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CHARACTERIZATION OF METHANOL EXTRACTS FROM *Quercus hartwissiana* WOOD AND BARK

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The MeOH extracts of wood and bark from Quercus hartwissiana *have been investigated by GC-MS after derivatization, as well as by classical spectroscopic methods. The results for the free compounds revealed that ellagic acid, catechin, gallic acid, quercitol, and also long chain fatty acids, sugars, and sitosterol were the essential compounds in wood and bark, most of them being present in differing amounts. Quercitol, a characteristic compound for the oak wood tannin, was also recognized and determined in oak bark extracts in this study. Amounting to 1/4th to 1/3rd of the free compounds, the bark had the highest catechin content. While the content of sugars, such as fructose and glucose, increased in sapwood and bark extracts remarkably, the amounts of these compounds decreased in extracts of heartwood. The profile of the bound compounds contained sugars (i.e., arabinose, xylose, and, above all, glucose), ellagic and gallic acids, quercitols, and inositols. Compared with the composition of free compounds, the hydrolyzed extracts showed relatively higher amounts of sugars, especially glucose, gallic acid and quercitol.*

Keywords: bark, condensed tannin, ellagic acid, gallic acid, inositols, lipophilic extracts, *Quercus hartwissiana*, sugars.

The genus *Quercus* is represented in Turkey by eighteen species. One of them, *Quercus hartwissiana*, has a limited distribution in the world and grows naturally in Southern Bulgaria, Northern Thrace, and the Black Sea region of Turkey up to Trans-Caucasia [1]. Its wood is used in the furniture and slicing veneer industry, and its technological properties have been investigated by Dundar [2, 3].

Oak woods contain hydrolyzable tannins, mainly ellagitannins (as the main polyphenols castalagin, vescalagin, and to a lesser extent castalin and vescalin), and gallotannin. However, catechin, gallocatechin, and various procyanidin dimers were also found in the oak's barks [4–6].

Articles on the tannin composition of oak species generally report on the analysis of tannins by spectroscopic and, especially, liquid-chromatographic methods. However, using HPLC in analyses, the number of detected compounds is few and rather restricted. Therefore, many authors prefer GC-MS, which enables them to identify a great number of compounds even when present in trace amounts [7–11].

On the other hand, the oak woods are used to make barrels for aging of wines and spirits, and there are also several articles dealing with the composition of volatile, tasty flavoring compounds and some polyphenols in oak woods by using GC-MS [9, 10, 12]. Furthermore, by using classical spectroscopic methods, tannin composition, including the content of total phenol, proanthocyanidins (condensed tannins), and ellagitannins in other oak species, was reported [13–17].

However, no research has been conducted to date on the tannin-containing extracts of the species *Q. hartwissiana*, and this was the reason why we decided to examine the tannin composition of this oak species by different methods. The aim of this study was then to determine especially the tannin composition and low-molecular-weight phenolic compounds of wood and bark from the species *Quercus hartwisssiana* using both GC-MS and classical spectroscopic methods.

A previous work dealt with the study of the tannin composition of *Ceratonia siliqua* wood using GC-MS [8]. The same extraction and isolation procedure applied to the Carob wood was also used in this research to characterize *Q. hartwissiana* extracts.

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TABLE 1. Extract Yields and Spectroscopically Determined Polyphenols of Woods and Barks

Samples	MeOH/water extract yield, %	Ether extract		Aqueous extract, mg/g		
		yield, %	Σ phenols, mg/g	Σ phenols	ellagitannins	proanthocyanidins
Heartwood **1**	4.98	0.87	2.57	30.22	39.12	0.16
Heartwood **2**	6.27	0.98	2.01	34.21	50.96	0.26
Trans. zone **1**	4.95	0.72	1.13	18.09	31.87	0.1
Trans. zone **2**	5.55	0.62	1.05	18.72	30.53	0.15
Sapwood **1**	3.39	0.57	0.41	3.95	N.d.	N.d.
Sapwood **2**	3.50	0.60	0.43	4.75	N.d.	N.d.
Bark **1**	8.81	1.57	2.87	28.21	21.4	5.53
Bark **2**	10.47	1.62	4.02	41.74	29.5	10.25

1: 60 year old tree, **2**: 100 year old tree, N.d.: not determined.

Spectroscopic Results. The contents of total phenolics, condensed tannins, and ellagitannins of different sections from *Q. hartwissiana* are given in Table 1. The same table lists also the solubility yields in methanol–water and diethyl ether solvents.

Regarding the MeOH-water solubility of the samples, the highest extract yields were obtained from barks. The yields decreased in the sequence heartwood, transition zone, and sapwood, though the difference between the solubility value of heartwood and that of the transition zone was quite low.

Total phenolic extractives in the *Q. hartwissiana* heartwoods were similar in quantity to other oak species (heartwoods: *Q. petraea* 39.3 mg/g, *Q. robur* 62.6 mg/g [13]). Furthermore, it is apparent from Table 1 that the extractive and tannin content increased somewhat in older *hartwissiana*. Thus, the extractive and tannin yields of the samples seemed to exhibit some dependence on the age of the tree.

While the amount of ellagitannins was the highest in the heartwood, showing some decrease in the transition zone, these compounds were not detectable in the sapwood. This means that the concentration of these phenolic extractives decreases slowly from heartwood to transition zone, but then they diminish in the sapwood abruptly. Somewhat lower than heartwood, the bark extracts contained again significant amounts of ellagitannins. On the other hand, the GC-MS analyses delivered quite low percentages of ellagic acid in the bark extracts, a fact that was not in accordance with the spectroscopic estimations. Therefore, perhaps due to some interfering compounds, the spectroscopic method for the estimation of ellagitannins might not be quite suitable for the correct estimation of these compounds in the barks.

The proanthocyanidins were present in trace amounts in the heartwood and in the transition zone, and they were absent in the sapwood. But in contrast to the *Q. hartwissiana* wood, the bark of this tree was rich in condensed tannins.

GC-MS Results. The four fractions of MeOH–water extracts were analyzed in GC-MS for the estimation of free and bound phenolics, sugars, and other compounds. Tables 2 and 3 show basically that the identified compounds summed up to 52–80% for free compounds and 69–87% for bound compounds. Excluding from the sum calculations, five unidentified compounds were also added to the Tables (three to Table 2 and two to Table 3) since these compounds occurred in appreciable amounts in some fractions.

Free Compounds (fractions 1 and 2). Table 2 lists the composition of free compounds found in fractions 1 and 2 obtained by successive ether and ether–MeOH extractions. The main compounds in these extracts were ellagic acid, gallic acid, catechin, quercitol, malic acid, some sugars, C16, C18 fatty acids, and sterols or triterpenes. Furthermore, low- molecular-weight phenolic compounds such as vanillin, syringaldehyde, coniferyl aldehyde, sinapaldehyde, vanillic acid, syringic acid, and some other benzene derivatives were also identified in the extracts, being enriched towards the sapwood.

The articles related to volatile compounds of oak woods include such low-molecular-weight phenolic compounds that are important for the quality of wine stored in oak barrels [11]. Although the natural oak wood contains a small amount of these compounds, their content can be increased intentionally when the wood is modified by seasoning or toasting. During these processes many compounds are also generated from cellulose, hemicelluloses, and lignin. Therefore, it is difficult to compare our results with those of the above studies where these processes have been applied.

On the other hand, quercitol occurred in the heartwood, transition zone, and bark extracts in pronounced amounts. It was reported that this compound was only found in the oak wood, and therefore it is a characteristic compound of the oak species [18, 19]. Some monosaccharides and polyalcohols in commercial oak wood tannin were studied by Sanz et al. [19].

TABLE 2. Chemical Composition of Free Compounds (bold: percentage > 1%)

Compound	R. time	Heart 1	Heart 2	Trans 1	Trans 2	Sap 1+2	Bark 1	Bark 2
Lactic acid	6.94	0.68	**2.95**	**1.14**	0.49	0.12	0.21	0.48
Glycolic acid	7.20	0.09	0.22	0.28	0.26	0.06	0.14	0.54
Laevulic acid	8.02	0.06	0.34	0	0.23	0.05	0.03	0.14
Octanol	8.09	0.16	0.65	0.27	0.86	0.24	0.05	0.30
2-Furancarboxylic acid	8.15	0.32	0.33	0.10	0.17	0	0.03	0.06
Pyruvic acid	8.82	0.17	0.60	0.10	0.37	0.06	0.09	0.19
Malic acid dimethyl ester	10.83	0.08	0.12	0	0	0	0.04	0.03
Glycerol	11.76	0.08	0.53	0.55	0.91	0.33	0.81	**2.66**
Succinic acid	12.02	0.14	0.42	**1.30**	**3.53**	0.93	0.48	0.81
Glyceric acid	12.62	0	0	0.04	0.12	0.06	0.06	0.19
Fumaric acid	12.68	0	0	0.13	0.40	0.07	0.04	0.14
Dimethoxybenzoquinone	14.91	0.28	0.37	0.65	0.11	0.21	0	0
Malic acid	15.04	0.56	**3.07**	**5.21**	**16.14**	**4.09**	**1.55**	**3.51**
Vanillin	15.19	0.45	0	**1.05**	0.17	1.19	0.31	0.12
Threitol	15.67	0	0	0	0	0	0.08	0.28
p-Hydroxybenzoic acid	16.60	0	0	0	0	0.10	0.17	0
Vanillyl alcohol	16.91	0.02	0	0.18	0.15	0.32	0.05	0.07
L-Arabinopyranose	17.10	0.02	0.19	0.01	0	0.02	0.04	0.07
Lauric acid	17.12	0.10	0.16	0.19	0	0.18	0.09	0.04
L-Rhamnose	17.27	0.03	0.47	0.03	0.05	0.06	0.08	0.22
Syringaldehyde	17.45	0.51	0.70	0.89	0.97	**1.00**	0.08	0.08
Arabinopyranose isomer	17.49	0.06	0.20	0.01	0	0.02	0.04	0.14
L-Rhamnose isomer	18.31	0.03	0	0	0	0	0.04	0.21
Vanillic acid	18.67	0.55	0.16	**1.46**	0.39	**1.30**	0.26	0.06
Xylitol	18.89	0	0	0	000	0	0.21	0.50
Azelaic acid	19.23	0.14	0	0.60	0	**1.05**	**1.34**	0.29
D-Xylopyranose	19.38	0.08	0.39	0	0	0	0	0
Coniferyl aldehyde	19.64	0.35	0.16	0.34	0	0.72	0.06	0
Vanillyl propanol	19.69	0.15	0	0.05	0	0	0	0
3,4-Dihydroxybenzoic acid	19.88	0.17	0	0.20	0	0.33	0.53	0.34
Citric acid	20.19	0.07	0.23	0.08	0	0.06	0.14	0.40
Quercitol	20.29	0.34	**2.17**	0.56	**2.48**	**1.17**	**1.29**	**2.93**
Tetradecanoic acid	20.29	0.26	0.40	0.57	0.31	0.06	0.18	0.07
2-Ketogluconic acid	20.46	0.07	0	0	0.85	0.81	0.71	**1.53**
2-Ketogluconic acid isomer	20.60	0.07	0	0	0.77	**1.32**	0.73	**1.46**
D-Fructose	20.80	0	0	0.25	0.76	**2.09**	**1.24**	**2.64**
Syringic acid	21.11	0.90	0.59	**1.27**	0.85	1.05	0	0.23
Sebacic acid	21.14	0	0	0.38	0	0.66	0.39	0
Me-Gallic acid	21.37	0.16	0	**3.27**	**1.28**	0.29	0.04	0
D-Glucopyranose anomer	22.39	0.17	0.40	0.56	**1.28**	**1.96**	**1.29**	**2.10**
Sinapaldehyde	23.07	0.26	0	0.42	0	0.59	0	0
Gallic acid	23.25	**5.25**	**9.89**	**8.82**	**11.45**	**1.08**	**1.57**	**1.76**
Glucitol (Sorbitol)	23.83	0	0	0	0.79	0	0.14	0.33
Glucitol isomer	24.01	0.09	0	0	0.79	0	0.80	0.10
Palmitelaidic acid	24.28	0.19	0	0	0.12	0.75	0.26	0.18
Palmitoleic acid	24.44	0	0	0	0.	0.42	0.06	0
D-Glucopyranose anomer	24.85	0.17	0.40	0.47	**1.36**	**2.15**	**1.38**	**2.15**
Palmitic acid	25.07	**3.47**	**5.43**	**7.96**	**9.95**	**14.05**	**3.10**	**0.99**
Methyl palmitic acid	27.58	0	0	0.17	0	0.38	0.13	0
9,12-Octadecadienoic acid	29.10	**2.64**	**3.55**	**3.52**	**4.49**	**5.37**	0.26	0
Linolenic acid	29.19	0.20	Tr.	0.92	**1.08**	**3.10**	0.06	0
Oleic acid	29.31	**1.16**	**1.92**	**1.03**	**1.47**	**1.10**	0.49	Tr.
Stearic acid	30.08	0.57	**1.07**	0.79	**1.31**	**1.51**	0.52	0.12
Eicosanoic acid	34.77	0.08	0	0.23	0	0.22	0.23	0
Docosanol	37.38	0.23	0	0	0	0.34	0.18	0
Guiacyl glycol	38.27	0.19	0.50	0.98	0.91	0.58	0.14	0.08
Monopalmitin	38.34	0.09	0	0.23	0	0.55	0.15	0
Guiacyl glycol isomer	38.61	0.10	0.40	**1.12**	**1.77**	0.82	0.10	0.10

TABLE 2. (continued)

Compound	R. time	Heart 1	Heart 2	Trans 1	Trans 2	Sap 1+2	Bark 1	Bark 2
Docosanoic acid	39.09	0.28	0.48	0.37	0	0.37	0.32	0
Sucrose	41.09	0	0	0.32	**1.06**	0.60	**1.44**	**2.81**
Monolinolein	41.66	0.21	0	0.65	0.25	**1.41**	0	0
Monostearin	42.30	0.28	0	0	0	0.31	0	0
Squalene	42.61	0.30	0.23	0	0	0.12	0	0
Tetracosanoic acid	43.13	0.41	0.71	0.32	0	0.28	0.13	0
Catechin	44.82	0	0	0.29	0	0.87	**24.25**	**33.79**
Catechin isomer	46.92	0	0	0.38	0.00	0.07	**1.35**	0.39
O-Me-Ellagic acid	53.92	0.91	0.37	0.33	0.31	0.37	0	0
Ellagic acid	54.89	**48.61**	**15.81**	**23.35**	**6.05**	**1.88**	**1.19**	**1.29**
Keto amyrin	55.41	0	0	0	0	0	**1.21**	0
Sitosterol S	55.49	**3.57**	**5.38**	**4.25**	**3.40**	**5.09**	0	0.92
Sitostanol S	55.82	0.86	1.57	0.74	0.62	1.20	0	0
Keto amyrin isomer	56.52	0	0	0	0	0	**2.83**	**1.61**
β-Amyrin	57.63	0	0	0	0	0	**8.53**	0
β-Amyrin isomer	58.59	0	0	0	0	0	**7.43**	0
Σ ident. compounds		74.41	51.72	79.79	80.39	67.58	60.07	62.72

They detected some inositols such as *muco*-inositol, *scyllo*-inositol, and *chiro*- and *myo*-inositol in their sample. However, we could not detect any inositols among free compounds.

Monosaccharides such as arabinose, xylose, rhamnose, fructose, and glucose were also determined in all samples. The amount of these free sugars tended to decrease from the bark to the heartwood.

Belonging to hydrolyzable tannins, ellagic and gallic acids were abundant in all extracts. The content of ellagic acid was the highest in the heartwood (up to 50%), while the amounts of gallic acid and methyl gallic acid reached significant levels in the transition zone.

Catechin as a proanthocyanidin precursor was shown to be distinct compound in sapwood and bark extracts. With about 34%, this compound reached the highest percentage in the bark extracts.

It was conspicuous that the amounts of gallic acid and ellagic acid were considerably low in bark extracts compared to wood extracts. Moreover, the bark extracts contained a higher amount of sugars than that present in the heartwood and the transition zone.

The distribution of tannin-related compounds in the transversal section of *Q. hartwissiana* wood can be evaluated briefly as follows (Fig. 1A): the ellagic acid content decreases steadily from the heartwood to the bark; the gallic acid content increased first in the direction from the heartwood to the transition zone, then it showed a remarkable decrease in the sapwood and the bark; the catechin content could be determined in the sapwood and transition zone in only very amall amounts, but it was most the abundant compound of the bark.

Some triterpenes, keto-amyrin and β-amyrin isomers, were found only in bark extracts. Since they were not detected in wood extracts, they are obviously specific compounds for the bark.

Bound Compounds (fractions 3 and 4). The remaining aqueous extracts were then hydrolyzed and derivatized, and the chemical composition of the fractions were examined by analyzing them in GC-MS. These chemical compounds, bound in wood, are given in Table 3. The carboxyl groups of some acids, and the hydroxyl groups of polyalcohols and sugars might be partly methylated during the hydrolysis treatment in strong acidic medium with a surplus of methanol. Though many of same compounds listed in Table 2 occurred also in fractions 3 and 4, the profile of the bound compounds shows remarkable differences from the free compounds. In all hydrolyzed extracts the sugars, hexoses and pentoses, ellagic acid, gallic acid, and inositols were dominating. The presence of malic acid and its methylated derivatives after hydrolysis is due to the fact, that this compound could not be effectively removed by diethyl ether extraction, and a part of it remained in the hydrolyzed extracts.

Inositols were certainly the most characteristic compounds in extracts 3 and 4 since they were not detected among the free compounds. They were probably components of hydrolyzable tannins in wood and bark. Representing three isomers, quercitol was encountered in the hydrolyzed extracts in much higher amounts than in the free extracts. Quercitol occurred in all extracts in higher percentages than inositols too. As a polyalcohol in chain form, glucitol (sorbitol) was also detected in wood and bark extracts.

TABLE 3. Chemical Composition of Extracts after Hydrolyses (bold: percentage > 1%)

Compound	R. time	Heart 3	Heart 4	Trans 3	Trans 4	Sap 3	Sap 4	Bark 3	Bark 4
Lactic acid	6.94	0.26	0.88	0.21	0.26	0.17	0.05	0.06	0.11
Glycolic acid	7.20	0.06	0.12	0.09	0.07	0.15	0.04	0.06	0.04
Laevulic acid	8.02	0.49	0.49	**1.75**	0.83	**5.15**	0.59	0.31	0.04
Malic acid dimethyl ester	10.83	0.10	0.07	**1.83**	0.80	**1.39**	0.19	0.71	0.05
Glycerol	11.76	0.54	**4.40**	0.65	0.58	**1.34**	0.66	**2.85**	**1.08**
Glyceric acid	12.62	0.04	0.27	0.10	0.19	0.18	0.16	0.12	0.13
Malic acid monomethyl ester	13.03	0.26	0.30	**4.26**	**2.83**	**6.02**	**1.61**	0.91	0.12
Malic acid monomethyl ester	13.35	0.09	0.09	**2.08**	**1.27**	**2.56**	0.64	0.55	0.06
Malic acid	15.04	0.45	0.34	**3.14**	**2.32**	**3.92**	**1.71**	0.51	0.08
Vanillin	15.19	0.04	0.08	0.05	0.04	0.06	0.02	0.02	0.01
Citric acid	15.53	0.05	0	0.39	0.25	0.53	0.16	0.46	0.05
Erythrose	15.67	0.05	0.08	0.04	0.10	0.05	0.06	0.20	0.18
Me-L-Arabinoglycoside	16.06	0.40	0.34	0.18	0.18	0.26	0.23	**1.13**	0.63
Me-L-Ara-glycoside isomer	16.20	0.05	0.04	0.05	0.05	0.13	0.10	0.67	0.30
Me-Fucose glycoside	16.51	0.13	0.10	0.14	0.11	0.36	0.23	**1.36**	0.30
p-Hydroxybenzoic acid	16.60	0.03	0	0.03	0	0.03	0.02	0.51	0.04
Me-Fucose glycoside isomer	16.68	0.03	0.01	0.03	0.01	0.06	0.04	0.26	0.09
Vanillic acid methyl ester	16.85	0.15	0.11	0.22	0.16	0.26	0.12	0.24	0.04
L-Arabinose	17.10	0.31	0.64	0.10	0.25	0.07	0.14	0.31	0.45
L-Rhamnose	17.27	0.31	0.26	0.26	0.26	0.21	0.15	0.36	0.25
Syringaldehyde	17.45	0.10	0.06	0.08	0.05	0.15	0.05	0	0
L-Arabinose isomer	17.49	0.77	**1.34**	0.55	0.75	**1.06**	0.84	**1.00**	0.74
Benzoic acid, 3,4-dihydroxy	18.07	0.05	0.04	0.08	0.05	0.08	0.02	0.87	0.07
Levoglucosan	18.12	0.09	0.14	0.17	0.21	0.12	0.12	0.10	0.09
L-Rhamnose	18.31	0.10	0.17	0.11	0.12	0.05	0.05	0.25	0.13
Vanillic acid	18.67	0.16	0.13	0.21	0.17	0.35	0.10	0.13	0
D-Xylitol	18.89	0.10	0.32	0.12	0.23	0.21	0.24	0.90	**2.25**
D-Xylopyranose	19.38	0.31	0.43	0.34	0.48	0.36	0.41	0.25	0.16
Vanillyl propanol	19.69	0	0	0.11	0	0.21	0.05	0	0
Me-D-glucopyranoside	19.73	0.26	0.22	0.13	0.23	0.28	0.37	0.77	0.49
Dihydroxybenzoic acid	19.88	0.06	0	0.04	0	0.05	0	0.75	0.11
Quercitol isomer (I)	20.29	**12.25**	**17.70**	**8.16**	**16.75**	6.63	**11.26**	**6.84**	**14.07**
Me-D-Galactopyranoside	20.46	0.44	0.82	0.29	0.64	0.76	**1.02**	0.94	**1.35**
Me-D-Gal-pyranose, isomer	20.60	0.17	0.12	0.21	0.26	0.26	0.47	0.47	0.21
Syringic acid	21.11	0.12	0.10	0.16	0.10	0.16	0.09	0.05	0.01
Me-Gallic acid	21.37	**7.55**	**4.94**	**5.83**	**3.77**	**1.27**	0.61	**5.35**	0.70
Me-D-Glucopyranose, *iso*	21.87	**7.07**	**7.40**	**8.46**	**10.85**	**21.16**	**22.72**	**16.93**	**18.04**
Quercitol isomer (II)	22.02	0.57	**1.82**	0.29	0.85	0.25	0.61	0.29	0.78
D-Glucopyranose anomer	22.39	**4.58**	**7.21**	**5.09**	**8.98**	**16.88**	**21.16**	**13.89**	**18.33**
D-Galactopyranose	22.76	0.17	0.36	0.11	0.35	0.16	0.44	0.20	0.65
Gallic acid	23.25	**4.56**	**2.99**	**3.03**	**2.17**	0.49	0.33	**2.89**	0.45
muco-Inositol	23.47	0.19	0.73	0.17	0.83	0.13	0.59	0.13	0.69
D-Glucitol	23.83	0	0.20	0	0.10	0.03	0.09	0.28	**2.29**
D-Glucitol isomer	24.01	0.06	0.17	0	0	0	0.09	0.05	0.24
Quercitol isomer (III)	24.28	**1.41**	**4.36**	0.70	**2.65**	0.43	**1.28**	0.56	**2.06**
chiro-Inositol	24.44	0.08	0.37	0.04	0.35	0.03	0.25	0.04	0.25
D-Glucopyranose anomer	24.85	**2.92**	**5.44**	**3.07**	**8.44**	**6.24**	**12.80**	**5.37**	**10.82**
Palmitic acid	25.07	0.49	0.08	0.47	0.10	0.34	0.05	0.26	0.10
scyllo-Inositol	26.24	0.25	**3.16**	0.11	**2.12**	0.08	0.92	0.08	**1.25**
myo-Inositol	27.89	0.20	3.20	0.09	1.92	0.08	1.33	0.10	1.74
Stearic acid	30.08	0.22	0.05	0.11	0.07	0.06	0	0.06	0.02
Ellagic acid	54.89	**37.95**	3.64	**32.24**	**1.29**	**2.16**	0.45	**6.05**	0.04
Σ ident. compounds		86.83	76	85.93	74.95	81.92	83.67	68.62	72.49

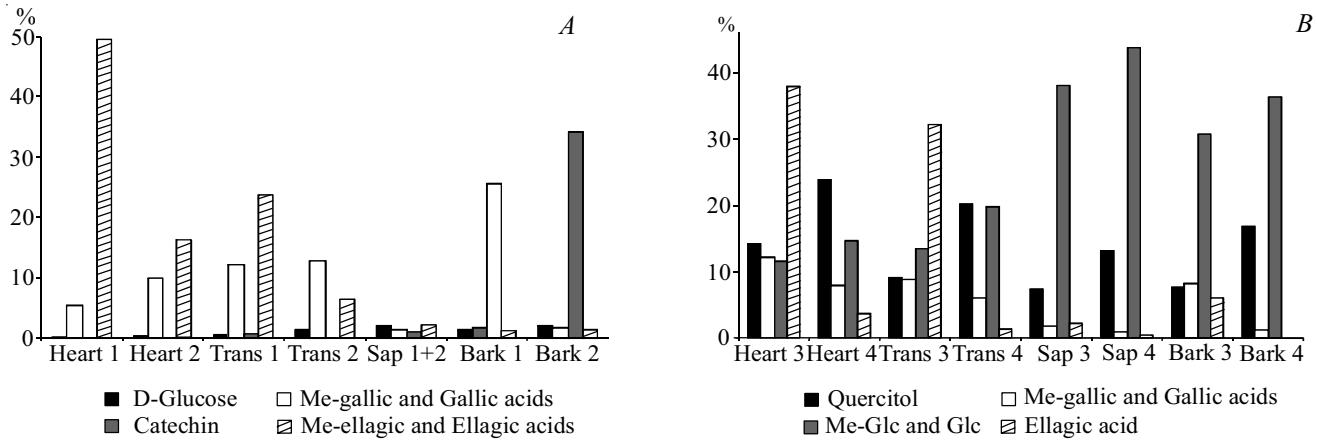

Fig. 1. Distribution of the four main free compounds (*A*) and four bound compounds (*B*) in transversal direction of the oak stem.

Regarding the monosaccharide composition, arabinose, rhamnose, xylose, fucose, glucose, and galactose were found in all extracts, with different percentages. D-glucose anomers occurred as the most abundant sugars in the extracts, being concentrated in sapwood and bark (up to 44%). Furthermore, the arabinose was present in heartwood and bark extracts preferentially.

Ellagic acid and gallic acid were the most abundant phenolic compounds in extracts after hydrolysis, occurring in significantly different contents in fractions. The heartwood exhibited the highest percentage of ellagic acid. The methyl gallic acid, perhaps partly produced during the methanolysis, was found especially in the heartwood, transition zone, and bark extracts in higher proportions.

The results of GC-MS analyses after acid hydrolysis are summarized in Fig. 1B.

The heartwood extracts contained mainly ellagic acid, followed by D-glucose and lesser amounts of gallic acid as well as significant amounts of quercitol.

The transition zone extracts were characterized by a higher amount of glucose and lower amounts of gallic and ellagic acids and quercitol than those in heartwood.

The highest content of glucose was determined in sapwood extracts, which have also the lowest amount of ellagic and gallic acids and quercitol.

On the other hand, bark extracts showed relatively high amounts of D-glucose and quercitol. Sanz et al. [19] reported that *myo*-inositol and quercitol were the most abundant compounds in oak tannin isolated from wood. In this study the presence of inositols in the bark of a *Quercus* species was recognized for the first time.

EXPERIMENTAL

Plant Material. Wood samples were obtained from the trunks of *Quercus hartwissiana* trees grown in a mixed hardwood forest in Eastern Thrace (District Demirkoy). Due to limited official permission, only two trees could be felled, one of them being about 60, the other ca. 100 years old. Four discs from each tree were cut from the stems in about equal intervals. By visual observation of discs, three different regions in the wood were distinguished in the transversal direction: sapwood, heartwood, and the transition zone between sap- and heartwood.

Isolation of Phenols. Two trees were analyzed separately. Each fraction was ground and sieved (40–100 mesh), then the same extraction-isolation and colorimetric estimation steps were followed as previously described in detail [8]. Briefly, samples were extracted with methanol–water-mixture (4:1) and soluble matter was fractionated into two parts as diethyl ether-soluble and water-soluble extractives. Total phenols were estimated in both fractions by the Folin-Ciocalteu method, and water extracts were analyzed for proanthocyanidins by the acid-butanol assay with catechin as a standard, and for ellagitannins by the nitrous acid method [13, 20–22].

The same milled wood and bark samples of two trees were mixed equally. Free and bound compounds were isolated using the following method [8]. The wood or bark meal (1 g) was soaked in methanol–water mixture (4:1 v/v) 3 times for 6 hours at room temperature. After the mixture was filtered, methanol was removed under light vacuum. The remaining aqueous

phase was first extracted with diethyl ether (2 × 25 mL) and then with diethyl ether–methanol mixture (9:1, 2 × 25 mL). After the isolation of these two fractions, the remaining extract was hydrolyzed with 6 M HCl in MeOH at 100°C for 8 h. The acid in the hydrolysate was evaporated repeatedly by adding distilled water each time until the hydrolysate was neutral. The neutral hydrolysate was extracted successively with diethyl ether–methanol mixture (9:1, 2 × 25 mL) and water. The four fractions obtained are designated as 1,2 (free compounds) and 3,4 (hydrolysable bound compounds).

GC-MS Analysis. All four fractions were silylated with trimethylchlorosilane and BSTFA (1:3) and injected into a GC-MS instrument (Shimadzu, model QP 5050A) fitted with a nonpolar, 30 m long DB-1 capillary column (diameter 0.25 mm, film thickness 0.25 μm). The temperature program was at 90°C for 2 min, increased to 290°C with heating rate 20°C/min, then 5 min at 290°C, increased to 310°C at 4°C/min, and finally 10 min at 310°C. The carrier gas was helium with flow rate 1.5 mL/min; the split ratio was 1:10; the injector and interface temperatures were 280 and 300°C, respectively. In the mass spectrometer, electron impact (EI) spectra were recorded at the ionization energy of 70 eV in full scan mode within the range 30–850 *m/z* with 0.5 s/scan.

Phenolic compounds and monosaccharides were identified by comparison of the mass spectra of their TMS derivatives with those in the Wiley 229, NIST 21, NIST 107. Besides sugars and sugar alcohols (such as sorbitol, inositol, quercitol), catechin and gallic and ellagic acids were purchased from Merck and Sigma and derivatized to TMS ethers in order to compare their retention times and spectra with those from extracts. Four inositols (*muco*-, *chiro*-, *scillo*- and *myo*-inositol) were recognized according to the retention series by Sanz et al. [19], who separated several inositols in a nonpolar column.

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