similar to each other in terms of microscopic structure and physical properties, the native wood of Hornbeam (*Carpinus betulus* L.) and the yakal wood (*Shorea astylosa* Foxw.) endemic to the Philippines, which is still little understood, were investigated. Wood is a material composed mainly of polymers called lignocellulose which includes: (cellulose, lignin and hemicelluloses), but also contains many extractive and mineral substances. . The extractive content of wood in temperate climates is approximately for up to 5% by weight of wood. Their identification is difficult because of the wide variety and multiplicity of compounds present. However, extractive compounds are important for the resistance of trees and wood to biological agents such as fungal infestation or insect infestation. Usually the zones of dead wood (heartwood or cored wood) are characterized by a higher content of extractives. The understanding of the chemical composition of extractives in wood can contribute to the development of wood preservative products and environmentally friendly and would be part of the green chemistry policy.

Keywords: wood extractives, GC-MS, yakal, hornbeam

INTRODUCTION

Wood is a natural polymeric material based on structural compounds including substances such as celluloses and hemicelluloses, which form chain structures responsible for the tensile strength of wood, and lignin, which is an amorphous polymer that penetrates structures made of cellulose and hemicelluloses. Lignin is mainly responsible for the compressive strength of wood. In addition to structural compounds, minerals and extractives are present in wood. These substances include compounds such as tannins, resins, waxes, essential oils, resins, gums, dyes and protein substances[Krutul 2002]. These compounds have different polar character. Therefore different organic and non-organic solvents and their mixtures are used for their determination. [Ralph et al. 2008; Rowell et al. 2005]. Due to the different nature of the polarity of extraction compounds, they affect the colour of the wood, its resistance to biological corrosion (action of fungi and insects that can feed on the wood and decompose wood tissue), its stickiness, buffer capacity, etc. [Król et al. 2017; Bockel et al. 2019].

"Domestic common hornbeam (*Carpinus betulus* L.) wood and exotic yakal (*Shorea astylosa* Foxw.) are structurally similar Species. Both species belong to hardwood species with diffuse-porous wood, characterized by vessels of similar size and distribution in the whole annual ring and the lack of a clear border in the annual ring between the early and late wood." [Antczak, Sierant 2020]. Hornbeam wood is heavy, hard and therefore difficult to split, it is

heartless and greyish-white in color, with an average density of 790 kg/m3. It is used, among other things, for machine parts, tools, particle board and also as firewood [Krzysik 1978].

The content of extractives in wood depends on the species, the habitat of the tree, the climate in which it occurs, but also on the location on the tree cross-section itself, the zone of the trunk (heartwood zone or sapwood zone), the height in the trunk. The content of extractives in wood varies from approx. 1% to approx. 20%. Exotic tree species have a higher extractive content than temperate species. This is already visible in the colour of the wood itself and its resistance to biological agents. Wood from tropical zones is also characterised by a higher density and lower variation in mechanical strength at individual anatomical sections of wood than wood from temperate zones [Kozakiewicz et al. 2021; Krzysik 1978; Prosiński 1984; Krutul 2002].

Extraction substances are mixtures of tannins, terpenes, rosins and aromatic phenolic compounds. The compounds found in wood in extraction substances are used in various fields. Vanillic acid, for example, is used as a fragrance compound. Some groups of compounds have very different properties, for example phenolic compounds are used as analgesics, antiarrhythmics, antiedematous, antifungal, anti-inflammatory, anesthetics, although some of the neolignans (phenols) have toxic properties including cytotoxicity. Flavonoids (included in phenols), occurring in wood, through oxidative dimers, are responsible for intense red pigmentation of some resins. A wide range of compounds belonging to the group of terpenes, are a great source of essential oils and are also used as various solvents, have insecticidal properties [Gottlieb 1980].

Using gas chromatography coupled to mass spectrometer (GC-MS), extracts in these wood species were analyzed. Compounds from several classes such as phenols, fatty acids and their derivatives, and sterols were identified. The identification of the compounds detected during the study is discussed along with the estimation of the content in the wood of the studied species. The use of an appropriate instrumental method for sample analysis allowed the detection of compounds with low boiling points. The analysis of the composition of extractives of two similar species, growing in significantly different habitat conditions, can help in further studies and analyses of their properties, colour or even its applicability.

The aim of this study was to analyse with an attempt to identify substances extracted from the wood of two species, with a mixture of chloroform:ethanol, using a gas chromatograph coupled with mass spectrophotometry (GC-MS).

MATERIALS AND METHODS

Before conducting the analysis, the research material should be properly prepared, subjected to adequate processing, so as to be able to analyze these samples with a gas chromatograph.

The sample of common Hornbeam wood (*Carpinus betulus* L.) was taken from the Mazovia region (Forest Inspectorate of Wyszków) from a tree of average age with a diameter of about 22cm, while the sample of exotic yakal wood (*Shorea astylosa* Foxw.) came from the southern part of the island of Luzon, in the province of Batangas in Philippines from a tree of similar age (about 30 years) and only part of heartwood was used for each wood species.

Three samples of about 100 grams were prepared for each species, which were grounded using a Retsch SM100 laboratory mill and sieved. The chips of 0.43 ÷ 1.02 mm fraction were

used to extract the substances. The extraction material used was 7.7% moisture content for hornbeam wood and 7.5% moisture content for Yakal wood.

Extraction was carried out using Soxhlet apparatus with a mixture of chloroform and ethanol at a ratio of 93:7 by weight [Antczak et al. 2006] using the method described by Krutul [2002]. Regardless of the studied species, the extraction time was 10 hours[Antczak, Sierant 2020]. Then, about 1 cm^3 each of the obtained solution of solvents and extraction substances was taken for further study.

The content of non-structural constituents in the hornbeam test wood sample was 1.97% (including 1.45% extractives), while the yakal test wood sample contained 3.13% non-structural constituents, including 1.79% extractives[Antczak, Sierant 2020].

The qualitative composition of the extractives was studied on a GCMS-QP2010 chromatograph (Shimadzu, Kyoto, Japan) coupled to a mass spectrometer. A ZB-5ms capillary column with a length of 30 meters, a diameter of 0.25 mm, and a 0.25 μm bed was used. The temperature program started at 50 degrees Celsius, which was maintained for 7 minutes, after which it was increased by 10 degrees Celsius per minute until it reached 320 degrees, a temperature that was maintained for 10 minutes. The carrier gas used was helium 6.0, with a flow rate of 0.8 cm^3/min. Sample injection was performed directly on the column, and the injection temperature was 250 degrees Celsius. And the detector voltage was set at 0.8 kV. Samples were introduced onto the column using an AOC-20i autosampler. The resulting chromatographs were analyzed using dedicated software GCMSolution software version 2.72. Single waveform smoothing was used using the implemented Savitzky-Golay method with a smoothing width of 2 s. Individual peaks were identified by comparing the spectrum with the NIST11, NIST11b spectrum library. The most likely matches were verified against literature data on the occurrence of individual compounds in plants.

RESULTS AND DISCUSSION

The chloroform:ethanol mixture used with a mass ratio of 93:7, selected based on Hansen's three-component solubility parameter system [Hansen 1967], may cause a difference in the amount of extractives compared to literature data where other solvents were used. It was decided to change the solvent from the formerly popular mixtures due to the carcinogenic benzene that these mixtures contained. The difference of the contained extractives with respect to the literature may be caused by the different environmental conditions of the growing trees.

The highest number of peaks, at the same time with the highest relative intensity, was identified in yakal (*Shorea astylosa* Foxw.) wood extract for retention times above 18 min.

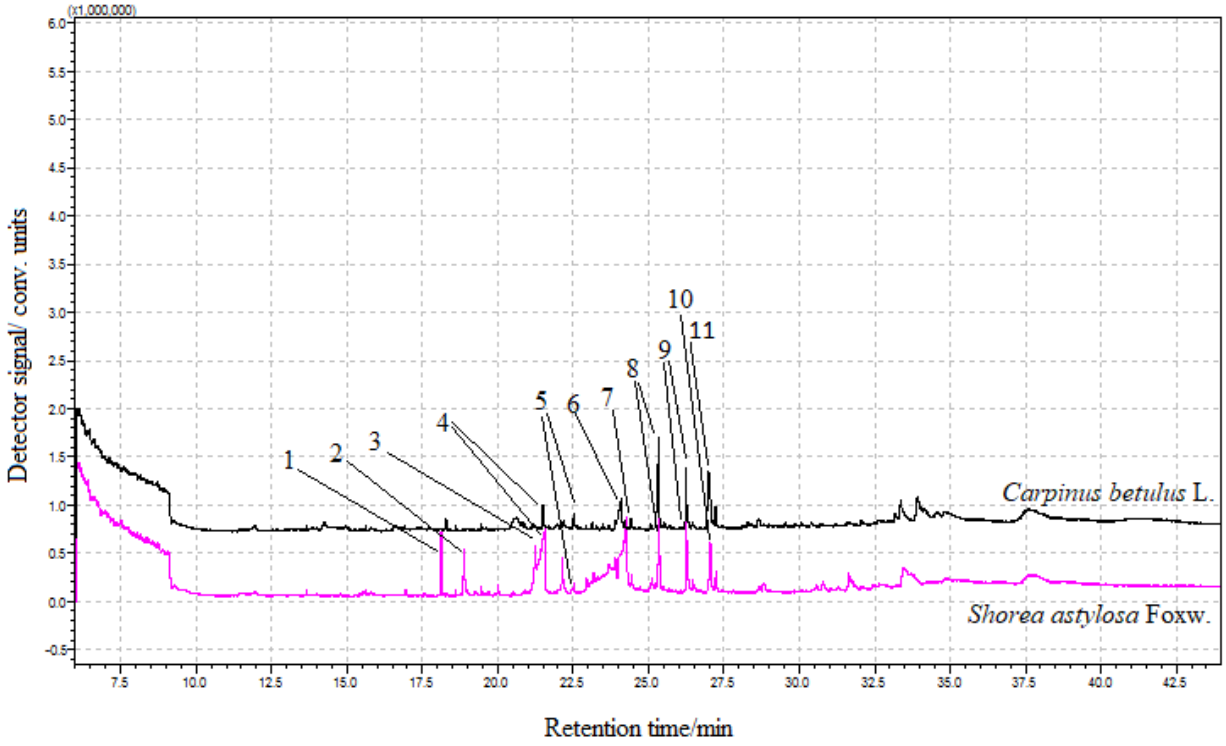
The individual substances are separated sufficiently in the chromatograms to be identified in individual samples. The compounds in the extractives were best identified for the exotic species. Results are discussed in order of increasing retention times.

Occurring in natural oils (for example, from seeds) are a number of lucrative fatty acids that are used as ingredients in the manufacture of cosmetics and personal care products, including lubricants, soaps and hair shampoos. [Muhamed et al. 2017] The fatty acids determined during the analysis, such as linoleic acid, were also determined by Felegyi-Tóth [2021] in her study in extraction substances found in hornbeam leaves.

Despite the presence of some of the same compounds in the extractives of both species, the compositions are significantly different, which can be seen immediately on the

chromatographs (Figure 1). Changes in analysis parameters, such as temperature or column changes, can affect the analysis.

Figure 1. Shows the two chromatographs, with the substances with the largest peaks identified



1- octanoic acid,
2- vanillin,
3- vanillic acid 1,
4- vanillic acid 2,
5- phenol, 2,6-dimethoxy-4-(2-propenyl)-,
6- syringic acid 1,
7- syringic acid 2,
8- n-hexadecanoic acid,
9- 9-octadecen-1-ol, (Z)-,
10- cis-vaccenic acid
11- linoleic acid

Marked substances found in samples of both species include (noted on the chromatographs):

- Vanillin (number 2 in Figure 1.) is most common phenolic substance. Suspected of inhibiting the enzymatic hydrolysis of wood. [Walker, Wilson 1991]
- Vanillic acid (number 3 and 4 in Figure 1.) is a phenolic acid, a derivative of vanillin.
- Phenol, 2,6-dimethoxy-4-(2-propenyl)-, (number 5 in Figure 1) it is a phenolic component of wood. [Walker, Wilson 1991]
- Syringic acid (number 6 and 7 in Figure 1) it is syringaldehyde derivative, a potential fermentation inhibitor [Lu et al. 2002]
- n-Hexadecanoic acid (number 8 in Figure 1.) is hexadecenoic acid, called palmitic acid, It is a component of vegetable fats is used as a fragrance compound.

- 9-Octadecen-1-ol (number 9 in Figure 1.) is probably a derivative of oleic acid. The most common fatty acids found in plants are monounsaturated (oleic acid) and saturated (stearic acid and palmitic acid) [Alireaza 2012]
- cis-Vaccenic acid (number 10 in Figure 1.) is a stereoisomer, of vaccenic acid, this stereoisomer is a monounsaturated fatty acid of the omega-7 group found in sea buckthorn (Hippophae rhamnoides L.) oil, among others) [Vuorinen 2015]
- Linoleic acid is the fatty acid most preferred for use in cosmetics and personal care products because it cannot be synthesized by the body and is very often used as a moisturizer for skin, nails and hair. Linoleic acid deficiency can lead to dry and scaly skin, hair loss or cracked nails [Vermaak et al. 2011]

Table 1. The list of identified compounds in the wood extracts of both tree species by average retention time

Name	Hornbeam	Yakal
	Avg. R [min]	Avg. R [min]
Furan, 2-pentyl-	11,45	-
Hexanoic acid	11,97	11,95
Hexane, 1,1-diethoxy-	13,65	13,66
Nonanal	14,00	-
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14,94	-
Dodecane	15,62	15,56
Octanoic acid	-	16,33
5-Hydroxymethylfurfural	16,56	-
Nonanoic acid	16,92	16,49
2-Methoxy-4-vinylphenol	-	17,36
Ethanol, 2-(2-butoxyethoxy)-, acetate	-	17,94
Heptanoic acid, 2-methylbutyl ester	18,28	-
n-Decanoic acid	-	18,29
Tetradecane	18,62	-
Hexadecane	-	18,62
Vanillin	-	18,88
1-Pentene, 3,3-dimethyl-	-	19,26
Isoeugenol	19,45	19,46
.gamma.-Dodecalactone	-	19,70
Apocynin	-	20,01
2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	-	20,51
.beta.-D-Glucopyranose, 1,6-anhydro-	20,58	-
Dodecanoic acid	-	20,83
Heptadecane	21,19	-
Vanillic acid 1	-	21,24
Vanillic acid 2	21,49	21,52
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	-	22,14
Vanilic acid hydrazide	-	22,45

Table 1. continued The list of identified compounds in the wood extracts of both tree species by average retention time

Phenol, 2,6-dimethoxy-4-(2-propenyl)-	22,52	22,53
(1)Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	-	22,94
2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-	-	23,09
Tetradecanoic acid	23,13	23,18
3,5-Dimethoxy-4-hydroxyphenylacetic acid	23,28	23,30
2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	23,68	23,66
(2)Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	-	23,90
Syringic acid	24,08	24,25
Pentadecanoic acid	24,22	-
1-Hexadecanol	24,41	24,43
9-Hexadecenoic acid	25,06	-
n-Hexadecanoic acid	25,33	25,34
Hexadecanoic acid, ethyl ester	25,51	-
3,5-Dimethoxy-4-hydroxycinnamaldehyde	25,66	25,68
9-Octadecen-1-ol, (Z)-	26,24	26,25
1-Octadecanol	26,46	-
Linoleic acid	26,98	-
7-Tetradecenal, (Z)-	27,02	-
cis-Vaccenic acid	-	27,04
Stearic acid	27,21	-
Octadecanoic acid	-	27,23
cis-9-Hexadecenal	28,65	-
Eicosanoic acid	29,00	-
Heptadecanal	29,82	-
Octadecanal	30,10	-
Octacosanol	-	33,43

Table 2. List of individual compounds identified in Yakal wood, along with the average percent peak area and peak height

Name	Avg. R [min]	% areas		% heights		% ext. part
		Avg.	SD	Avg.	SD	Avg.
Hexanoic acid	11,95	0,64	0,061	0,60	0,031	0,64
Hexane, 1,1-diethoxy-	13,66	0,57	0,276	0,99	0,361	0,56
Dodecane	15,56	0,43	0,151	0,62	0,240	0,43
Octanoic acid	16,33	1,90	3,015	3,42	5,352	1,90
Nonanoic acid	16,49	0,53	0,250	0,72	0,144	0,53
2-Methoxy-4-vinylphenol	17,36	0,48	0,186	0,55	0,270	0,48
Ethanol, 2-(2-butoxyethoxy)-, acetate	17,94	5,63	5,812	6,57	6,307	5,62
n-Decanoic acid	18,29	0,19	0,103	0,35	0,058	0,19
Hexadecane	18,62	0,39	0,110	0,76	0,104	0,39
Vanillin	18,88	10,67	4,538	8,47	2,000	10,65
1-Pentene, 3,3-dimethyl-	19,26	0,41	0,089	0,44	0,078	0,41
Isoeugenol	19,46	1,60	0,812	1,92	0,575	1,60
.gamma.-Dodecalactone	19,70	0,43	0,153	0,54	0,085	0,43
Apocynin	20,01	1,25	0,400	1,51	0,246	1,24
2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	20,51	0,40	0,272	0,50	0,218	0,40
Dodecanoic acid	20,83	0,25	0,146	0,43	0,110	0,25
Vanillic acid 1	21,24	4,48	1,028	4,59	0,758	4,47
Vanillic acid 2	21,52	18,95	1,810	10,93	1,416	18,91
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	22,14	5,31	1,089	6,13	1,515	5,30
Vanilic acid hydrazide	22,45	0,55	0,083	0,64	0,075	0,55
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	22,53	1,28	0,696	1,83	0,619	1,27
(1)Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	22,94	1,39	0,365	1,77	0,098	1,38
2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-	23,09	0,31	0,128	0,49	0,106	0,31
Tetradecanoic acid	23,18	0,89	0,114	1,44	0,072	0,88
3,5-Dimethoxy-4-hydroxyphenylacetic acid	23,30	0,33	0,185	0,50	0,137	0,33
2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	23,66	0,69	0,386	1,05	0,240	0,69
(2)Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	23,90	2,07	0,558	2,66	0,298	2,07
Syringic acid	24,25	10,30	3,366	9,55	0,781	10,28
1-Hexadecanol	24,43	1,22	0,115	1,61	0,310	1,22

Table 2. continued List of individual compounds identified in Yakal wood, along with the average percent peak area and peak height

Name	Avg. R [min]	% areas		% heights		% ext. part
		Avg.	SD	Avg.	SD	Avg.
n-Hexadecanoic acid	25,34	8,62	1,807	9,51	0,914	1,22
3,5-Dimethoxy-4-hydroxycinnamaldehyde	25,68	0,53	0,451	0,61	0,271	8,60
9-Octadecen-1-ol, (Z)-	26,25	7,56	4,103	8,66	3,833	0,53
cis-Vaccenic acid	27,04	4,12	1,127	5,76	1,037	7,55
Octadecanoic acid	27,23	1,26	0,435	2,10	0,442	4,12
Octacosanol	33,43	4,55	2,803	2,14	0,869	1,26

Table 3. List of individual compounds identified in Hornbeam wood, along with the average percent peak area and peak height

Name	Avg. R [min]	% areas		% heights		% ext. part
		Avg.	SD	Avg.	SD	Avg.
Furan, 2-pentyl-	11,45	0,30	0,134	0,51	0,120	0,29
Hexanoic acid	11,97	2,57	0,541	1,35	0,066	2,49
Hexane, 1,1-diethoxy-	13,65	0,94	0,035	1,54	0,304	0,91
Nonanal	14,00	0,40	0,156	0,71	0,064	0,39
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14,94	1,34	1,104	1,21	0,893	1,30
Dodecane	15,62	0,56	0,193	1,06	0,259	0,55
5-Hydroxymethylfurfural	16,56	3,31	2,121	1,93	0,813	3,22
Nonanoic acid	16,92	0,27	0,071	0,60	0,007	0,26
Heptanoic acid, 2-methylbutyl ester	18,28	2,24	0,276	2,91	0,087	2,18
Tetradecane	18,62	0,79	0,252	1,36	0,315	0,77
Isoeugenol	19,45	1,10	0,140	1,64	0,170	1,07
.beta.-D-Glucopyranose, 1,6-anhydro-	20,58	5,63	5,340	1,64	1,101	5,47
Heptadecane	21,19	0,43	0,156	0,98	0,191	0,42
Vanillic acid	21,49	4,70	1,521	5,77	1,250	4,56
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	22,52	2,50	0,396	3,83	0,628	2,43
Tetradecanoic acid	23,13	0,89	0,287	1,53	0,305	0,86
3,5-Dimethoxy-4-hydroxyphenylacetic acid	23,28	0,76	0,453	0,98	0,276	0,74
2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	23,68	0,84	0,474	0,93	0,382	0,81
Syringic acid	24,08	10,19	5,862	6,72	1,689	9,91
Pentadecanoic acid	24,22	0,95	0,117	1,45	0,141	0,92
1-Hexadecanol	24,41	2,10	0,588	2,86	0,201	2,04
9-Hexadecenoic acid	25,06	0,94	0,662	1,03	0,470	0,91
n-Hexadecanoic acid	25,33	31,10	9,977	24,13	2,194	30,23

Table 3. continued List of individual compounds identified in Hornbeam wood, along with the average percent peak area and peak height

Name	Avg. R [min]	% areas		% heights		% ext. part
		Avg.	SD	Avg.	SD	Avg.
Hexadecanoic acid, ethyl ester	25,51	0,35	0,175	0,82	0,217	0,34
3,5-Dimethoxy-4-hydroxycinnamaldehyde	25,66	0,63	0,231	0,69	0,066	0,61
9-Octadecen-1-ol, (Z)-	26,24	14,32	4,535	15,96	3,042	13,91
1-Octadecanol	26,46	0,67	0,064	1,05	0,007	0,65
Linoleic acid	26,98	3,61	0,044	6,15	0,283	3,51
7-Tetradecenal, (Z)-	27,02	1,57	0,912	3,10	1,089	1,52
Stearic acid	27,21	2,70	0,749	4,23	0,583	2,62
cis-9-Hexadecenal	28,65	2,15	0,571	1,71	0,136	2,09
Eicosanoic acid	29,00	0,81	0,057	0,94	0,085	0,79
Heptadecanal	29,82	0,46	0,150	0,89	0,259	0,45
Octadecanal	30,10	0,81	0,062	1,17	0,152	0,79

About 10% of the peak area in yakal wood chromatograms and about 8% of the peak area in hornbeam wood chromatograms were unidentified. The quantitative analysis of the substances, as a proportion relative to the dry weight of the wood is shown in Table 2 and 3.

Differences in identified extractives in the two wood species depend on species and wood growth habitat, although to the fact that the content of extractives in both species is similar, as well as their physical parameters - density and structure of the vascular structure.

The GC-MS method makes it possible to detect and identify extractives in wood regardless of the individual growth site of the tree. This knowledge can be useful for a more accurate understanding of the chemical composition of wood and their influence on the durability of the wood material. This knowledge can enable the extraction of valuable compounds from wood biomass finding application e.g. in pharmacy, cosmetic industry or medicine.

CONCLUSION

The aim of this study was to qualitatively and quantitatively analyze the composition of extractives in wood of two species that are similar in microscopic structure, including for a very poorly studied of yakal wood (*Shorea astylosa* Foxw.).

The species, despite similar physical properties and microscopic structure, differ in their extractives.

To define better the composition of extractives in both wood species, experiments would need to be conducted using solvents of different polarity.

The results obtained do not fully satisfy the detailed information on the still poorly studied yakal species , conducting further studies would fill these gaps and preserve information on endemic species of the Philippines.

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Streszczenie: Celem pracy była analiza ilościowa i jakościowa składników ekstrakcyjnych wyekstrahowanych z drewna grabu *(Carpinus betulus L.)* i drewna jakalu *(Shorea astylosa Foxw.)*. Badaniom poddano dwa podobne do siebie pod względem budowy mikroskopowej i właściwości fizycznych gatunki drewna, rodzime drewno grabu (*Carpinus betulus* L.) oraz drewno endemicznego gatunku występującego na Filipinach yakalu (*Shorea astylosa* Foxw.), który jest wciąż mało poznany. Drewno jest materiałem składającym się głównie z polimerów zwanych lignocelulozą, w skład których wchodzą: (celuloza, lignina i hemicelulozy), ale zawiera również wiele substancji ekstrakcyjnych i mineralnych. . Zawartość ekstraktów w drewnie w klimacie umiarkowanym wynosi około do 5% suchej masy drewna. Ich identyfikacja jest trudna ze względu na dużą różnorodność i mnogość występujących związków. Związki ekstrakcyjne są jednak ważne dla odporności drzew i drewna na czynniki biologiczne, takie jak infekcje grzybów lub owadów. Zazwyczaj strefy martwego drewna (twardziel lub drewno rdzeniowe) charakteryzują się większą zawartością ekstraktów. Poznanie składu chemicznego substancji ekstrakcyjnych w drewnie może przyczynić się do opracowania produktów do konserwacji drewna, które byłyby przyjazne dla środowiska i wpisywałyby się w politykę zielonej chemii.

Słowa kluczowe: substancje ekstrakcyjne, GC-MS, yakal, grab

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