Chemical Composition and Antioxidant Activity of Extract from the Wood of *Fagus orientalis*: Water Resistance and Decay Resistance against *Trametes versicolor*

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Extracted wood samples of *Fagus orientalis* with different solvents were analyzed for their antifungal activity against white-rot fungus (*Trametes versicolor*) in an agar plate. The most active extract was analyzed for its antioxidant activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, and the chemical composition were analyzed using gas chromatography-mass spectrometry. After the solvent removal of extractives, especially using chloroform (Chl) and Chl-water mixture, losses in the resistance of beech wood had occurred. However, Chl extract was found to be the most active antifungal agent, and the weight loss (30.38%) of specimens after 14 weeks of incubation was higher than that of other extracted specimens. At the concentration of 0.016 mg/mL, the highest activity observed was by Chl extract (89.45%), which was lower than the value of vitamin C (96.63%) at the same concentration. The lowest weight loss values obtained from the decay test were 0.35% and 0.64% for ethanol and ethanol-water extracted beech wood samples, respectively. The highest weight loss values were 30.38% and 23.98% for Chl and Chl-water extracted samples, respectively.

Keywords: Fagus orientalis wood; Chloroform extract; Antifungal and antioxidant activity; GC/MS; Trametes versicolor

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INTRODUCTION

Biodegradation of wood by fungi and some species of termites can incur heavy economic loss (Istek *et al.* 2005; Gonçalves and Oliveira 2006). Basically, deterioration of wood by fungi is related to three categories of decay resulting in different patterns of fungal degradation of the cell wall: soft rot, brown rot, and white rot (Fackler *et al.* 2007; Olfat *et al.* 2007; Folmann *et al.* 2008). Decay is one of the most significant problems facing the wood and wood products industry. Several scientific studies have evaluated wood degradation by fungi causing decay damage to trees and wood, which causes the simultaneous degradation of lignin and cellulose (Goktas *et al.* 2008, 2010; Olfat 2014). The white-rot fungi initially degrade lignin, followed by the carbohydrates; therefore, defibrillated materials remain through the dissolution of the middle lamella compounds (Eaton and Hale 1993; Schmidt 2006; Bari *et al.* 2015a).

Oriental beech (*Fagus orientalis* Lipsky) is one of the most important commercial species used in Iran's wood industries (Madhoushi *et al.* 2014). The highest weight loss

in wood of *F. orientalis* occurs after 16 weeks of colonization by the wood-rotting Basidiomycetes, *Coriolus versicolor* (47.5%), in comparison with 10 weeks (13.2%) (Olfat 2014). Recent research in north-east Iran found that more than 90 percent of wood decay in standing and felled trees, including oriental beech (*Fagus orientalis*), is caused by white-rot fungi (Bari *et al.* 2015a).

Trametes versicolor (white-rot fungus) is among the most destructive wood-decaying fungi to trees and wood (Schmidt 2006). This fungus produces white rot and attacks birch, hornbeam, and particularly beech (Bari *et al.* 2015a).

This fungus has considerable ability in decomposing lignified cells of wood and wood products, as well as causing serious damage to roots and standing tree trunks, and finally destroying the mechanical strength of wood (Gautam 2013). Bari *et al.* (2015b) reported that there were no significant differences in wood mass loss and chemical composition between wood affected by *Pleurotus ostreatus* and *T. versicolor* at the end of incubation periods (30, 60, and 120 days). After each incubation period, the cell wall thickness decreased to the same extent. The accumulation of hyphae as well as the rupture of cell walls was also similar for both fungi, and the occurrence of hyphae, cavities in the pits, and vessel walls followed nearly the same patterns.

Tannins as phenolic compounds have been reported to increase the durability of beech wood against *T. versicolor*, *Serpula lacrymans*, and molds (Jeloková and Šindler 1997). The phenolics act mostly as protection against invading pathogens and competing species (Rüdiger and Lohaus 1987; Schultz and Nicholas 2000). Previous study on analyses of the wood extract of *F. orientalis* has yielded the presence of 1,2-benzenedicarboxylic acid-4-hydroxy (43.53%), 6-(2-formylhydrazino)-N,N'-bis(isopropyl)-1,3,5-triazine-2,4-diamine (9.50 %), 2,3-dihydro-benzofuran (6.08%), 4-morpholineethanol (5.11%), *p*-xylene (4.94%), 1,1'-oxybis-cyclohexane (3.35%), 4-(3-hydroxy-1-propenyl)-2-methoxy- phenol (3.15%), 2,6-dimethoxy-phenol (3.13%), 2-ethyl-1-hexanol (3.13%), and 6-methyl- bicyclo[4.1.0]heptan-2-one (2.00 %) (Liu *et al.* 2008).

To the best of our knowledge, there have been no reports on the antifungal and antioxidant activity of extracts from *F. orientalis* wood. Therefore, this novel approach investigated the antifungal activity of extracted wood of *F. orientalis* by different solvents. The antioxidant activity and chemical composition of the most active solvent extraction was analyzed by the DPPH method, as well as by gas chromatography-mass spectrometry (GC/MS), respectively.

EXPERIMENTAL

Plant Materials

Beech wood (*Fagus orientalis* Lipsky) was selected according to TS 2470 (Anon. 1976), as defect-free, whole, knotless, normally grown wood (without zone lines, reaction wood, decay, insect damage, or fungal infection) from Kelardasht, located at 1250 meters above sea level in Iran. The mean annual precipitation of this area is approximately 450 mm. The log was sawn in a traditional flat-sawn scheme to obtain exterior boards with the main face in the tangential direction and boards closer to the pith in the radial direction. The thickness of each cut was set at 2.5 cm.

All boards were air-dried. The final moisture content was between 8% and 10%. The boards were then stored in a conditioned room maintained at 20 °C and 65% relative

humidity to an equilibrium moisture content of 8 ± 2% before further use. The density of samples from each log, determined according to ASTM D 2395-93 (1999), was 570 kg/m^3.

Sample Preparation for Decay Resistance

Boards, measuring 2.5 cm × 30 cm × 100 cm, were selected to prepare the samples. The samples measured 20 mm × 20 mm × 20 mm (T × R × L). The samples were then planed and successively sanded with sanding paper. Thirty-two sanded specimens were oven-dried at 103 ± 2 ºC for 24 h to reach 0% moisture content, and 32 sanded specimens containing moisture were prepared for extraction with solvents.

Removal of Extractives

The samples were extracted with various combinations of solvents, including hexane:ethanol (1:1 *v/v*), ethanol, chloroform, diethyl ether, and water, according to modified ASTM standards (see below). Hexane is a nonpolar solvent and is traditionally believed to be capable of opening up and penetrating wood cell walls. Thus, when used for extraction, it would be expected to merely recover the extractable materials located within the cell lumen (Ajuong and Breese 1998). Previous reports indicate such substances as consisting of long chain fatty acids, fats, resins, waxes, terpenes, and phytosterols (Laks 1991; Anon. 1999).

Ethanol is a good bulking agent and can swell wood structures by 83% more than water (Laks 1991; Ajuong and Breese 1998). Thus, ethanol would be expected to remove materials from within the wall structure, including, among others, condensed tannins, flavonoids, and phenolics (Laks 1991). When applied, hot water extraction recovers condensed tannins and water-soluble low-molecular weight carbohydrates (Ajuong and Breese 1998). Water extraction was conducted according to ASTM D 1110-96 (ASTM 1999). Solvent extractions were conducted according to ASTM D 1105-96 (ASTM 1996). The main modification was related to the fact that solid wood samples were used in the extraction process instead of the wood (powder) recommended in the standards. The extraction time was consequently increased to remove more extractives from the specimens.

Preliminary tests to determine the appropriate extraction time suggested that an extraction cycle of 72 h was sufficient. Conditioned samples were divided into eight groups. The first group was extracted with a mixture of hexane:ethanol (1:1 *v/v*) (Hex-Eth) for 72 h, the second group with hexane:ethanol for 72 h and then extracted with water for 72 h (Hex-Eth + Water), the third group with ethanol (Eth) for 72 h, the fourth group with ethanol for 72 h and water for 72 h (Eth + Water), the fifth group with chloroform (Chl) for 72 h, the sixth group with Chl for 72 h and then extracted with water for 72 h (Chl + Water), the seventh group with diethyl ether (Dieth) for 72 h, and the eighth group with Dieth for 72 h and then extracted with water for 72 h (Dieth + Water).

Four samples were extracted for each treatment from beech species. The total amount of extractives removed (Table 1) was calculated by the weight difference of the moisture-free samples before and after extractions, as recommended by the standards ASTM D 1107-96 (ASTM 1996). Extracted specimens were stored in the dark in the conditioning room while awaiting further tests.

Specimens were divided into two matching halves (rectangular to the annual rings or parallel to the ray parenchyma) measuring 10 × 20 × 20 mm each. One half was used in decay testing, and the matching half was used in the measuring of water absorption

(WA%) from the first day to the end of the fourteen days of measurement. Four specimens for each extraction type were exposed to cultures of the white-rot fungus *Trametes versicolor* according to BS 838 (1961).

Decay Test

Fungus culture

Decay testing was conducted in accordance with BS 838 (1961) as applied by exposure to *T. versicolor* in Petri dishes for 14 weeks. The fungus was grown and maintained on malt extract agar (MEA). To do so, the medium was autoclaved and sterilized for 30 min at 105 kPa and 125 °C and cooled to room temperature before inoculation. After cooling the medium, purified Turkey Tail fungus was transferred to Petri dishes containing malt extract agar under a sterile hood using sterile pincers. The dishes were kept at 23 °C for one week until the culture medium was fully covered by the fungus. The cultured fungus was transferred into Petri dishes containing the culture medium, which was incubated for one week at 23 °C.

Trametes versicolor beech wood colonization

Eight wood samples of *F. orientalis* wood (10 × 20 × 20 mm) from two treatments, for example, four extracted Eth wood specimens and four extracted Eth + water wood specimens, were mounted over four 3-mm platforms and placed in the Petri dishes. The dishes containing *T. versicolor* and wood specimens were incubated for 14 weeks at 23 °C and 75% relative humidity until the specimens were heavily colonized by the test fungus as mentioned in Kolleschale's method according to BS 838 (1961). All decay tests were performed on specimens from each group of extracted samples with different solvents.

Weight loss

At the end of the exposure period, the test blocks were removed from the Petri dishes and their surfaces carefully brushed. The blocks were then dried to constant weight at 103 ± 2 °C for 24 h. The blocks were weighed to the nearest 0.01 g to determine the decayed weight (W_2). Weight loss was calculated as percentage of the initial weight of the specimen dried in the oven (W_1) using the following equation:

$$\text{Weight loss (\%)} = [(W_1 - W_2)/W_1] \times 100 \quad (1)$$

Free radical scavenging activity by DPPH assay

The free radical scavenging activities of the chloroform extract of the wood samples were determined using the DPPH method (Karau *et al.* 2013). Serial dilutions were carried out from the stock solutions (1 mg/mL) of the tested extract to obtain concentrations of 0.0005, 0.001, 0.002, 0.004, 0.008, and 0.016 mg/mL. For this method, a working solution was prepared by dissolving 2.4 mg of DPPH free radical in 100 mL of methanol. Then, 1250 µL of the working solution was combined with 250 µL of the Chl extract (1 mg/mL) which was the most active extract. The reaction mixture was mixed for 10 s and left to stand for 30 min in a dark place at room temperature. The experiment was performed in triplicate, and the average absorbance was recorded for each concentration. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer.

Ascorbic acid and butylated hydroxytoluene (BHT) were used as the reference standards and were dissolved in methanol to make stock solutions with the same

concentration (1 mg/mL). The control samples were prepared with the same volume of solution, without test compounds and the referenced standards. Pure methanol (Sigma-Aldrich, Germany) was used as a blank. The DPPH free radical scavenging activity (%) was calculated using the following equation,

$$\text{Inhibition} = 100(Ac - As)/Ac \quad (2)$$

where the percentage inhibition value was calculated from the absorbance of the control, *Ac*, and of the sample, *As*. The controls contained all the reaction reagents except the extract or positive control substance. The values presented are the means of triplicate analyses.

Identification of extract components

The pure extractives obtained were separated and dried by nitrogen gas to give brown-yellowish extracts in 5.97% yields from the beech wood. To obtained trimethylsilylated derivatives and thus identify of extracts, approximately 1 mg of the solid extract obtained was mixed with 30 µL of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), 10 µL of 1% trimethylchlorosilane (TMCS) reagent, and approximately 15 µL of pyridine inside a test tube. Afterwards, the test tube was placed into a Ben Mary bath at 70 °C.

The analyses of the extracts were performed using GC/MS on an Agilent 7890A gas chromatograph (Santa Clara, CA 95051, USA), equipped with a split injector (1:150) and a 5975C mass selective detector (MSD). The column oven was programmed as follows: chromatography was performed on a DB-1 capillary column (SGE, 30 m, 0.25 mm) with helium as the carrier gas, with 1 mL/min speed and a temperature program between 40 to 240 °C, increasing the temperature by 4 °C/min.

The individual compounds in the extractives were identified by their retention time relative to known compounds, and further by comparison of their mass spectra with either the known compounds or published spectral data. Individual components were identified using Wiley 275 L and NIST05 a.L database matching, and by comparing the retention times and mass spectra of constituents with published data (Julian and Konig 1988; Adams 1995, 2001).

Statistical procedure

To evaluate changes in density, extractive content, and weight loss of extracted beech samples, one-way analysis of variance (ANOVA) was used. The effects of the extraction solvents on the weight loss and density of the prepared samples were determined. Duncan's test was used to determine if there was a significant difference between the groups.

RESULTS AND DISCUSSION

Density, Extractives Content, Maximum Water Absorption, and Weight Loss Measurements

There was no significant difference between the densities of the beech wood specimens before and after the extraction with different solvents, nor were the maximum water absorptions significantly different. The highest extractives values obtained from the extracted specimens were 9.49% and 8.58% for Eth + Water and Chl + Water extracted

samples, respectively, and the lowest values obtained from the extracted beech wood specimens were 2.95% and 5.97% for Eth and Chl, respectively (Table 1).

The highest weight loss values obtained from the decay test were 30.38% and 23.98% for Chl and Chl + Water extracted samples, respectively. The lowest weight loss values obtained from the decay test were 0.35% and 0.64% for Eth and Eth + Water extracted beech wood samples, respectively (Table 1).

Table 1. Average and Standard Deviation of Density, Percentage of Extractives Removed (Dry Weight Basis), Maximum Water Absorption, and Weight Loss Measurements for Beech Wood Samples

Extraction Solvents	Density (g/cm^3)	Extractives content (%)	Weight loss (%)	Maximum water absorption (%)
Control	0.58 ± (0.01)*	5.3 ± (0.55)† bc	23.74 ± (0.72) b	128.12 ± (3.12)
Hex-Eth	0.56 ± (0.03)	6.89 ± (0.76) ab	4.79 ± (1.22) cd	129.30 ± (2.49)
Hex-Eth + Water	0.56 ± (0.04)	8.28 ± (0.36) a	6.77 ± (1.53) c	117.49 ± (13.46)
Eth	0.58 ± (0.02)	2.95 ± (1.36) e	0.35 ± (0.24) e	127.28 ± (7.26)
Eth + Water	0.57 ± (0.03)	9.49 ± (2.33) a	0.64 ± (0.75) fe	128.68 ± (6.08)
Chl	0.57 ± (0.03)	5.97 ± (0.90) bc	30.38 ± (3.12) a	125.51 ± (8.51)
Chl + Water	0.55 ± (0.01)	8.58 ± (1.48) a	23.98 ± (5.33) b	129.21 ± (5.80)
Dieth	0.59 ± (0.01)	6.27 ± (1.06) ab	1.12 ± (0.79) f	124.49 ± (4.97)
Dieth + Water	0.55 ± (0.02)	6.26 ± (0.35) ab	2.68 ± (0.59) e	125.29 ± (4.28)

*Means with the same letter within the same column are not significantly different. Values in parentheses are standard deviation (SD).
† Total extractives content for control samples was measured according to ASTM D 1105-96 (1996).

After solvent removal of extractives, especially using Chl and Chl + Water, losses in the natural resistance of beech wood occurred; this made them more susceptible to decay (Ohmura *et al.* 2000; Taylor *et al.* 2006; Oliveira *et al.* 2010). It was reported that Chl solvent can extract the active chemical groups like terpenoids (Ayafor *et al.* 1994), flavonoids (Perrett *et al.* 1995), and alkaloids (Ali *et al.* 2013).

According to the results of a one-way ANOVA test applied to this variable, the significance level obtained from the average values was found to be lower than the selected significance level (0.05) (Table 1). The Duncan's test revealed that of the mean values present in Table 1, extractives and weight loss of wood samples extracted with any of the eight different combinations of solvents after extraction were statistically significant, while the density values were not significantly affected.

The Willeitner scale showed distinct evidence of fungal colonization (100%) on the surface of wood samples (Willeitner 1984). However, the Chl and Chl + water extractives of beech samples were relatively suitable for preventing fungal growth, as shown in Fig. 1 where after extraction and exposure to *T. versicolor,* the fungal growth reached 100%. Based on these findings, it was concluded that the Chl solvent extracted the most active compounds in beech wood in comparison with other solvents, which could act as a barrier to growth of *T. versicolor* on beech wood.

Microscopic evaluations at 15-day intervals for 120 days indicated that *P. ostreatus* and *T. versicolor* degraded marginal ray parenchyma at the early stages of decomposition, causing a drastic loss in impact bending strength of Oriental beech wood (*F. orientalis* Lipsky) (Bari *et al.* 2015b,c). Measurements of mechanical and chemical properties of Iranian beech wood provide a more sensitive insight into property changes

from fungal degradation compared with mass loss percentages (Madhoushi *et al.* 2014; Malakani *et al.* 2014). Decreases in mass loss and fiber efficacy of beech wood are evident after incubation periods with *Coriolus versicolor* and other decay fungi, which affect the physical properties of wood (specific gravity) (Hatakka 2001; Schmidt 2006; Fackler *et al.* 2007; Schwarze 2007; Olfat 2014).

Fig. 1. Mycelium growth of *T. versicolor* fungus in the extracted samples with different groups of solvents

In the present study the mass loss of control samples was 23.74% after 14 weeks, whereas in the study of Olfat (2014), the highest weight loss of *F. orientalis* wood colonized and degraded by *T. versicolor* occurred after 16 weeks (47.5%) in comparison with 10 weeks (13.2%). Goktas *et al.* (2010) found that beech wood specimens had weight losses ranging between 12.00% and 30.29% as affected by *T. versicolor*. The percents of the three cell wall components of Oriental beech wood (cellulose, lignin, and hemicellulose) were decreased by *T. versicolor* (Bari *et al.* 2015b).

Beech wood has medium to heavy-weight density (Fukasawa *et al.* 2011); because of this, as well as for its low durability, beech wood is well suited for impregnation (Olfat 2014). Subsequently, one of the most limiting factors for the commercial utilization of beech wood is its low resistance to fungi, especially in the semi-arid and sub-humid tropics (Istek *et al.* 2005). Bari *et al.* (2015b) found that *T. versicolor* completely destroyed the parenchyma cells and produced white rot in Oriental beech wood.

Natural durability is the inherent resistance of heartwood timber to decay. Thus, in the research of Olfat (2014) on the natural durability ratings in *Fagus orientalis* degraded by wood-rotting Basidiomycetes *Coriolus versicolor*, the durability rating was 47.5%. This was very low (non-durable) according the Findlay methods, which was in accordance with experiments of Scheffer and Morrell (1998) and Van Acker *et al.* (2003).

Low nitrogen content of the wood, which was considered a major factor contributing to complete delignification of the wood, could be taken into consideration. As high nitrogen concentrations stimulate polysaccharide breakdown, low nitrogen levels could promote selective lignin removal (Fenn and Kirk 1981; Reid 1983). In the present study and the study of Bari *et al.* (2015b), the conditions of growth media were favorable for a simultaneous white rot and for the formation of bore holes and can thus be considered. Figure 2 shows the water absorption of extracted wood with different

combinations of solvents. At day 14, the highest WA% values were observed in the hexane-ethanol (129.30%) and chloroform + water (129.21%) extracts, whereas the lowest values were observed in the hexane-ethanol + water extracts (117.49%) (Table 1).

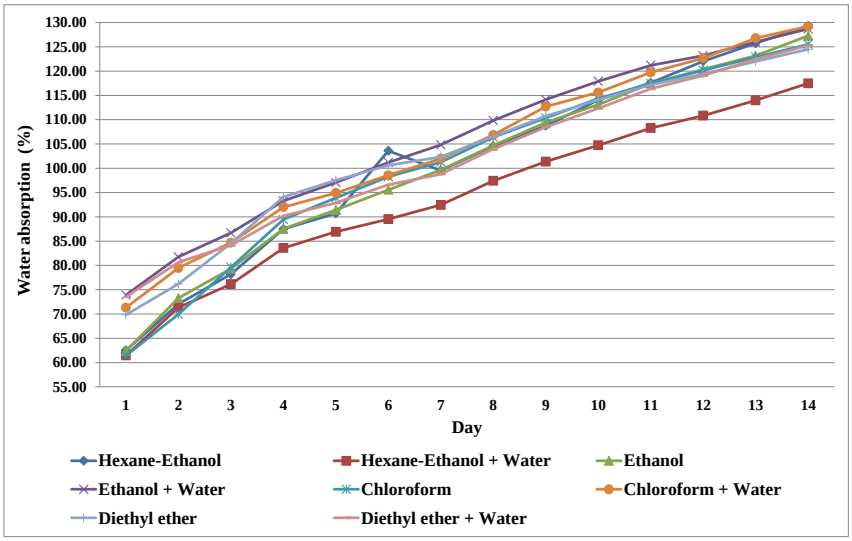

Fig. 2. Water absorption of wood samples after extraction by different combinations of solvents at the end of 14 days

Antioxidant Activity

The chloroform extracts exhibited favorable antioxidant activity overall (Table 2). The lowest antioxidant activity (14.45%) was observed at the concentration of 0.0005 mg/mL, which was lower than vitamin C (89.88%) at the same concentration. The highest activity observed by chloroform extract (89.45%) at 0.016 mg/mL, which was also lower than the value of vitamin C (96.63%) at the same concentration. The same trend was observed with the reference, BHT. Beech wood extractives with proven bioactive properties for commercial extraction are considered to have antioxidant activities (Malterud *et al.* 1985; Dubeler *et al.* 1997; Välimaa *et al.* 2007; Liu *et al.* 2008).

Table 2. Mean±SD of Antioxidant Activity (%) as Affected by Concentration of Wood Extractives of *F. orientalis* Compared with BHT and Vitamin C

Treatment	Concentration (mg/mL)					
	0.0005	**0.001**	**0.002**	**0.004**	**0.008**	**0.016**
Beech Wood Chl Extract	14.45± 0.78 a	25.20± 0.59 b	49.41± 2.54 d	72.27± 0.78 e	88.48± 1.76 f	89.45± 0.00 fg
BHT	26.33± 11.41 a	41.44± 7.73 c	70.44± 0.28 e	90.33± 1.93 fgh	91.16± 0.00 fgh	93.37± 1.66 fgh
Vitamin C	91.14± 4.35 fgh	95.50± 0.23 gh	95.63± 0.69 gh	95.63± 0.00 gh	95.63± 0.69 gh	96.56± 0.23 h

Chemical Composition of Wood Extract

Chloroform extract was found to be the most active of the fractions against the decay of wood caused by *T. versicolor*, and it also possesses good antioxidant activity. The chemical components identified in the chloroform solvent extract from wood of *Fagus orientalis* are presented in Table 3.

The main constituents were trimethylsilyl (TMS) derivatives of hexadecanoic acid or palmitic acid (13.28%) (Kubel and Weissmann 1988; Zule and Može 2003), *trans*-caryophyllene (12.88%), TMS-3,5-dimethoxy-4-(TMS-oxy) benzoate (11.69%), eicosane (6.96%), hexadecane (6.79%), octadecane (6.29%), isovanillic acid 2TMS (5.75%) (Mounguengui *et al.* 2007), homovanillyl alcohol 2TMS (3.77%), (1-methyldodecyl)-benzene (3.70%), hexadecyloxytrimethyl-silane (3.31%), trimethyl(1-methylethoxy)-silane (3.19%), β-selinene (3.10%), β-bisabolene (3.01%), α-selinene (2.19%), and 3,5-dimethoxy-4-TMS-oxybenzaldehyde (2.14%). The TMS derivative of glucose was found in low ratio (1.70%) (Irmouli *et al.* 2002). Glycerol with minor concentration was found (0.30%) (Zule and Može 2003). Some derivatives of benzaldehyde were reported (Challinor 1996).

Table 3. Analysis of the Composition of the Chemical Constituent (Area %) in the Wood Extractives of *Fagus orientalis*

Component	Retention time (min)	Area (%)	Component	Retention time (min)	Area (%)
3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl-	13.243	0.57	Caryophyllene oxide	33.194	1.54
TMS ether of glycerol	23.808	0.30	Hexadecane	33.870	6.79
Tridecane	24.114	0.23	3,5-dimethoxy-4-TMS-oxybenzaldehyde	36.183	2.14
α-Copaene	26.779	0.92	Trimethyl(1-methylethoxy)-Silane	36.390	3.19
β-Elemene	27.204	0.77	Homovanillyl alcohol 2TMS	36.941	3.77
Tetradecane	27.558	0.23	Isovanillic acid 2TMS	38.408	5.75
trans-Caryophyllene	28.212	12.88	Octadecane	39.531	6.29
α-Caryophyllene	29.273	1.85	TMS 3,5-dimethoxy-4-(TMS-oxy)benzoate	41.892	11.69
Isoeugenol-monoTMS	29.539	0.19	(1-methyldodecyl)-Benzene	42.177	3.70
β-Selinene	30.300	3.10	Hexadecyloxytrimethyl-silane	43.725	3.31
α-Selinene	30.614	2.19	Eicosane	44.649	6.96
Pentadecane	30.792	0.62	Glucose 5-TMS	45.296	1.70
β-Bisabolene	30.921	3.01	Hexadecanoic acid-TMS ester	45.691	13.28
3-methoxy-4-[(TMS)oxy]-Benzaldehyde	31.231	1.77	Caryophyllene oxide	33.194	1.54
α-Cadinene	31.357	0.85	Hexadecane	33.870	6.79

A relatively low amount of extractives, *i.e.*, 3% and 5% (Wagenführ 1996), is characteristic of beech wood (Rowe and Conner 1979; Kubel and Weissmann 1988). The contents of extractives (fatty acids and their derivatives) soluble in petrol ether and diethyl ether are also relatively low (Kubel and Weissmann 1988). Like in our study with Chl and Chl + Water, more compounds are extractable by more polar solvent mixtures (Kubel and Weissmann 1988). Typical wood sugars such as glucose, galactose, arabinose, fructose, xylose, mannose, and rhamnose have been reported (Dietrichs 1964; Kubel and Weissmann 1988; Irmouli *et al.* 2002; Vek *et al.* 2013, 2015). These sugars are mostly responsible for growth of fungi and other deteriorated agents that could not be extracted completely by Chl and Chl + Water, while ethanol and diethyl ether were able

to extract sugar compounds of wood surface and inner sections of wood. Also, as shown in Table 3 the most identified compounds are hydrocarbon and phenolic compounds, sesquiterpenes and fatty acids which were reported to have good biological activity (Salem *et al.* 2014, 2016). Beech and spruce woods showed resistance against *T. versicolor* and *Serpula lacrymans* with increasing the tannins in their woods (Jeloková and Šindler 1997), but the presence of sugar contents had a negative effect on the wood durability of beech wood (Jeloková and Šindler 2001).

CONCLUSIONS

1. No significant difference was found between the densities and the maximum water absorptions of the beech wood specimens before and after the extraction with different solvents.
2. The highest extractives content were 9.49% and 8.58% for Eth + Water and Chl + Water extracted samples, respectively, while the lowest values obtained from the extracted beech wood specimens were 2.95% and 5.97% for Eth and Chl, respectively.
3. The highest weight loss percentages obtained from the decay test were 30.38% and 23.98% for Chl and Chl + Water extracted samples, respectively, in comparison with the control treatment. The lowest weight loss values were 0.35% and 0.64% for Eth and Eth + Water extracted beech wood samples, respectively.
4. The main constituents of Chl extract were TMS derivatives of hexadecanoic acid (13.28%), trans-caryophyllene (12.88%), TMS-3,5-dimethoxy-4-(TMS-oxy) benzoate (11.69%), eicosane (6.96%), hexadecane (6.79%), octadecane (6.29%), and isovanillic acid 2TMS (5.75%).
5. At 0.016 mg/mL, the highest antioxidant activity was observed for chloroform extract (89.45%), which was close to the value of vitamin C (96.63%).

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