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Pinus brutia extractives analysis for sustainability

Dimitris Birbilis[1], *Kostantinos V.* Kakavas[2] *and M.* Chavenetidou[3]

[1]University of Thessaly, Department of Forestry - Wood Science and Design, Karditsa, Greece
[2] University of Thessaly, Department of Environmental sciences, Laboratory of environmental chemistry and human health, 41500, Larisa
[3]Aristotle University of Thessaloniki, Department of Forestry and Natural Environment, Thessaloniki, Greece

Abstract. The aim of this study was to determine all the extractives from different samples were collected from heartwood, sapwood, bark and needles and branches of Pinus brutia. A Soxlet device with two solvents (water and ethanol) were used to collect the extracts. Analytical chemical analyses were conducted with Gas C1hromatography-Mass Spectrometry (Agilent 5975C). The greater percentage of the extracts in water and ethanol showed that the greater percentage of them is found in the branches and needles of the trees. Especially the results showed significant amounts of the chemical compounds, such as Borneol, Tetradecane, 6,6-Biquinoline, Butyl citrate, Isopropyl palmitate and Isopropyl myristate, which can be used for sustainability, environmental integration at the aspect of more effective use of natural resources and have many uses in novel – functional food, green chemistry and pharmaceutical industries. Finally, the greater percentage of the extracts in water and ethanol showed that the greater percentage of them is found in the branches and needles of the trees.

1 INTRODUCTION

The reduction of raw materials leads to the necessity of sustainable development. The primary goal is the more effective use of natural resources and the reduction of forest waste after logging, in the form of wood residues. Extractives are compounds contained in large amount at the material mentioned above. Countries around the world, such as Poland, Canada, are focused on exploiting wood waste in order to produce energy or other materials from the extractives of forest residues [1, 2] and production of wooden barrels [3]. All forest species contain a large amount of extractives in their wood but also at their branches, bark, needles, cones and roots as well. Extractives are chemical compounds inside cell walls, more rarely in cell cavities, with various synthesis, such as aromatic phenolic compounds, aliphatic compounds, terpenes, terpenoids and others. Since there is no chemical attachment to the wood, extractives can be removed from it with the use of various solvents, for example hot water, methanol, ethanol, dichloromethane and others, without causing transformation of it [4-9]. Of the genus Pinus several species are native in Greece, such as *Pinus brutia*, *Pinus nigra*, *Pinus pinea, Pinus leucodermis, Pinus peuce and others* [10, 11].

The aim of this study was the quantitative and qualitative estimation of the extracts from the species *Pinus brutia* for further use, at the frame of the most effective exploitation of wood, wood residues and other wood products.

Previous researches [12] determined the extractive content of *Pinus brutia* wood around 7.76 ± 2.43% (w/w). Kivrak et al (2013) using an ultrasonic extraction method with 50% ethanol solution, revealed 15 compounds in *Pinus brutia* bark: gallic acid, gentisic acid, protocatechuic acid, 4-hydroxy benzoic acid, catechin hydrate, vanillic acid, caffeic acid, vanillin, p-coumaric acid, ferulic acid, myricetin, resveratrol, luteolin, naringenin, kaempferol [13]. Cretu et al (2013) also consider that *Pinus brutia* bark extracts are useful in dietary supplements industry because of high free radical scavenging and 15-lipoxygenase inhibitory effects [14]. Ulukanli et al. (2014) found some remarkable antimicrobial, insecticidal, phytotoxic and antioxidant activities of Mediterranean *Pinus brutia* resin essential oils, giving a perspective for use in the formulation of ecofriendly and biocompatible pharmaceuticals [15].The antibacterial activity of *Pinus brutia* bark extracts by restriction of pathogenic bacteria in the intestines and simultaneous protection of commensal or beneficial ones, probably due to the phenolic components, was also confirmed by Demirtaş (2020) [16].

2 MATERIALS AND METHODS

2.1. Experimental procedure

The research material originated from the University Forest of Aristotle University of Thessaloniki in

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Taxiarchis, Chalkidiki (North Greece) (Figure 1.a). From each tree, wood discs were taken from breast height. Each disc was cut and came up a longitudinal strip of wood from pith to bark. The disks were divided into three different protions: bark, sapwood and heartwood (Figure 1.b). All the specimens were cut with the use of a sharp blade into smaller pieces and passed through the Willey mill in order to produce particles smaller than 0,1mm.

Figure 1.(a). Map of the sample collection area.

Figure 1.(b). Pinus brutia discs and samples of bark, needles and branches, from which the research material originated.

2.2. Chemicals and Reagents

All the reagents used were extra pure. Al_2O_3, MgO_3Si, Na_2SO_4 and 1-bromo-2-nitrobenzene were purchased from Fluka.

2.3. Apparatus and separation conditions

A Soxhlet-type device of big size made from glass was used for the extractions. A wood sample from the Willey mill of 2g was placed in the Soxhlet device as well as a glass filter (Figure 2 a, b). The dry weight of the samples and the glass filter was calculated by weighing after drying in an oven at 103 ± 2 °C, before each extraction. The extractions with water lasted six hours, while with ethanol lasted four hours. For each extraction and each solvent a different sample was used. Four complete cycles of solvents were repeated according to the ASTM Standards [17, 18]. At the end of the extraction the samples were left at room temperature for many hours (more than 24) and then placed in an oven at 103°C for an additional 24 hours. Furthermore, the samples were weighed to determine the dry mass of the wood, after removing the extracts (1).

$$Percentage\ of\ extractives\ (\%) = \frac{Dry\ weight\ before\ extraction - dry\ weight\ after\ extraction}{Dry\ weight\ before\ extraction} \quad (1)$$

Figure 2. (a). Rotary evaporator and glass Soxhlet type device.

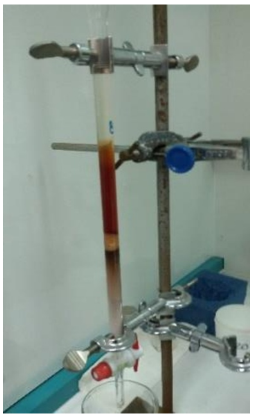

Figure 2. (b). Use of Al2O3 and florisil for bark extract filtering.

Qualitative extractives analysis was conducted with an Agilent 5975C GC-MS instrument. The solvents together with the extracts were condensed to about 2 ml final volume, with the use of rotary evaporator instrument (Bùchi Rotavapor R-215, Bùchi Heating bath B-491). The rotation speed of the vial ranged between 80 and 120 rpm and the temperature from 15-20 ºC (for ethanol and dichloromethane extractions, according to their boiling point). The condensation process was completed using pure nitrogen gas vapor until the appropriate final volume was reached. A glass chromatography column (1 cm internal diameter) was used to clean the samples that could damage the chromatography column. For this reason Florisil (MgO_3Si) 2.5g, Al_2O_3 3.5g and Na_2SO_4 1.5g were used to absorb moisture (Figure 2.b). The mass spectrometer was equipped with quadrupole non-polar capillary column DB-5ms, 30m length and 0.25mm internal diameter, film thickness 0.25μm, filler 5% phenyl polysiloxane and 95% methyl polysiloxane. The conditions of Gas chromatography Agilent 7890A for the compounds identification and quantification were: flow rate 0.99333 mL/min and pressure 11.656 psi and use of Helium as a carrier gas. The Library used for the identification of the compounds was the one applied in the Gas chromatography and the identification of the compounds based on the largest percentage of appearance and previous knowledge of *Pinus* extracts, according to literature. Two temperature programs were applied, for better resolution. The first temperature program was: Initial temperature at 70ºC, for 4 minutes, raising rate 50ºC/min and final temperature up to 280ºC for 10 minutes. The last temperature program was: Initial temperature at 60ºC, for 4 minutes, raising rate 50ºC/min and final temperature up to 240ºC for 5 minutes. Compounds identification was based on mass spectrometry and each peak of diagrams conducted.

3 RESULTS

The greater percentage of water and ethanol soluble extractives was found in the branches and needles of the trees (Figure 3). More especially chemical analysis showed significant amounts of chemical compounds, such as Borneol, Tetradecane, 6,6-Biquinoline, Butyl citrate Isopropyl palmitate and Isopropyl myristate.

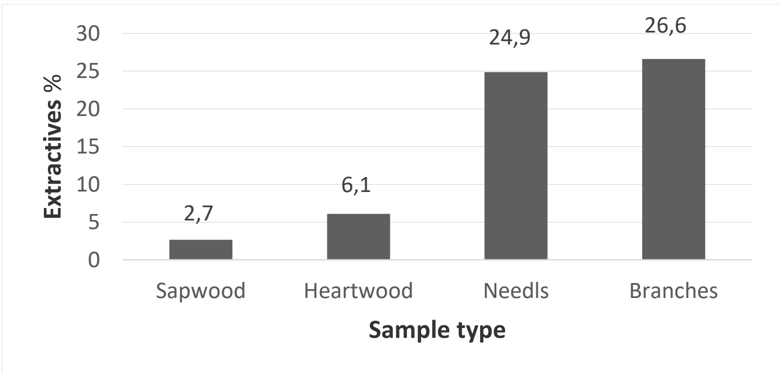

Figure 3. Extracts amounts from different parts of Pinus Brutia with water.

The chemical compound 1-bromo-2-nitrobenzene was used as Internal Standard (IS) for the estimation of the quantity sapwood GC-MS analysis revealed the results below [19, 20]. Table 1 shows that in sapwood there are great amounts of 2-propenoic acid, ethyl oleate, ethyl ester linoleic acid, 2-dodecene (Z), diethoxydimethoxy-silane, borneol and 2,5-bis-(1,1-dimethylethyl) Phenol.

Table 1. Chemical compounds found at Pinus brutia sapwood specimens (ethanol extracted specimens)

Type of chemical compounds	Integration area/internal standard area	Type of chemical compounds	Integration area/internal standard area
2,2-diethoxy-Ethanol	0.015	3-methyl-2 phenylethyl ester Butanoic acid	0.062
1-butoxy-2-Propanol	0.002	Pentadecane	0.070
Benzaldehyde	0.023	2,5-bis (1,1-dimethylethyl) Phenol	0.172
diethoxydimethoxy-Silane	0.210	7-Hexadecene,(Z)-	0.015
1-(2-methoxypropoxy)-2-Propanol	0.048	Neomethylamine	0.060
2-Propenoic acid	0.357	Benzophenone	0.065
Benzyl alcohol	0.092	Longofolenaldehyde	0.055
1-Octanol	0.046	Tetradecanoic acid	0.018
1-ethyl-2,3-dimethyl-Benzene	0.013	Terbuthilazine	0.249
Undecane	0.030	Octadecane	0.223
Nonanol	0.041	Isoromadendrene epoxide	0.074
Borneol	0.117	Isopropyl myristate	0.014
2-Dodecene(Z)-	1.120	(-)-trans-Pinene	0.025
Benzothiazole	0.103	Eicosane	0.118
2-propenyl Cyclohexane	0.003	2-alpha - Pyrrolidine	0.047
Caprolactam	0.012	N,N-Dimethylindo-aniline	0.064
3,6,6,-trimethyl-2-Norinanol	0.066	ethyl ester Linoleic acid	0.230
Tridecane	0.175	Ethyl oleate	0.349
1-bromo-2-nitro Benzene	**1.000**	Myclobutanil	0.184
1-Tetracosanol	0.069	1-(hexadecyloxy-)2-Propanol	0.096
6-Tetradecene	0.139	2-Chloropropionic acid, octadecyl ester	0.197
Isophytol	0.069	6-methoxy-2-methyl-3-Quinoline-4-ol	0.085
1-methyl-Piperidine	0.107	Bis (7-methyloctyl) ester Phthalic acid	0.069
Tetratriacontane	0.042		

The chemical compound 1-bromo-2-nitrobenzene was used as Internal Standard (IS) for the estimation of the quantity heartwood GC-MS analysis revealed the results below. According to the Table 2 below, in heartwood the chemical compounds in large quantities are borneol, piperidine, benzothiazole, isopropyl myristate, isopropyl palmitate and 6,6 biquinoline. Compared to sapwood, in heartwood are found larger amounts of benzaldehyde, borneol, caprolactam, isopropyl myristate and benzophenone.

Table 2. Chemical compounds found at Pinus brutia heartwood specimens (ethanol extracted specimens)

Type of chemical compounds	Integration area/internal standard area	Type of chemical compounds	Integration area/internal standard area
d-Glycoheptose	0.172	Homovanillyl alcohol	0.102
2,2-diethoxy-Ethanol	0.020	Phytol	0.042
Sec-Butyl nitrtite	0.006	2,5,-bis (1,1-dimethylethyl) Phenol	0.226
Morpholine	0.020	Diethyl phthalate	1.110
Benzaldehyde	0.436	Benzophenone	0.095
Tetraethyl silicate	0.175	Isooctylmercaptoacetate	0.276
3-Carene	0.018	2,6- Diisopropyl naphthalene	0.019
Benzyl alcohol	0.107	1-Adamantene ethanol	0.081
3-methyl-Phenol	0.036	Octadecene	0.084
Nonanol	0.059	Isopropyl myristate	0.825
Borneol	2.910	Triexadecyl borate	0.044
Benzothiazole	0.453	Isoamyl laurate	0.552
Caprolactam	0.077	Longifoleraldehyde	0.059
Cinnamaaldehyde	0.085	Monomenthyl salicylate	0.200
Tridecane	0.332	Dibutyl phthalate	0.121
Terpin hydrate	0.278	Piperidine	0.405
Bacchotricuneatin C	0.098	Isopropyl palmitate	0.969
1-bromo-2-nitro Benzene	**1.000**	Tributyl acetylcitrate	0.660
Cyclotetradecane	0.199	6,6-Biquinoline	3.483
3,5-Dimethoxybenzaldehyde	0.275		
d-Glycoheptose	0.172	Homovanillyl alcohol	0.102
2,2-diethoxy-Ethanol	0.020	Phytol	0.042
Sec-Butyl nitrtite	0.006	2,5,-bis (1,1-dimethylethyl) Phenol	0.226
Morpholine	0.020	Diethyl phthalate	1.110

As it is shown in Table 3, needles contain large quantities (+)-4-careen, butyl citrate, cyclotetradecane, tridecane, diphenyl-Pyrazole, megastigmatrienone and aminosalicylic acid.

Table 3. Chemical compounds found at Pinus brutia needles specimens (ethanol extracted specimens)

Type of chemical compounds	Integration area/internal standard area	Type of chemical compounds	Integration area/internal standard area
3,4,4-trimethyl-3-pentanol	0.165	alpha-hydroxy-Benzene acetic acid	0.784
3-methyl-3,5-pentanetriol	0.016	ethoxymethoxy-Cyclohexane,	0.137
1-butoxy-2-propanol	0.024	3,5-bis(1,1,-dimethyl-ethyl) Homovanillyl alcohol	0.188
1,6-dideoxygalactitol	0.002	Phenol	0.140
Benzaldehyde	0.088	Isocyclocitrol	0.150
Tetraethyl silicate	0.161	Dodecanoic acid	0.148
1H-pyrrole-2-carboxaldehyde	0.089	Megastigmatrienone	0.310
N-butyl-Acetamide	0.265	Diethyl phthalate	0.655
Benzyl alcohol	0.105	Thiophene, tetrahydro-2-methyl	0.064
1-(1H-pyrrol-2-yl)-Ethanone	0.012	Aminosalicylic acid	0.334
2-pyrrolidinone	0.025	cis-11-tetradecen-1-ol	0.228
2-methoxy-Phenol	0.149	Tetradecanoic acid	0.305

Phenylethyl alcohol	0.219	tetrahydro-2-methyl-Thiophene	1.928
Borneol	0.068	Butyl citrate	1.511
3-ethyl-Cyclohexene	0.759	Tricosane	0.036
1,2,3-trimethyl-Cyclohexane	0.085	7-oxodehydroabietic acid, methyl ester	0.269
4- (2-propenyl)-Phenol	0.140	5-amino-1,3-Hexadecanamide	0.180
Trans-shisool	0.037	diphenyl-Pyrazole	0.302
Tridecane	0.199	Semperiverene	0.497
2-methoxy-3-vinylphenol	0.011	1,6-dien-3-ol Humulane-	0.119
Bacchotricuneatin C	0.161	Squalene	0.087
1-bromo-2-nitro Benzene	**1.000**	Bromoacetic acid, octadecyl ester	0.180
(+)-4-careen	0.340	3-methyl-9-chloro-acridine	0.228
Cyclotetradecane	0.386	8-methyl-octan-hydrocoumarin	0.100

Bark specimens extracted with ethanol appeared to have great amounts of bis-(trimethylsilyl)-Mercaptoacetic acid, tetradecane, triethyl borate and hexadecane.

Table 4. Chemical compounds found at Pinus brutia bark specimens (ethanol extracted specimens)

Type of chemical compounds	Integration area/internal standard area	Type of chemical compounds	Integration area/internal standard area
Triethyl borate	4.092	Naphthalene (isomers)	0.070
1R-alpha-Pinene	0.008	Eudesma-4(14),11-diecene	0.013
alpha-Pinene	0.005	Pentadecane	0.042
Diisoamylene	0.070	Butylated Hydroxytoluene	0.051
Decane	0.044	Tetratriacontane	0.009
3-Carene	0.022	Hexadecane	1.252
2-methylpropyl-Hydroxylamine	0.026	45-diamino-2(1H)-Pyrimidine-ethienone	0.018
5-methyl-Undecane	0.002	1-(1-oxobutyl)-Pyrrolidine	0.021
Iridomyrmecin	0.007	Heptadecane	0.034
Camphor	0.042	Methyl tetradecanate	0.044
Azulene	0.024	Anthracene	0.017
Dodecane	0.568	Tetradecanoic acid	0.018
4-methoxy Benzaldehyde	0.012	Isopropyl palmitate	0.026
Estragole	0.015	2,5-dimethyl-Thiazole	0.442
1-methyl- n-Butyric acid, 2-ethylhexyl-ester Naphthalene	0.053	Eicosane	0.384
	0.067	Methyl abietate	0.027
1-bromo-2-nitro Benzene	**1.000**	Squalene	0.124
Biphenyl	0.006	bis (trimethylsilyl)-Mercaptoacetic acid	0.501
Tetradecane	1.222		

In all cases, many extractives of scientific and commercial interest were found As shown in figure 4, Borneol and 6,6 Biquinoline are two extracts found in the heartwood and in the highest concentration, alpha-hydroxy-Benzene acetic acid, Butyl citrate and tetrahydro-2-methyl-Thiophene are three extracts found in the needles and in the highest concentration.

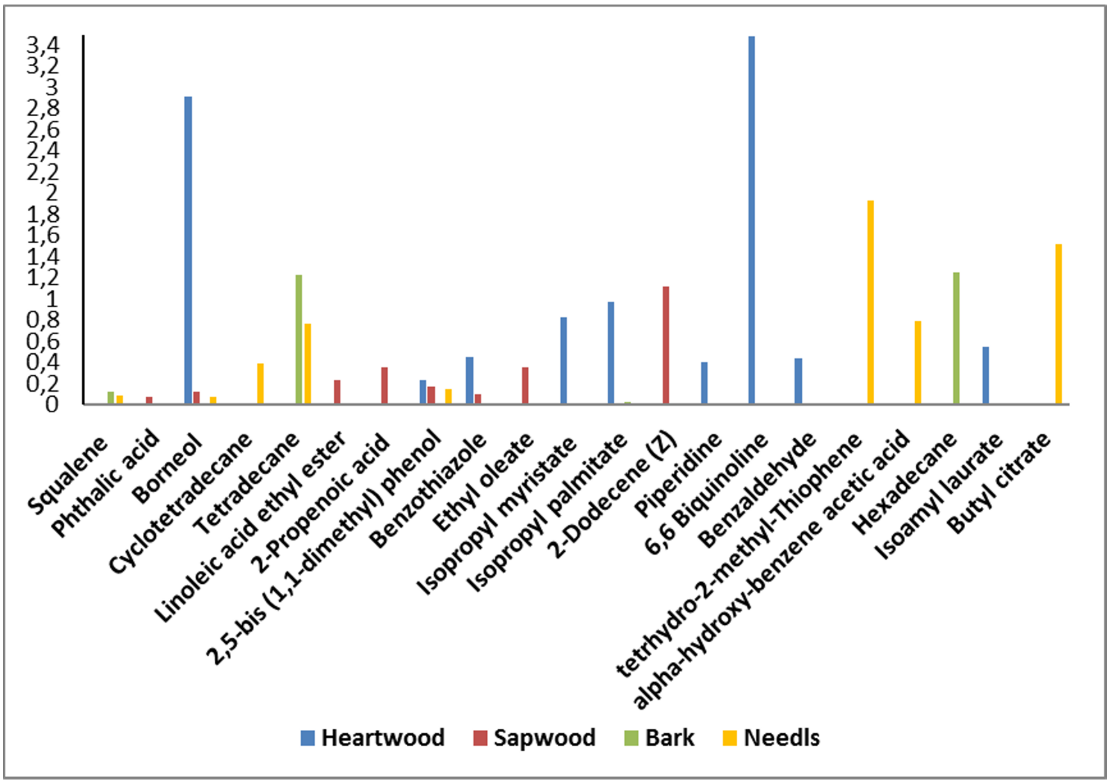

Figure 4. Quantity and type of Pinus brutia extracts.

4 Conclusions

From all the above data, it is obvious that the largest number of extractives was found in needles and branches. The results compared with other *Pinus* species are in agreement with those of other researchers [13, 14]. Furthermore, many compounds detected in this research were found from previous researchers as well [14, 15]. In this work an attempt was made to extract the ingredients of the species *Pinus brutia* using green techniques and solvents that help in the production of green products and help in sustainable development. In summary, all the extracts which were found can have many applications in human life, in the aspect of the optimization of natural resources utilization, taking into consideration the green growth, circular economy and sustainable development, since new uses of natural resources are suggested.

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