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A QUALITATIVE AND QUANTITATIVE ANALYSIS OF EXTRACTIVES FROM THE SPECIES *Quercus conferta* IN THREE DIFFERENT SOLVENTS

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Abstract:
This research deals with the quantitative and qualitative analysis of extractives of the species Quercus conferta Kit (Quercus farnetto Ten.). Samples were collected not only from the wood (separately heartwood and sapwood), but from the bark, leaves and branches as well. Extractions were carried out with a Soxhlet device and three different solvents (water, ethanol, dichloromethane). Chemical analyses were conducted with gas chromatography and mass spectrometry. The results revealed significant amounts of the chemical compounds, such as D-limonene, myrtanol, phytol, megastigmatrienone, caryophyllene etc, found in the specimens, which have multiple applications in chemical, food and pharmaceutical industries.

Key words: *extractions; gas chromatography; mass spectrometry; oak.*

INTRODUCTION

All wood species contain a large amount of mainly organic compounds, which can be removed from the wood without transforming their structure. These compounds are called extractives. Extractives consist of gums, fats, resins, oils, alkaloids, tannins etc. which don't participate in the structure of the cell walls, but are laid between them and in the cell cavities. They can be removed by using various solvents, such as hot water, alcohol, benzene, dichloromethane and others, without changing the structure of the material. Besides wood, extracts are found in other parts of the tree, like roots, bark, branches and foliage (Hillis 1962, Tsoumis 1994, Grigoriou 1996).

Quercus conferta (Q. frainetto) grows in Balkan peninsula, extending northwards to N.W. Romania. In Greece, it is found almost throughout the country (Athanasiadis 1986, www.reherb.eu). Greek *Quercus* forests are of a great interest, since they provide food for the local flocks (sheep, goats etc.). Also, their nuts' extract, contain great amounts of tannins, which are used at the treatment of leathers and for the production of dyes (www.greenapple.gr). Furthermore, oak is a tree known from ancient times for its healing effects and nowadays some of its extractives, like quercetin, have many applications in pharmaceutical industry (www.greenapple.gr, www.reherb.eu).

As far as the chemical analysis is concerned, Santos and others (2010) identified in the species *Quercus suber*, after water and methanol extraction, fifteen phenolic components. The most abundant compound was ellagic acid, followed by gallic and protocatechuic acid. Additionally, some of the reported compounds were identified for the first time as cork components, namely salicylic acid, naringenin, quinic acid and hydroxyphenyllactic acid.

Pereira (1988) reported after analysis of *Quercus suber* cork that the total extractives were 14.2%. In more detail, the amount of extractives was estimated 5.4%, 4.8% and 4% when $CHCl_2$, ethanol and water respectively were used as solvent.

OBJECTIVE

The objective of this research was the extraction of important compounds from the species *Quercus conferta* using three different solvents, namely hot water, ethanol and dichloromethane (CH_2Cl_2). Moreover, the analysis was aiming in the identification of the differences in the extractives content between the samples that originated from various tree tissues (sapwood, heartwood, bark, leaves, branches).

MATERIALS AND METHODS

The studied material originated from the Aristotle University of Thessaloniki Forest at the area of Cholomontas, Chalkidiki (North Greece)(Fig. 1, a, b). From each tree, disks (Fig. 1, c) were taken from the

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chest height and then a longitudinal strip was cut from pith to bark. Afterwards, the bark, sapwood and heartwood were separated, so as to be treated apart from one another. All samples - bark, heartwood, sapwood, leaves (Fig. 1, d), branches - were initially cut into smaller piece by hand with a sharp blade and then were trimmed with a mill (Wiley's mill), in order to create particles with approximately the same dimensions, smaller than 0,1mm.

The experiments were conducted on two or three samples in each case, based on the standards which were followed, in case a large difference in measurements was observed between two samples.

The quantitative estimation of extractives soluble in hot water, ethanol and dichloromethane were conducted according to ASTM Standards (ASTM D1107-96, D1108-96, D1110-84). The stages of the extraction of the samples are illustrated in Fig. 2.

For the extractions, a glass Soxhlet type device with the appropriate size was used, so as a 2g specimen and glass filter with medium porosity to be fit. Before each extraction, the dry weight (DW) of each specimen and of each glass filter was calculated by double weighing, after being dried in the oven at 103±^{0}C for 24 hours (Table 1).

Each hot water extraction lasted almost 6 hours and almost 4 hours for each of the other two solvents and 4 cycles of the solvent were repeated per hour. After the procedure, the specimens were removed from the Soxhlet device and left at normal conditions of temperature and humidity (approximately 25^{o}C and 55%) for 24h, before they were put in the oven at 103±^{0}C for another 24 hours, until the total drying of the material. In the end, they were weighed to determine the dry weight of the extracted (wood) material, after the removal of the extractives (Chavenetidou 2009).

Qualitative analysis of extractives was conducted with gas chromatography and mass spectrometry. The solvents containing the extractives after extraction was reduced with the use of rotary evaporator up to 1-2ml, in order to trace very small amounts of the chemical compounds of interest. In most cases, at the final stage of the procedure the reduction was reached with the use of nitrogen gas stream.

In all cases, specimens were filtered in a chromatographic column with the use of the following materials: Florisil (MgO_3Si) 2.5g, Al_2O_3 3.5g and Na_2SO_4 1.5g to absorb moisture.

For the identification of the compounds and the quantification of the results gas chromatograph Agilent 7890A was used, provided with non-polar capillary column DB-5ms, 30m length and 0.25 internal diameter, film thickness 0.25μm and as a filler 5% phenyl polysiloxane, 95% methyl polysiloxane, using helium as a carrier gas (flow 0.99333 mL/min, pressure 11.656 psi) and mass spectrometer with quadrupole Agilent 5975C). Finally, the mass spectrometer with quadrupole Agilent 5975C was also implemented and 1-bromo-2-nitrobenzene was used as internal standard for the estimation of the quantity (Tziouvalekas 2011).

Two temperature programmes were applied, in order to succeed better analyses. The temperature programs which were applied where:

1. Initial temperature: 600^{0}C – for 4 minutes
Final temperature: 2400^{0}C with raising rate 50^{0}C/min - for 5 minutes
2. Initial temperature: 700^{0}C – for 4 minutes
Final temperature: 2800^{0}C with raising rate 50^{0}C/min - for 10 minutes

Identification was based on mass spectrometry and the results came from the substances recognized at each peak of diagrams conducted by mass spectrometer.

Fig. 1.
The stages of the collection of the samples:
a - The Aristotle University of Thessaloniki Forest at the area of Cholomontas, Chalkidiki (North Greece); b – logs; c - tree discs; d – leaves.

Fig. 2.
The stages of the extraction of the samples:
a - starting material after trimming with Wiley's mill; b - glass Soxhlet type device; c - rotary evaporator; d - gas chromatography; e - concentrated extracts in three different solvents.

RESULTS AND DISCUSSION

Quantitative analyses

In Table 1 the dry weight (DW) of the samples before and after the extraction is presented. Moreover, the following table as well as Fig. 3 show the percentage of hot water, ethanol and dichloromethane soluble extractives from sapwood, heartwood, bark, leaves and branches of *Quercus conferta*, as estimated with the above mentioned procedure.

At all samples, a variation in the amount of extractives is observed related to the origin of the samples from the various parts of the tree. More specific, the quantity of extractives is higher at the needles/leaves and the branches of the species, lower at the bark, even less at heartwood and the least at sapwood.

Table 1

Hot water, ethanol and dichloromethane soluble extractives from sapwood, heartwood, bark, leaves and branches of Quercus conferta

Quercus conferta	DW before extraction, g	DW after extraction, g	Extractives, %
Hot water			
Sapwood	1.908	1.844	3.354
Heartwood	1.878	1.682	10.437
Bark	1.971	1.741	11.652
Leaves	1.946	1.405	27.718
Branches	1.896	1.253	18.660
Ethanol			
Sapwood	1.858	1.813	2.422
Heartwood	1.888	1.745	7.577
Bark	1.950	1.800	7.681
Leaves	1.891	1.500	2.676
Branches	1.637	2.344	1.011
Dichloromethane			
Sapwood	1.000	0.987	1.300
Heartwood	1.929	1.894	1.814
Bark	2.061	1.979	3.945
Leaves	2.039	1.876	8.016
Branches	1.892	1.833	3.085

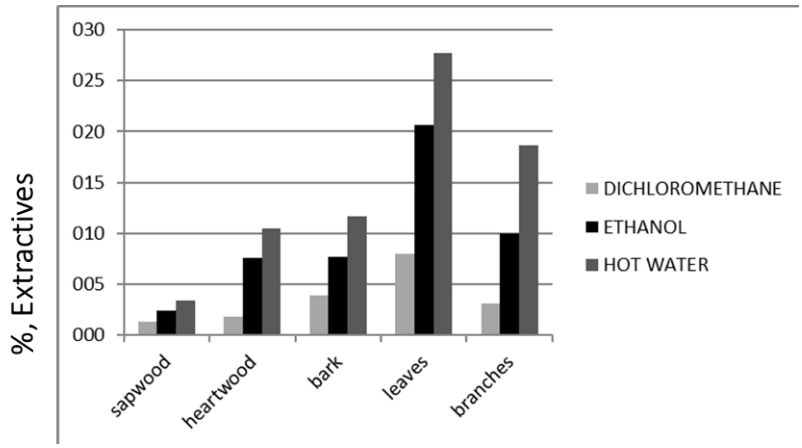

Fig. 3.
Hot water, ethanol and dichloromethane soluble extractives of Quercus conferta.

Qualitative analyses

The results of the analysis are presented in detail in the following tables (Tables 2, 3, 4, 5 and 6), which contain data from all the three solvents applied, since the interest of this essay was to discover the chemical compound found in the species at first and then at a following research to discriminate and quantify in more detail. From the detailed processing of the data it is obvious that:

- Heartwood contained larger amount of **benzyl alcohol** than sapwood, and almost the same as bark and leaves;

- **Caprolactam** appeared in almost the same quantity at heartwood and sapwood, whereas at leaves was larger;

- Heartwood and sapwood contained almost the same amount of **phenol, 3,4,5-trimethoxy-**

- **Benzophenone** appeared in larger quantity at sapwood than heartwood, while it was almost the same in heartwood and branches;

- Leaves contained larger amount of **eugenol** than branches;

- **Isopropyl palmitate** appeared in larger quantity at sapwood than heartwood, even smaller amount in branches and the smallest amount in bark;

- **Isoamyl laurate** appeared in larger quantity at bark, smaller at leaves and the smallest in branches;

- Sapwood contained larger amount of **ethyl oleate** than bark, and heartwood contained even less;

- **Squalene** appeared in almost the same quantity at heartwood and sapwood, whereas at bark was much larger;

- **Linoleic acid ethyl ester** appeared in larger quantity at heartwood than sapwood, smaller at branches and the smallest at leaves;

- Bark contained a great amount of **beta.humulene,** whilst leaves had less and branches even fewer;

- **Azulene** appeared in larger quantity at branches than bark, whilst it was relatively small in both;

- All samples contained different types of **phenols** in various concentrations and with various substituents.

Table 2

Chemical compounds found at Quercus conferta heartwood specimens

Quercus conferta heartwood chemical compounds	Integration area/internal standard area	*Quercus conferta* heartwood chemical compounds	Integration area/internal standard area
Morpholine	0,029	1-methyl-naphthalene	0,044
5-methyl-2-Furancarboxaldehyde	0,036	1-bromo-2-nitro- benzene	1,000
2-Benzylpiperazine	0,031	2,5-bis (1,1-dimethylethyl) phenol,	0,341
1,2,4-trimethyl-Benzene	0,004	3,4,5-trimethoxy-phenol,	0,139
Benzyl Alcohol	0,141	Benzophenone	0,328
4-ethyl-1,2-dimethyl-Benzene	0,011	Isopropyl Myristate	0,067
2-methyl- trans-Decalin	0,008	Galaxolide 1	0,010
Phenylethyl Alcohol	0,022	isobutyl octyl ester Phthalic acid	0,404
1,2,4,5-tetramethyl-Benzene	0,016	Isopropyl Palmitate	0,053
decahydro-2-methyl-Naphthalene	0,014	ethyl ester Linoleic acid	1,404
Borneol	0,009	Ethyl Oleate	0,972
2-bromo-Benzenamine	0,124	Butyl citrate	0,400
Benzothiazole	0,049	Squalene	0,468
Caprolactam	0,042	4,8-Methanoazulen-9-ol	0,761

Table 3

Chemical compounds found at Quercus conferta sapwood specimens

Quercus conferta **sapwood** chemical compounds	Integration area/internal standard area	*Quercus conferta* **sapwood** chemical compounds	Integration area/internal standard area
2-methyl-Adenosine	0,004	1,8-dimethyl-Naphthalene	0,015
Benzyl Alcohol	0,090	Propylthiane	0,047
2-methoxy-(mequinol) Phenol,	0,008	2-methoxy-4(1-propenyl) Phenol	0,035
1,2,4,5-tetramethyl Benzene,	0,010	3,4,5-trimethoxy-Phenol	0,174
Imidazolidine	0,000	Benzophenone	0,477
Benzothiazole	0,017	Hinesol	3,385
Caprolactam	0,047	Ispropyl palmitate	0,027
1-bromo-2-nitro- Benzene,	1,000	Mercaptoacetic acid bis	0,186
Linoleic acid ethyl ester	1,087	Ethyl oleate	1,721
Tributyl acetylcitrate	0,064	squalene	0,416
Mandelic acid	0,136		

Table 4

Chemical compounds found at Quercus conferta bark specimens

Quercus conferta **bark** chemical compounds	Integration area/internal standard area	*Quercus conferta* **bark** chemical compounds	Integration area/internal standard area
Sorbitol	0,002	Azulene	0,024
Dipropylene glycol	0,017	Hinesol (.beta.guainene)	0,363
Benzyl alcohol	0,139	Isopropyl myristate	0,226
4-bromo-Benzeneamine	0,141	Isoamyl laurate	0,556
Benzothiazole	0,029	Isopropyl palmitate	0,126
1-bromo-2-nitro- Benzene	1,000	Ethyl oleate	1,094
Naphthalene	0,026	Tributylacetylcitrate	1,125
2,5-bis(1,1-dimethylethyl) Phenol	0,334	Squalene	4,406
Aziridine	0,094	batilol	1,197
Sorbitol	0,002	beta.humulene	6,829
Dipropylene glycol	0,017	Naphthalene	0,026
Benzyl alcohol	0,139	2,5-bis (1,1-dimethylethyl) Phenol	0,334
4-bromo-Benzeneamine	0,141	Aziridine	0,094
Benzothiazole	0,029	**1-bromo-2-nitro- Benzene**	1,000

Table 5

Chemical compounds found at Quercus conferta leaves specimens

Quercus conferta **leaves** chemical compounds	Integration area/internal standard area	*Quercus conferta* **leaves** chemical compounds	Integration area/internal standard area
5-methyl-2-furancarboxaldehyde,	0,060	Phenol	0,141
1-methyl-4-piperidinone,	0,202	Bacchotricuneatin C	0,756
Acetamide, N-butyl-	0,306	**1-bromo-2-nitro-Benzene**	1,000
Benzyl alcohol	0,140	Eugenol	0,022
2-myristynoil pantetheine	0,014	Pyridinecoarboxamide	0,139
2(3H) – furanone	0,017	Silane, trichlorodocosyl	0,056
Phenylethyl alcohol	0,035	3,5-bis(1,1-dimethylethyl) Phenol	0,367
3-pyridinemethanamine	0,001	1H-imidiazole	0,216
Guanidineacetic acid	0,012	Boroxin	1,349
Valeric acid	0,138	Phytol	0,342
Benzaldehyde	0,215	Isoamyl laurate	0,474
2-furancarboxaldehyde	1,330	Homosalate	0,152
Caprolactam	0,132	Phthalic acid	1,077
Dibutyl phthalate	1,528	Kaur-16-ene	0,083
Silane	0,434	Linoleic acid ethyl ester	0,914
beta.humulene	2,201		

Table 6

Chemical compounds found at Quercus conferta branches specimens

Quercus conferta **branches** chemical compounds	Integration area/internal standard area	*Quercus conferta* **branches** chemical compounds	Integration area/internal standard area
2-furancarboxaldehyde	0,067	Caryophyllene	0,067
2-propyl-Thiophene	0,054	Maleamic acid	0,059
Valine	0,449	5-bromovaleric acid	0,051
2,3-dihydro-Thiophene	0,211	.alpha.-farnesene	0,030
4-bromo-Benzeneamine	0,037	Diethyl phthalate	0,986
Benzothiazole	0,299	Benzophenone	0,318
4-mercaptophenol	0,611	Azulene	0,133
1-bromo-2-nitro- Benzene	1,000	Isopropyl myristate	0,187
Eugenol	0,064	Isoamyl laurate	0,358
Bacchotricuneatin C	0,517	Imidiazolidine-1-	0,060
1,4-methanoazulene	0,087	Isopropyl palmitate	0,081
Linoleic acid ethyl ester	1,074	Chola-5,22-dien-3-ol	0,044
.beta.humulene	0,051		

In comparison to other studies related to *Quercus suber*, since studies conducted on *Quercus conferta's* chemical composition were not found, showed that the percentage of the extractives was approximately the same as found in Pereira study (1988). Furthermore, many phenolic compounds were identified at the present research, a fact in agreement with the observations of Santos and others (2010).

CONCLUSIONS

Quercus conferta contains a significant amount of extractives, which appear to have notable variation and, as a result, multiple applications in various sectors of industry. This research showed a large amount of water, dichloromethane and ethanol soluble extractives especially in the bark, leaves and branches with a tendency to diminish in the heartwood and then in the sapwood. At all samples, a variation in the amount of extractives is observed related to the origin of the samples from the various parts of the tree. More specific, the quantity of extractives is higher at the leaves and the branches of the species lower at the bark, even less at heartwood and the least at sapwood. As the chemical analysis is concerned, bark and branches appeared to have larger amounts of extractives and gteater chemical variation, in comparison with heartwood. Sapwood is very poor in chemical compounds, especially in quantity.

Further investigation needs to be conducted, so as to utilize the maximum of the products nature has to provide.

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