

Original article

## Chemical compounds of oak (*Quercus petraea* (Matt.) Liebl.) species with different medicinal properties in eastern Black Sea region

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### Abstract

Oak (*Quercus petraea* (Matt.) Liebl.) has many medicinal values. It was observed that many researchers have reported about anti-inflammatory, wound healing, anthelmintic, antibacterial and antioxidant effects of this plant. In our study, the quantities of chemical composition of heartwood, sapwood and bark of Strendzha oak (*Quercus hartwissiana*), Sessile oak (*Quercus petraea*) and eastern Black Sea oak (*Quercus pontica*) have been determined by means of standard methods. Additionally, chemical components of the heartwood, sapwood and bark of Strendzha oak (*Q. hartwissiana*) were analyzed by GC-MS. As a result of GC-MS analyses, the compounds identified in heartwood with higher ratio were octacosane (5.50%), heptacosane (4.44%) and hexacosane (2.76%) while those in sapwood were found octacosane (7.09%), triacontane (6.50%) and heptacosane (4.72%). In addition to this, vitamin E (5.38%), stigmastan-3,5-dien (2.53%) and campesterol (1.85%) were found in higher ratio in the bark of Strendzha oak.

**Key words:** Oak (*Quercus petraea* (Matt.) Liebl.), GC-MS, chemical compounds, different medicinal properties

### 1. Introduction

Oak (*Quercus petraea* (Matt.) Liebl.) grows in large forests in temperate, tropical regions and high mountainous parts of northern hemisphere with more than two hundred species, many subspecies, varieties and natural hybrid. There are 450 varieties of *Q. petraea* on this planet. Turkey has 18 varieties of *Q. petraea* species that grow naturally, four of them are endemic. Oak species (Strendzha oak-*Q. hartwissiana*; Sessile oak-*Q. petraea* and east Black Sea oak-*Q. pontica*) grow extensively in eastern Black Sea region of Turkey (Hedge and Yaltirik, 1982; Yaltirik, 1984; Todaro *et al.*, 2013; Demeter *et al.*, 2014; Keten *et al.*, 2015; Sen *et al.*, 2016; Yilmaz, 2017).

Sessile oak (*Q. petraea*) is a forest tree which can grow longer up to 30 m. They have long leaf and their length is 12 cm. Moreover, its acorns sit on the twigs without stem. On the other hand, Strendzha oak can grow longer up to 25 m. Its leaf length is in the range of 8-15 cm. In addition to this, its berry stem has 2-7 cm length and there are 3-4 berries in a stem. Eastern Black Sea oak has also 3-5 m. length. Its leaves length is 20-30 cm and they have short stem

(Yaltirik, 1984; Ansin and Özkan, 1999; Ansin and Terzioglu, 2001).

In our country, hardwood of oak tree is usually used in timber, construction and furniture industry. Additionally, fuelwood and cork are used for insulation and erosion control. Also, acorns and leaves of *Quercus* is an important food source for animals and wildlife (Cypert and Burton, 1948; Bainbridge 1986; Lieutaghi, 1998.). However, it is known that oak (*Q. petraea*) is also used medically in public. Some species (*Q. robur*, *Q. cerris* and *Q. ithaburensis* subs.) of oak are used against throat diseases, stomach and constipation discomforts (Baytop, 2001). Furthermore, it is indicated that oak barks and acorns have astringent and antiseptic effects. Moreover, it is included in ointments used as wound healing agents (Bremness, 1994).

In addition to what we have already mentioned above, the *Quercus* tree (oak) has a long history of medicinal uses. It is anti-inflammatory, antiseptic, astringent, decongestant, haemostatic, bronchitis, diabetes, tonsillitis, as well as gonorrhoea and tonic. The bark is the part of the plant that is most commonly used, though other parts such as the galls, seeds and seed cups are also sometimes used. In general, a decoction of the bark is useful in the treatment of chronic diarrhoea, dysentery, intermittent fevers, haemorrhages, *etc.* Externally, it is used to bathe wounds, skin eruptions, sweat feet, piles, *etc.* It is also used as a vaginal douche for genital inflammations and discharge and also as a wash for throat and mouth infections; besides in the treatment of disorders of spleen and gall bladder

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(Popovic *et al.*, 2013; Petrovska, 2012; Shah *et al.*, 2014; Orszaghova *et al.*, 2015, Lorzadeh *et al.*, 2016).

## 2. Materials and Methods

### 2.1 Plant material

Species, region, diameter and altitude information of samples are shown in Table 1. Because fiber length and chemical compounds in different parts of oak trees can be different, samples were collected from bottom, middle and top of oak trees.

### 2.2 Sample preparation for chemical analysis

Woods and barks were collected from sample trees and wood chips were prepared by hand as size of matchstick and then dried in clean and airy place. They were ground by Wiley grinder according to Tappi 257 om-2002. The samples were stored in glass jars (Tappi, 2002).

**Table 1:** Information of samples

Tree species	Region	Diameter (cm)	Altitude (m)	Symbol
Sessile oak ( <i>Q. petraea</i> (Matt.) Liebl.)	Gumushane	27	710	I1
		28	720	I2
		34	725	I3
Strendzha oak ( <i>Q. hartwissiana</i> )	Trabzon	42	350	H1
		45	355	H2
		47	370	H3
Eastern Black Sea oak ( <i>Q. pontica</i> )	Rize	13	1700	P1
		14	1735	P2
		11	1760	P3

### 2.3 Hot water solubility

Hot water solubility test was done according to Tappi, 207 om-1999 standard. Two gram of dried samples (heartwood, sapwood and bark) were transferred to a 250 ml Erlenmeyer flask and 100 ml of hot distilled water was added. Then, the solution was boiled at 100°C for 3 h. under the reflux condenser. The contents of the flask were transferred to a tared filtering crucible which has been previously dried to a constant weight at  $105 \pm 2^\circ\text{C}$ , washed with 200 ml of hot water and dried to constant weight at  $105 \pm 3^\circ\text{C}$ . After samples were dried, they were placed in a desiccator and weighed. Amount of solute was calculated according to equation 1.

Hot and cold water solubility, % =  $[(A - B) / A] \times 100$

where, A: initial weight of the test specimen, g oven dry.

B: weight of the test specimen after extraction, g equation 1.

### 2.4 Cold water solubility

This test was also done according to Tappi 207 om-1999 standard. Two gram of dried samples (heartwood, sapwood and bark) were transferred to a 500 ml Erlenmeyer flask and 300 ml of distilled water was added. Then, the solution was kept at 23°C for 48 h. After 48 h., the procedure continued as hot water solubility test (Tappi, 1999).

### 2.5 Alcohol solubility

Tappi 204 om-1997 was applied for this test. Two gram of dried samples (heartwood, sapwood and bark) were extracted in 350 ml

ethanol by soxhlet apparatus for 6 h. After this time, extracts were dried to constant weight in oven. Then, they placed in a desiccator and amount of solute was calculated according to equation 1 (Tappi, 1997).

### 2.6 %1 NaOH solubility

According to Tappi 212 om-2002 standard, two gram of dried samples (heartwood, sapwood and bark) were transferred to a 200 ml Erlenmeyer flask, 100 ml of %1 NaOH solution was added. Then, Erlenmeyer was waited in 100°C water bath for 1 h. After 1 h., residue in Erlenmeyer was filtered by tared filtering crucible and then washed with 50 ml of %10 acetic acid and hot distilled water. The procedure continued as hot water solubility test.

### 2.7 Hexane solubility

Sixty gram of wood samples (heartwood, sapwood) and 120 g. of bark samples were extracted in 350 ml hexane by Soxhlet apparatus for 11 h. After 11 h, the procedure continued as %1 NaOH solubility test (Tappi, 2002).

### 2.8 Holocellulose analysis

Method which was applied by Wise *et al.* (1945), was used for this analysis. Five gram of dried samples (heartwood, sapwood and bark) which were extracted in alcohol, 160 ml distilled water, 1,5 g sodium chloride and 0.5 ml iced acetic acid were transferred to a 250 ml Erlenmeyer and waited in 78-80°C hot water bath for 1 h. After 1 h., 0.5 ml. iced acetic acid and 1.5 g. sodium chloride were added to the Erlenmeyer and this procedure was repeated three times. Then, the solution was filtered by glass crucible and washed with acetone and cold distilled water. They were dried at 103°C and weighed. The amount of holocellulose was calculated according to equation 2 (Wise and Karl, 1962; Browning, 1967).

$$\% \text{ Holocellulose} = (A)/(B) \times 100$$

where, A: initial weight of the test specimen, g oven dry

B: weight of the test specimen after extraction, g Equation 2

### 2.9 Cellulose analysis

Kurschner and Hoffer's method was used for the cellulose analysis. Two gram of dried samples (heartwood, sapwood and bark) which were extracted in alcohol were transferred to a flask. 10 ml of  $\text{HNO}_3$  and 40 ml of ethyl alcohol were added to the flask. This solution was boiled in water bath under the reflux condenser for one hour. After 1 h, the residue was filtered. Then, 10 ml of  $\text{HNO}_3$  and 40 ml of ethyl alcohol were added to residue. This solution was boiled again and this procedure was repeated three times (Eroglu, 1988; Han and Rowell, 1997). After this, the procedure continued as holocellulose analysis.

### 2.10 Lignin analysis

Lignin analysis was done according to Tappi 211 om-2002 standard method. 1 g. of dried samples (heartwood, sapwood and bark) which were extracted in alcohol, were transferred to a beaker. Then, 15 ml of 72% sulfuric acid was added to this beaker and waited for 2 h. at 12-15°C. After this, the solution was transferred to one litre Erlenmeyer. The solution had been diluted by distilled water until acid concentration was 3 % . Then, this mixture was boiled under the reflux condenser for 4 h. The residue was filtered and washed with hot water. The extract was dried at 103°C. The amount of lignin was calculated by equation 2 (Tappi, 2002).

### 2.11 Sample preparation for GC-MS analysis

Fifteen gram of dried samples (heartwood, sapwood and bark) were extracted in 350 ml hexane by Soxhlet apparatus for 11 h. After the extraction, the solvent was evaporated. Extract was dissolved in chloroform, filtered by 45 µm filtration and stored in 2 ml vial in deep freeze for GC-MS analysis (Hafizoglu, 1997; Kilic *et al.*, 2010).

### 2.12 GC-MS analysis

The GC-MS analysis was performed using an HP-Agilent 6890-5973 GC-MSD instrument equipped HP-5 capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Helium was used as carrier gas at 1.3 ml / min flow-rate. The column oven was programmed

starting from 60°C (2 min) to 260°C, at 5°C / min heating rate. The split-injector and MS-transfer line were held at 260 and 280°C, respectively. The MSD was operated in electron impact ionization mode at 70 eV (Hafizoglu and Reunanen, 1997).

## 3. Results and Discussion

### 3.1 Solubility tests

Solubility results of all samples are shown in Table 2. When looking at the Table 2, the highest solubility percentages are in %1 NaOH for all samples. On the other hand, the lowest solubility percentages are also in hexane. Moreover, the highest solubility in %1 NaOH, cold water, hot water, alcohol, and hexane percentages were observed in the East Black Sea oak bark as 41,88 %, 16,98 %, 23,81 %, 18,85 % and 1,49 %, respectively.

**Table 2:** Solubility results of heartwood, sapwood and bark of samples

Sample		Cold water sol. (%)	Hot water sol. (%)	Alcohol sol. (%)	%1 NaOH sol. (%)	Hexane sol (%)
Heartwood	I <sub>av.</sub>	5.84 ± 0.04	9.64 ± 0.01	6.20 ± 0.03	23.32 ± 0.01	0.56 ± 0.02
	H <sub>av.</sub>	10.76 ± 0.06	15.10 ± 0.01	10.80 ± 0.10	30.30 ± 0.01	0.49 ± 0.09
	P <sub>av.</sub>	8.17 ± 0.06	14.38 ± 0.07	7.17 ± 0.08	31.49 ± 0.07	0.60 ± 0.05
Sapwood	I <sub>av.</sub>	3.23 ± 0.10	5.47 ± 0.04	3.17 ± 0.05	19.29 ± 0.01	0.41 ± 0.02
	H <sub>av.</sub>	5.45 ± 0.07	9.03 ± 0.04	5.35 ± 0.13	26.41 ± 0.13	0.42 ± 0.01
	P <sub>av.</sub>	4.37 ± 0.02	8.72 ± 0.08	4.05 ± 0.06	27.03 ± 0.01	0.53 ± 0.02
Bark	I <sub>av.</sub>	8.63 ± 0.01	11.65 ± 0.05	8.66 ± 0.06	35.58 ± 0.01	1.35 ± 0.01
	H <sub>av.</sub>	11.99 ± 0.02	15.08 ± 0.05	14.13 ± 0.12	39.94 ± 0.01	1.43 ± 0.01
	P <sub>av.</sub>	16.98 ± 0.06	23.81 ± 0.01	18.85 ± 0.12	41.88 ± 0.01	1.49 ± 0.03

**av:** Average of 3 different tree samples

**I:** Sessile oak, **H:** Strendzha oak, **P:** East Blacksea oak

### 3.2 Chemical analysis

**Table 3:** Cell wall components of heartwood, sapwood and bark of samples

Sample		Lignin (%)	Holocellulose (%)	Cellulose (%)
Heartwood	I <sub>av.</sub>	25.07 ± 0.03	81.4 ± 0.05	48.08 ± 0.05
	H <sub>av.</sub>	23.04 ± 0.02	82.88 ± 0.06	46.43 ± 0.01
	P <sub>av.</sub>	22.19 ± 0.07	94.90 ± 0.07	46.10 ± 0.07
Sapwood	I <sub>av.</sub>	23.10 ± 0.01	84.04 ± 0.08	49.38 ± 0.13
	H <sub>av.</sub>	22.26 ± 0.01	81.47 ± 0.11	43.46 ± 0.02
	P <sub>av.</sub>	21.64 ± 0.04	83.28 ± 0.10	43.45 ± 0.04
Bark	I <sub>av.</sub>	32.06 ± 0.02	73.20 ± 0.08	36.29 ± 0.04
	H <sub>av.</sub>	18.03 ± 0.02	75.50 ± 0.08	37.54 ± 0.08
	P <sub>av.</sub>	32.57 ± 0.02	70.22 ± 0.17	34.12 ± 0.04

**av:** Average of 3 different tree samples.

**I:** Sessile oak, **H:** Strendzha oak, **P:** East Blacksea oak

Table 3 shows cell wall components of heartwood, sapwood and bark of samples. The investigation of Table 3 implies that the highest holocellulose amount was in East Blacksea oak heartwood

as 94.90 %. On the other hand, it is shown that East Blacksea oak bark has the highest lignin amount as 32.57 %. The highest cellulose amount was also Sessile oak sapwood as 49.38 %.

## 3.3 GC-MS analysis

**Table 4:** Per cent composition of chemical compounds of Strendzha oak (*Quercus hartwissiana*) heartwood, sapwood and bark

Nr.	R.T.	Compounds	Heartwood	Sapwood	Bark
1	5.94	Hexanoic acid	-	-	0.70
2	11.23	4-acetyl-Morpholine	-	-	0.15
3	11.65	Decanal	0.03	-	0.05
4	11.77	Ethyl ester, morpholine-4-carboxylic acid	-	0.08	0.14
5	12.09	Exo-2-Hydeoxycineole	-	-	0.09
6	13.22	Cyclic 1,2:3,4-bis (ethylboronate), $\alpha$ D Ribopyranose,	-	-	0.08
7	13.98	2,4-Decadienal, (E,E)	-	-	0.40
8	16.68	3-hydroxy-4-methoxy benzaldehyde	0.26	-	0.12
9	19.53	2,4-di-tert-butyl phenol	0.21	0.15	0.10
10	19.80	4-ethoxy- ethyl ester Benzoic acid	-	0.47	0.24
11	19.91	4-(acetyloxy)-3-methoxy Benzaldehyde	1.12	-	-
12	20.23	3,4-dihydro-8-hydroxy-3-methyl-, (R)-, 2 Benzopyran-1-one	-	-	0.17
13	21.14	Propano 3-methoxy-4-hydroxyphenone	0.11	-	-
14	21.37	$\gamma$ -Gurjunene	-	0.15	-
15	21.51	2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester, Propanoicacid	0.11	-	-
16	22.27	$\gamma$ -Eudosmol	-	0.11	-
17	22.51	2-isopropyl-5-methyl-9methylene- Bicyclo [4.4.0]dec-1-ene	-	0.32	-
18	22.56	4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-, 2-Cyclohexen-1-one	0.20	-	-
19	22.78	$\alpha$ -Cadinol	-	0.66	-
20	22.89	4-hydroxy-3,5-dimethoxy-, Benzaldehyde	0.32	-	-
21	23.63	Tetradecanyl ester acrylicacid	0.46	0.24	-
22	23.95	Benzoic acid,2,4-dihydroxy-3,6-dimethyl- methylester	-	-	0.23
23	24.08	Tetradecanal	-	0.15	-
24	24.38	4,6-Dimethoxysalicylaldehyde	0.60	0.10	-
25	24.44	1-Decene	-	-	0.18
26	25.89	Octadecane	0.42	-	0.08
27	26.80	6,10,14-trimethyl-, 2-Pentadecanone	0.51	0.16	0.51
28	27.02	4-Hydroxy-2-methoxycinnamaldehyde,1,2-Benzenedi carboxylic acid	0.06	-	-
29	27.27	Bis(2-methylpropyl) esterBenzenedicarbozylic acid	0.27	0.19	0.26
30	27.91	Nonadecane	-	-	0.07

31	28.27	Farnesyl acetone b	-	-	0.12
32	28.42	Methyl ester hexadecanoic acid	-	-	0.07
33	28.74	1,4-dimethyl-3-(2-methyl-1-propene-1-yl)-4-vinyl-Cycloheptene	-	0.16	-
34	29.11	Butyl 2-ethylhexyl ester, 1,2-Benzenedicarboxylic acid	-	0.21	-
35	29.83	Eicosane	0.08	3.28	0.08
36	30.22	Hexadecanal	-	-	0.11
37	31.17	Ethyl linoleate	0.09	-	-
38	31.38	1-Octadecene	0.31	-	0.86
39	31.56	8-Methyl-9-tetradecenoic acid	-	-	0.15
40	31.57	9,12-Octadecadienoic acid Methyl ester,	0.14	-	-
41	31.68	Heneicosane	0.30	0.30	-
42	32.47	9,12-Octadecadienoic acid	0.14	-	0.17
43	32.82	1-Methylbicyclo [3.2.1.] octane	-	-	0.14
44	33.08	9-Octadecenamide	-	-	0.07
45	33.45	Docosane	0.66	0.66	-
46	33.46	Heptadecane	-	-	0.12
47	33.88	1,19-Eicosadiene	-	-	0.27
48	34.95	1-Heptadecene	-	-	2.57
49	35.15	Tricosane	2.18	1.53	-
50	35.59	Methyletherbenzyl	-	-	0.21
51	35.82	Z-8-Methyl-9-tetradecenoic acid	-	-	0.17
52	36.02	4,8,12,16-Tetramethylheptadecan-4-olide	-	-	1.25
53	36.61	1-Eicosanol	0.77	0.09	0.41
54	36.79	Tetracosane	1.98	1.94	0.46
55	36.97	Octadecyl ester, Aceticacid	0.09	-	-
56	37.15	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-ethyl ester	0.16	-	-
57	37.89	LinoleicacidButyl ester	4.31	-	-
58	37.90	Isopropyl linoleate	-	-	6.29
59	38.23	1-Octadecanol	-	-	3.66
60	38.37	Pentacosane	2.58	-	-
61	38.50	Hexadecanoic acid,2,3-dihydroxypropyl ester	-	-	3.48
62	39.77	$\gamma$ -Sitosterol	-	-	1.77
63	39.88	Hexacosane	2.76	-	-
64	41.35	Heptacosane	4.44	4.72	-
65	42.58	1-ethenyloxy-Hexadecane	0.46	0.50	-
66	42.63	2,3-dihydroxypropylester,9,12-Octadecadienoic acid (Z,Z)-	0.96	-	-
67	42.81	Octacosane	5.50	7.09	1.01
68	43.26	2,6,10,15,19,23-hexamethyl-,2,6,10,14,18,22 Tetracosahexaene	1.79	2.26	3.44
69	43.73	6-fluoro-4,6-cholestadiene-3-ol	-	-	1.67
70	43.82	Nonacosane	1.93	1.62	1.12
71	44.28	13-Tetradecen-1-ol acetate	0.76	0.94	0.99
72	46.35	Stigmastan-3,5-dien	0.88	4.24	2.53
73	46.60	Triacotane	-	6.50	-
74	47.73	$\gamma$ -Tocopherol	-	-	1.05
75	49.41	5-ethenyl-, (5, $\beta$ )-, A-Norcholestan-3-one	-	-	0.79
76	49.96	Vitamin E	4.05	3.35	5.38
77	51.97	$\alpha$ -Tocopherol, D	0.23	3.18	-
78	52.44	Campesterol	-	-	1.85
79	53.52	Spinasterone	5.29	-	-

40 compounds in heartwood, 30 compounds in sapwood, 48 compounds in bark were identified by GC-MS (Table 4). The main compounds were observed as tricosane (2.18%), tetracosane (%1.98%), linoleic acid butyl ester (4.31%), pentacosane (2.58%), hexacosane (2.76%), heptacosane (4.44%), octacosane (5.50%), vitamin E (4.05%), spinasterone (5.29%) in heartwood. In sapwood, eicosane (3.28%), heptacosane (4.72%), octacosane (7.09%), 2,6,10,15,19,23-hexamethyl-,2,6,10,14,18,22 tetraco-sahexaene (2.26%), stigmastan-3,5-dien (4.24%), triacontane (6.50%), vitamin E (3.35%),  $\alpha$ -tocopherol, D (3.18%) were present in their highest amounts. On the other hand, 1-heptadecene (2.57%), Isopropyl linoleate (6.29%), 1-octadecanol (3.66%), hexadecanoic acid,2,3-dihydroxypropyl ester (3.48%), 2,6,10,15,19,23-hexamethyl-,2,6,10,14,18,22 tetracosahexaene (3.44%), stigmastan-3,5-dien (2.53%), vitamin E (5.38%) were found in bark.

#### 4. Conclusion

This study is one of the rare chemical analysis works which was done on heartwood, sapwood and bark of Strendzha oak (*Q.hartwissiana*), Sessile oak (*Q. petraea*) and Eastern Black Sea oak (*Q.pontica*), grown in Eastern Black Sea region. This present study shows that these oak species grown in Eastern Black Sea region have rich extractive content. Particularly, their barks have percentage of 8.66-18.85 % extractive component. Also, in heartwoods and sapwoods, there are percentages of 6.20-10.80 % and 3.17-5.35 % extractive component, respectively. On the other hand, in heartwoods, sapwoods and barks of Strendzha oak, 40,30 and 48 compounds were identified, respectively by GC-MS.

It is important to note that resolution values of barks of oak species grown in Eastern Black Sea region are higher than resolution values of heartwoods and sapwoods. When oak species were compared to angiosperm tree species, chemical compounds and resolution values of woods of Strendzha oak, Sessile oak and Eastern Black Sea oak were found quite high.

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#### Conflict of interest

We declare that we have no conflict of interest.

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