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QUANTITATIVE DETERMINATION OF EXTRACTIVES, INORGANIC INGREDIENTS AND PH OF CYPRESS TREES (*CUPRESSUS SEMPERVIRENS* L.) FROM DIFFERENT PROVENANCES

ABSTRACT

The objective of the present study was the determination of the extractives, acidity and ash content of three different provenances of *Cupressus semprevirens* wood. The specimens were collected from the sapwood, heartwood, bark and needles of the trees. The quantitative determination of the extractions was performed with two different solvents, water and ethanol.

The results showed no significant differences among the three provenances (P1, P2 and P3), except for the oldest, which also had the highest extract content, a fact that is attributed to the growth of the trees without competition. Furthermore, the needles and the bark, had, in all cases, the highest ash content, while heartwood appeared to have higher contents of extractives, compared to sapwood.

Keywords: cypress, ash, extractives, pH, bark, needles, sapwood, heartwood

INTRODUCTION

Evergreen cypress (*Cupressus sempervirens* L.) is a bare-seeded, conifer tree which belongs to the Cupressaceae family. (Athanasiadis, 1986). Cypress wood is yellow-red, hard, homogeneous, with odor and great natural duration, because of the oleorosin it contains. Furthermore, it exhibits considerable dimensional stability (Voulgaridis, 2007). It shows moderate mechanical strength,

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dries easily and can be easily treated. Due to the above-mentioned properties, it has many applications. (Tsoumis, 2000; Kakaras, 2015).

All parts of the tree give out a strong turpentine odor. The extractives of cypress wood contain essential oils, monoterpenes, oxides, alcohols, esters and are also found in great amounts in the branches, needles, bark and fruits (Tsoumis, 2000; Filippou, 2014). In recent years, a rekindled interest in cypress extractives is observed and many researchers have tried to address the specific issue. Al-Snafi (2016) reported the cypress physical-chemical properties and their possible applications in the fields of pharmacy and medicine, as it presented antibacterial, antifungal, antiviral, antiparasitic, therapeutic, anti-cancer, antioxidant, anticoagulant, among other, actions.

Milo et al. (1988), after analyzing the essential oils from fruits and needles of the Iranian provenance of cypress, identified 13 compounds, including a-pinene, δ-3-karen, terpenyl acetate and terpinolen. Emami et al. (2005, 2009) and Selim et al. (2014) reported in similar findings. According to their research, evergreen cypress contained alkaloids 0.7%, flavonoids 0.22%, tannins 0.31%, saponoids 1.9%, phenols 0.067%, various essential oils and biologically active ingredients. Furthermore, Emami et al. (2005) concluded that the essential oil of fruits and leaves contained mostly terpenes (79.4% and 73.3%, respectively). Leaves were richer in seskiterpenes compared to fruits (10.5% versus 1,7%). The main components in fruits as well as needles were α-pinene (39% and 40%), δ-3-karene (24% and 24%), terpenyl acetate (5.6% and 6.6%) and terpinolene (4.3% and 6.6%). Cypress extractives showed important antifungal and antibacterial properties, according to various studies. (Boukhris *et al*. 2012) Examination of the action of essential oils against various fungi and bacteria (Toroglu *et al*. 2007) by means of different solvents, such as water and chloroform (Emami *et al*. 2009), methanol, ethanol and ethyl acetate showed, in all cases, that extractives had an inhibitory effect on the growth of microorganisms. In addition, the extractives exhibited antiviral activity against certain specific viruses (Emami et al. 2009, Amouroux *et al*. 1998). Azzaz *et al*. (2019) ended up in similar results, pointing out the pharmaceutical importance and antifungal effect of cypress. With the conduction of chemical analyses and extractions of the needles, using ethanol and dichloromethane as solvents, identified 9.55% moisture, 7.54% proteins and 9.24% inorganic compounds. Additionally, alkaloids, phenols, saponins, terbines and tannins were detected as well as essential oils, such as citronella (5.04%), D-limonene (2.94%), α-citronellol (1.88%) and terpinolene (1.12%). Finally, the amount of inorganic content was studied by Liodakis et al. (2005), since it is regarded to be important due to the remains left in the soil after a fire. In accordance with their research findings, the residues left after burning at the temperatures of 600^0C, 800^0C and 1000^0C were 88.5%, 95.3% and 98.3%, respectively. The content of inorganic ingredients was 3.86%.

The aim of the study was the quantitative determination and the comparison of the extractives, the inorganic content and pH of cypress trees from three Greek provenances. Two provenances of the trees came from the native

vegetation of Peloponissos, S. Greece, while the third one from Central Macedonia, N. Greece.

MATERIAL AND METHODS

The specimens were prepared from the bark, sapwood, heartwood and needles of the chosen trees from the three provenances under examination. More specifically, samples coded as P1 were from a rural flat region at the area of Messinia (Peloponissos) and at an almost zero altitude. P2 samples were collected from the area of Ilia (Peloponissos), as a part of a native cluster of about 100 trees, at the altitude of 50 m. approximately and a slope of 10%. Finally, P3 samples came from the area of Thessaloniki, almost 1 km from the coastline and at a mean altitude of 15 m (Figure 1).

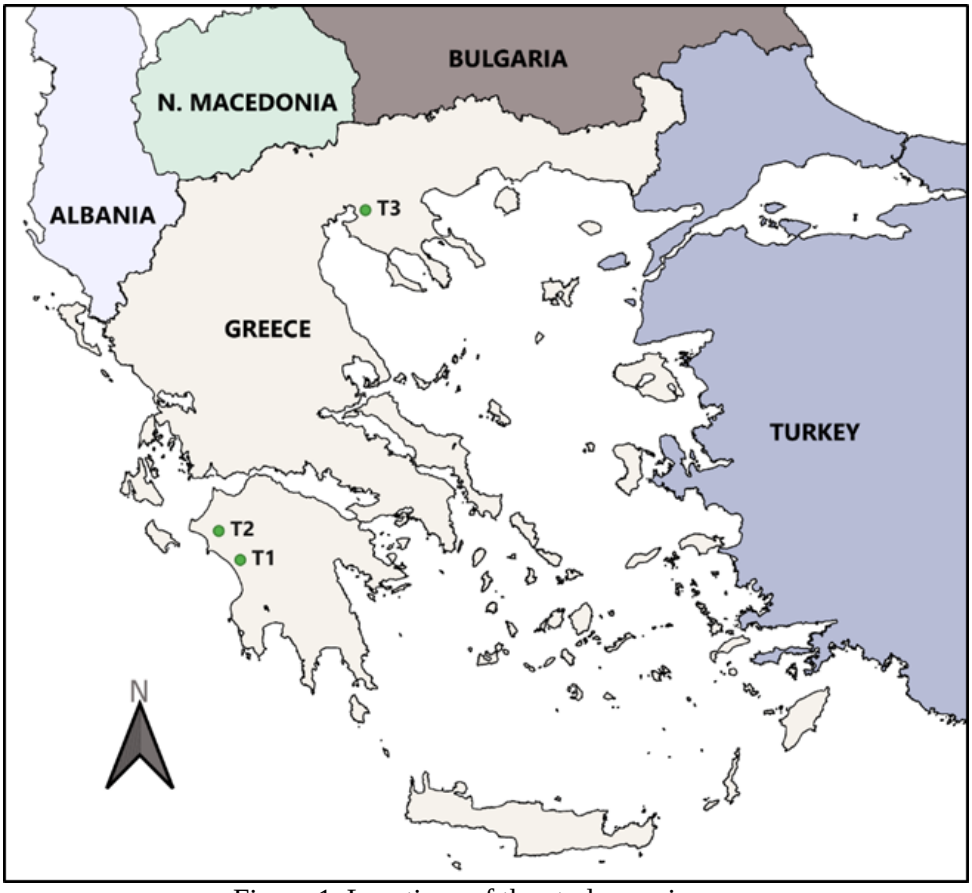

Figure 1. Locations of the study specimens

The determination of extractive properties was carried out according to ASTM Standards with specimens taken from each tree at the breast height. One disk was taken from each tree, which was later used to prepare a longitudinal strip

was cut from pith to bark. Then, the bark, sapwood and heartwood were separated, so as to be treated apart from one another. All specimens were cut by hand in small wood parts, which were trimmed by a mill into wood dust, in order to be used for the determination of the extractives, acidity (pH) and inorganic components (ash) (Picture 1) (Chavenetidou et al., 2020).

(a) (b)

Picture 1. (a) Transverse wood surface strips (b) Bark, sapwood and heartwood lumber.

Determination of the extractives

The quantitative determination of the extractives soluble in hot water and ethanol was done based on American Standards 1110-84 and ASTMD 1108-84, respectively (ASTM Standards 1961, 1984a, 1984c, Grigoriou 2008). For the extractions, a twin glass Soxhlet type device with the appropriate size was used, so as a 2.0 g 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 ± 2^0C for 24 hours. The rate of each extraction was set at four full cycles of the solvents per hour, which meant six hours for hot water extractions and approximately 4 hours for ethanol extractions, in accordance to the standards. After the extraction procedure, the specimens were removed from the Soxhlet device and left at room conditions of temperature and humidity (approximately 25^0C and 55%) for 24h, before they were put in the oven at 103 ± 2^0C for another 24 hours, until the total drying of the material. Finally, they were weighed so as to determine the dry weight of the extracted material, after the removal of the extractives (Chavenetidou et al. 2020).

The percentage of the extractives was calculated based on the absolute dry weight of the sample before and after extraction, with the use of Equation 1 (Tsoumis, 2000):

$$\boldsymbol{Extractives}\ (\%) = \frac{\boldsymbol{DW_b - DW_a}}{\boldsymbol{DW_b}} \times \mathbf{100} \qquad \text{(Equation 1)}$$

Where DW_b is dry weight before extraction and DW_a is dry weight after extraction.

Determination of inorganic components (ash)

The quantitative determination of the inorganic components contained in wood was conducted in accordance to the American Standard ASTM D 1102-84 (ASTM, 1984b), which suggests dry burning of approximately 2.0 g of air-dried wood dust (dry weight determination before burning) in incineration oven at 580-600 ^{0}C, until stabilization of the weight. Three repeatable weighings took place for the determination of inorganic contents humidity for each sample after 20-30 minutes at an electronic precision scale.

Inorganic contents were calculated using the Equation 2 (Grigoriou 2008):

$$\boldsymbol{Inorganic\ content}\ (\%) = \frac{\boldsymbol{Ash}}{\boldsymbol{DW_b}} \times \mathbf{100} \qquad \text{(Equation 2)}$$

Where *Ash* is the ash weight and DW_b is dry weight before extraction.

Determination of acidity (pH)

The quantitative determination of wood dust pH was held according to the standards (Grigoriou 2008) with the proportion 1:5 (wood:water) and with double measurements. At first, 1.0 g of wood dust from each sample (P1, P2, P3) and of each tree part (sapwood, heartwood, bark, needles) was placed into glass containers and then water was added gradually, in order to determine pH (picture 2). The measurements were conducted by an electronic pH meter.

Picture 2. Preparation of the samples for the determination of pH

Statistical analysis

Descriptive statistics were used to present the wood extractives content, acidity and inorganic content of the examined samples. Analysis of variance was used to test for significant differences between the extractives content for the two solvents used. All analyses were performed with SPSS ver 23.

RESULTS AND DISCUSSION

Extractives

Bark, heartwood, sapwood and needles content in extractive compounds soluble in hot water and ethanol is shown at Figure 2, which depicts the content of water soluble extractives, per provenance and tree part. P1 samples appeared to have larger concentration of extractives in its needles (19.504%) followed by the bark (8.269%), while the lowest was observed in sapwood. Simirarly, P2 needles (18.055%) and bark (14.737%) had the largest amount of extractives (18.055%), whereas the heartwood (0.866) contained the lowest of them. In regard to bark samples, P2 exhibited the highest concentration of extractives, (14.737%). Furtermore, compared to the other three parts, needles exhibited the highest concentration of extractives.

Unfortunately, no needles were available from P3 samples, so among the examined ones for the specific provenance, bark had the largest percentage of extractives. At both cases of P2 and P3, sapwood appeared to have higher percentage of extractives than heartwood.

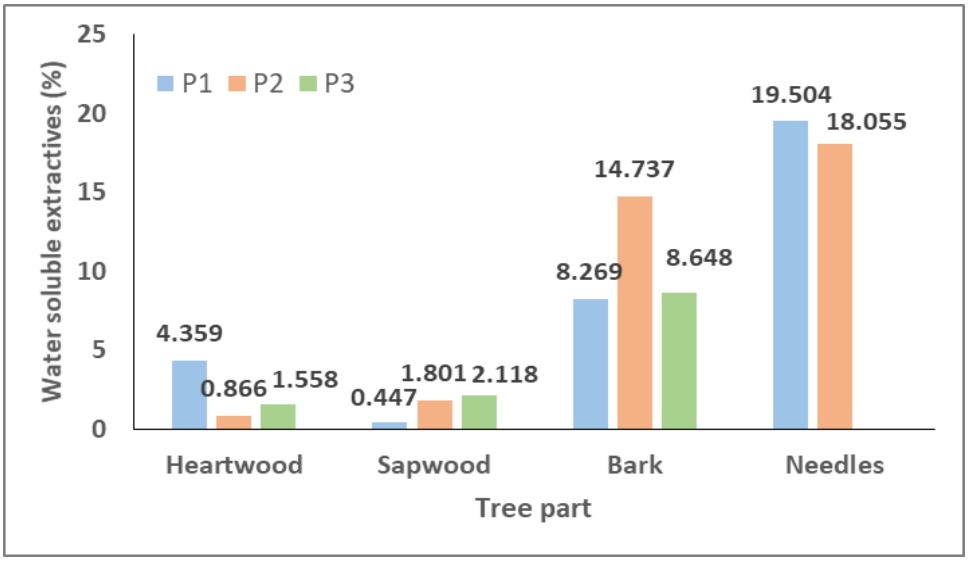

Figure 2. Determination of water soluble extractives per tree part

The use of ethanol as a solvent gave different results. Figure 3 depicts the content of ethanol soluble extractives, at the samples of bark, sapwood, heartwood and needles which were studied. As it is shown, among P1 samples, needles contained the largest percentage of the extractives (24.251%), followed by the bark (11.427%), while the minimum concentration was detected in sapwood (0.45%).

Table 1. Mean values of extractives percentage in the parts of the examined cypress trees

	Solvent	Mean	SD	F-value[1]	P-value[1]
Heartwood	Hot water	2.261	1.850	0.017	0.901
	Ethanol	2.431	1.250		
	Grand mean[2]	2.346	1.415		
Sapwood	Hot water	1.455	0.888	0.258	0.638
	Ethanol	1.112	0.762		
	Grand mean[2]	1.284	0.763		
Bark	Hot water	10.551	3.630	0.001	0.973
	Ethanol	10.463	2.313		
	Grand mean[2]	10.507	2.722		
Needles	Hot water	18.780	1.025	4.946	0.156
	Ethanol	22.663	2.246		
	Grand mean[2]	20.721	2.657		

[1] *F-value and P-value refer to the comparison between the two solvent types.*
[2] *Grand mean was estimated by aggregating all values per tree part.*

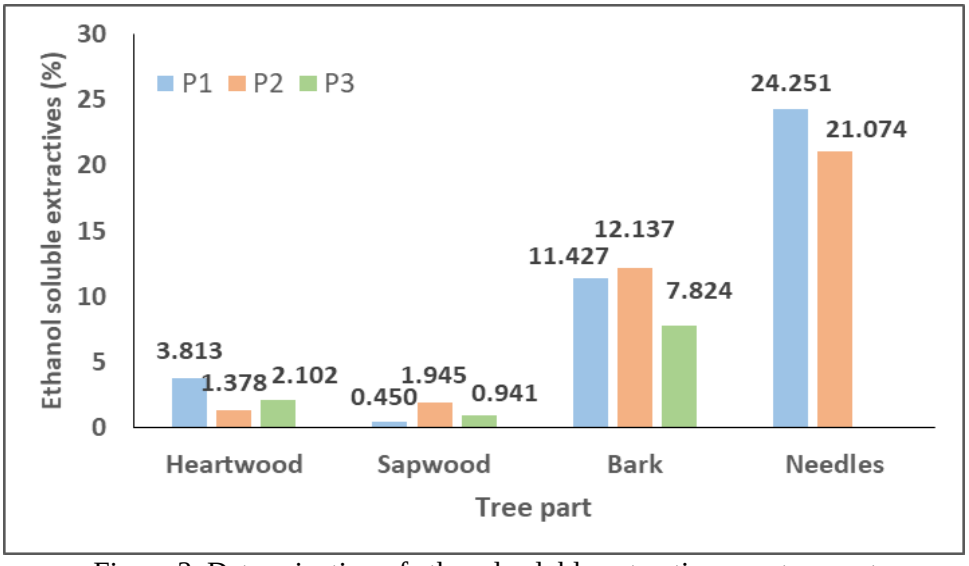

Figure 3. Determination of ethanol soluble extractives per tree part

A similar trend was observed in P2 specimens, where needles had the greatest amount of extractives as well (21,074%), followed by the bark (12.137%) and minimum concentration in sapwood (1.945%). Likewise, the highest percentage of extractive compounds among P3 samples was observed in the bark (7.824%) and the lowest in sapwood (0.941%). Our results agreed with

those of Palanti et al. (2021), who reported higher concentration of extractives in heartwood, compared to sapwood, who used ethanol as a solvent. Compared to the other two, P3 samples had the lowest amounts of extractives, in all cases, except for P1 sapwood.

However, all the reported differences were not significant. Thus, the grand means were 2.346% for heartwood, 1.284% for sapwood, 10.507% for bark and, finally, 20.721% for needles (Table 1).

Acidity (pH)

Generally, low acidity (pH) values of various woody species are attributed to free acids and acidic groups, acetic acids and acetyl groups, which are easily separated from the wood by extraction. Heartwood pH is slightly different from sapwood pH. Additionally, the time of the logging operations might slightly affect wood acidity. On the contrary, pH values might increase in case of storage at locations with high temperature and humidity (Grigoriou, 2008).

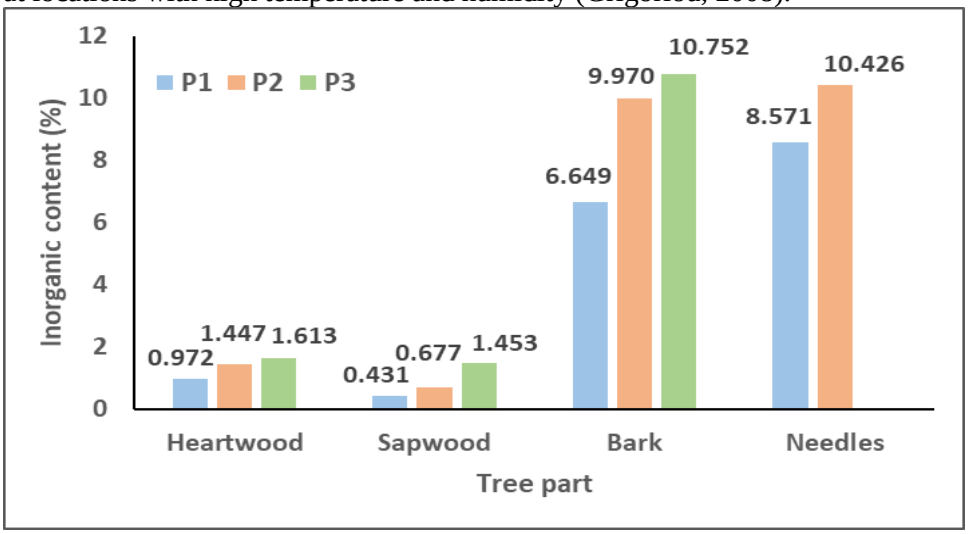

Fig. 4. Determination of bark, sapwood, heartwood and needles' pH per tree part

Table 2. Acidity (pH) values per cypress provenance and tree part

	Tree part			
Provenance	**Heartwood**	**Sapwood**	**Bark**	**Needles**
P1	5.725[a]	5.660[a]	5.560[b]	5.655[a]
P2	5.210[a]	5.585[b]	5.470[b]	5.145[a]
P3	5.805[a]	5.380[b]	5.610[c]	
Grand mean	5.580[a]	5.542[a]	5.547[a]	5.400[a]
SD	0.323	0.145	0.071	0.361

Different tree letters denote statistical significance at α=0.05 among tree parts within: 1) the same provenance values and 2) the grand mean values.

Table 2 and figure 4 show the acidity (pH) of sapwood, heartwood, bark and needles of all specimens.

Average pH values ranged from 5.145% to 5.805%, with P2 pH values being slightly lower than those of P1 and P3. The presented results are in line with the expected wood pH values at temperate climate locations within the range of 3.3-6.4 (Grigoriou, 2008).

Inorganic content (Ash)

The ash content of the examined cypress wood origins is presented in Figure 5. The above figure depicts the ash content of the examined cypress wood provenances.

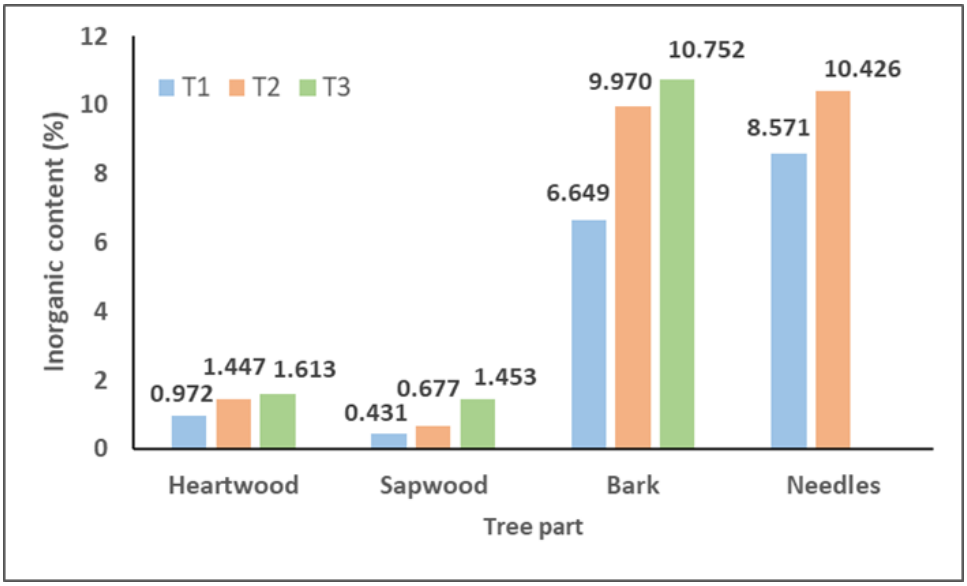

Fig. 5. Content of inorganic components (ash) per examined Cupressus provenance and tree part before extraction (%).

Based on the values which are presented, the smallest concentrations in all samples were located in the sapwood and heartwood, compared to the bark and needles. Between sapwood and heartwood, the percentage of the latter was slightly higher.

As far as the provenance of the samples was concerned, the highest values were observed at P3 samples, which originated from Thessaloniki followed by P2 and P1, from Peloponissos. In all cases, needles and then bark were rich in inorganic constituents, with values much higher (even up to 20 times) than those of wood. This result is in line with a previous study, in which cypress needles also had the high level of ash of 9.24% (Azzaz et al. 2019)

CONCLUSIONS

The objective of the study was the determination of the extractives, acidity and ash content of three different provenances of *Cupressus semprevirens* wood. According to our findings, no significant differences in extractives concentration were found among the three provenances. P1 samples, had the largest amount of heartwood, which resulted in the highest percentage of hot water-soluble extractives (4.3%) and ethanol soluble extractives (3.813%), compared to the other samples. T2 samples were rich in extractives, an expected result, due to their development without competition and due to their age.

Needles contained a much higher percentage of ash (high concentration of minerals), compared to the other tree parts, followed, in rank of magnitude, by the bark, heart wood and sapwood. Among all provenances, P3 samples had the highest ash percentage of 10.752%.

Average pH was practically the same, ranging from 5.145% to 5.805%, an expected rangewood pH values for temperate climate locations.

Future research should focus on the qualitative determination of cypress extractives, in order to investigate differences in terms of chemical composition among *Cupressus provenances*.

This will serve as a necessary further step towards the wider and more focused utilization of cypress extractives in industrial products.

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