Bartın Orman Fakültesi Dergisi
2013, Cilt: 15, Sayı: 1-2
ISSN: 1302-0943
EISSN: 1308-5875

LIPOPHILIC CONSTITUENTS OF SOME CONIFEROUS CONES

Ayben KILIÇ[1]*, Harzemsah HAFIZOGLU[1], Ilhami Emrah DONMEZ[2], Ibrahim TÜMEN[1], Hüseyin SİVRİKAYA[1], Jarl HEMMING[3], Markku REUNEN[3]

[1] Faculty of Forestry, Bartin University, Bartin, Turkey.
[2] Faculty of Forestry, Süleyman Demirel University, Isparta, Turkey.
[3] Laboratory of Wood and Paper Chemistry, Åbo Akademi University, Turku, Finland.
*Corresponding author
Bartin University, Faculty of Forestry, 74100, Bartin, Turkey
E-mail: ayben67@yahoo.com; Fax: 03782235062, Tel: 03782235089

ABSTRACT

The lipophilic constituents of cones, a renewable natural resource, from *Picea orientalis*, *Cedrus libani* L. and four different *Abies* species namely *A.equi-trojani*, *A.cilicica*, *A.nordmanniana* and *A.bornmülleriana*, were determined after n-hexane extraction. The amount of lipophilics, identified by GC and GC-MS from the cones of *A. cilicica* (154.6 mg g-1 g^{-1}) and *A. bornmülleriana* (131.8 mg g-1) was significantly different from others. The lowest value was obtained from the cones of *P.orientalis* (25.14 mg g-1). Abietic, neoabietic, sandaracopimaric and hydroxylated resin acids constituted the major compounds in the cones. Free fatty acids, resin aldehydes, resin hydrocarbons and sterols were the other chemical groups found in the composition.

Key words: Abies, *Cedrus libani* L, cone, lipophilics, *Picea orientalis.*

INTRODUCTION

Cone is the part protecting the seeds in the coniferous species till seeds are matured. The size and the shape of cone are changing from species to species. *Cedrus libani* L. (Lebanon cedar), the native species to south central Turkey, has 8-10 cm long and 4-6 cm wide cone with barrel shape. It matured in 24 months and the surface is resinous. More stable and smaller cone is belonging to *Picea orientalis* (oriental spruce) with 5-9 cm long and 1.5 cm broad size. It has smooth, stiff and dark brown rounded scales. Even after the maturation, scales are not disintegrated and keep attaching to axis. There is four different Abies species growing natively in Turkey; *A. equi-trojani* (Kazdagi fir), *A. cilicica* (Toros fir), *A. nordmanniana* (Silver fir) and *A. bornmülleriana* (Uludag fir). Fir has the biggest cone in between coniferous species. It is between 15-20 cm long and 4-6 cm wide. Matured fir cone is brown and has150-200 scales, each scale with two winged seeds. Like cedar, the surface of fir cones is resinous (Ansin and Ozkan 1993; Yaltırık 1993). Each year great amounts of cones are produced during conifer regeneration. Nonetheless this renewable natural product did find a limited industrial usage. The cones of *Pinus pinea* L. was tested in the production of medium density fiberboard with urea-formaldehyde and was proposed as an alternative biological formaldehyde catcher (Ayrilmis et al. 2009). Mainly the bioactivity of pine cone extracts and also its chemical composition are studied (Eberhard et al.1994; Eberhard et al.1996; Villagomez et al.2005; Barrero et al. 2005; Unaldi and Toroglu 2009. Kilic et al.2011).

Lipohilics, the non-polar compounds, are forming 1-3 % of wood extractives and can be in higher contents in tropical trees and some other parts of tree (e.g. in knots, heartwood). It composed mainly resin acids, some other diterpenoids, fats and steryl esters. The function of resin acids and other diterpeniods is to protect a tree against insect and fungal attacks. Fats and steryl esters are the energy sources and cell membrane components (Sunderberg and Holmbom 2005).

In the last decade there has been a big interest in the use of natural products not only in pharmacy but also in food industry. From this aspect could cones be a suitable natural source? The first step of the answer is to know the chemical composition of this natural resource. In the present paper, the chemical composition of lipophilic

*Yazışma yapılacak yazar:ayben_kilic@hotmail.com

Makale metni 01.08.2013 tarihinde dergiye ulaşmış, 10.08.2013 tarihinde basım kararı alınmıştır.

compounds from cones of *A. cilicica, A. bornmülleriana, A. nordmanniana, A. equi-trojani, C. libani and P. orientalis* which up to our knowledge has not been addressed before were studied.

MATERIAL AND METHODS

Plant material and Reagents

The study was carried out using the cones of *P. orientalis, C. libani* and four different *Abies* species (*A. equi-trojani, A. cilicica, A. nordmanniana, A. bornmülleriana*). Sampling data are listed in Table 1. For each species 5 kg of cones were collected from the trees just at the time of maturity and stored at -24 °C until analysis. After disintegrating into small pieces, the cones were freeze-dried and ground in a Wiley mill down to the size of 1 mm. n-Hexane, and pyridine were obtained in analytical grade purity from Merck (Turku, Finland). *N-O-*Bis(trimethysilytrifluoro-acetamide) (BSTFA), heneicosaic acid, heptadecanoic acid and trimethylchlorosilane (TMCS) were purchased from Fluka (Turku, Finland). Betulinol was obtained from a private paper factory in Finland.

Table 1. Species and sampling locations

Species	Sampling location (Turkey)
Abies bornmülleriana	Bartin
Abies cilicica	Adana
Abies equi-trojani	Edremit
Abies nordmanniana	Trabzon
Picea orientalis	Trabzon
Cedrus libani	Adana

Extraction

About 5 g of grounded cone samples were extracted with n-hexane in an accelerated solvent extractor (ASE; Dionex Inc.ASE 200) apparatus (solvent temperature 90^0C, pressure 13.8 MPa, 3x5 min. static cycles). 2 ml of internal standard (heneicosanic acid, heptadecanoic acid and betulinol) was added to aliquots of extracts and the mixtures were evaporated under nitrogen. 150 µl of pyridine:BSTFA:TMCS (1:5:1) mixture was added to the extracts and silylated at 70^0C for 35 min. Silylated samples were injected to GC and GC-MS for the determination of lipophilic constituent.

Chromatographic Conditions

Chromatographic analyses were performed with a PelkinElmer AutosystemXL gas chromatograph equipped with a long column of HP-1 (J&W) 25 m x 0.2 mm (0.11 µm film thickness); column and a flame ionization detector with H_2 as the carrier gas (0.8 ml min^{-1}). The temperature program was initiated at 120 °C min^{-1} with a ramp of 6 °C min^{-1} up to 320 °C, injector temperature was 260 °C and FID temperature 320 °C. 1 µl sample was injected (split ratio: 1 : 24). For Sterlyesters and triglycerides a short column (HP-1 (J&W) 5 m x 0.53 mm, 0.15 µm film thickness) was used with the temperature program of 80 °C (0.1 min), 110 °C (15 °C min^{-1}), 340 °C (7 min). The identification of the individual compounds was performed by HP 6890-5973 gas chromatograph/mass spectrometry instrument equipped also with an HP-1 capillary column. The temperature program was set to be the same as in the long column. Identification was based on both the mass spectra and the comparison of the samples to compounds present in the spectral library.

RESULTS

Silylated lipophilic extracts from oriental spruce, Lebanon cedar and four different fir species were analyzed by GC and GC-MS using short and long capillary columns. The composition of lipophilic extractives is presented in Table 2. The limit of quantification was 1/100 and precision of determination ± 5 %. The most predominant lipophilic compounds present in the cones were resin acids, followed by resin alcohols, resin aldehydes, fatty acids and sterols. Also, lower amounts of sterlyesters and triglycerides were determined with short column.

As mentioned before, resin acids are obtained mainly from coniferous species. The results of cone extractives in this study reveal this one more time. In the fir species 75 %, in Lebanon cedar 51 % and in spruce 23 % of total identified compounds was resin acids. The most abundant resin acid in all cones was the abietic acid (50.2-2.72 mg g^{-1}). Neoabietic acid was found to be more than 23 mg g^{-1} both in *A. cilicica* and *A. bornmülleriana* taking the place as the second important acid. However, in the cones of the other two fir species (*A. nordmanniana* and *A. equi-trojani*) and in the oriental spruce cone, hydroxylated resin acids, a group formed by crystalline resins, was the second dominant acid. The amount of this group was found to be very low in Lebanon cedar (0.18 mg g^{-1}). Sandaracopimaric, levopimaric and palustric acids were other detectable acids found in the cones. Isopimaric acid (1.80 mg g^{-1}) and cupressic acid (0.10 mg g^{-1}) was determined only in the Lebanon cedar. Abietol, the alcohol form of abietic acid, and abietal, the aldehyde form, were also important compounds in the lipophilic structure of cones especially in *A. cilicica* and *A. bornmülleriana*. Resin hydrocarbons e.g. abieta-8,11,13-triene and palustradiene, were detected abundantly in Lebanon cedar cones, while it was in trace amounts or even not detected in other species. Campestrol and sitosterol were the two sterols found in cones and campestrol was determined only in the cones of fir species.

The last group found in cones was free fatty acids and triglycerides. Generally, the structure of free fatty acids determined in the cones of fir species were the same despite various amounts. Triglycerides was determined by using a short column. A clear difference can be seen between fir species, Lebanon cedar and oriental spruce cones regarding to the total amount of triglycerides. The total amount of free fatty acids was found to be larger than the amount of triglycerides in fir cones. On the contrary, in oriental spruce and Lebanon cedar cones the amount of triglycerides was found to be higher (14.8 mg g^{-1} and 11.6 mg g^{-1}).

Table 2 Lipophilic constituents of cones (mg g $^{-1}$ in dry weight).

	A. equi-tro.	*A.cilicica*	*A.nordman.*	*A.bornmuller.*	*P. orientalis*	*C. libani*
Fatty acids						
16:0 +(Abieta-7,13-diene)*	0.32	2.09	0.33	0.98	0.24	2.34*
17:0	0.35	0.94	0.33	0.64	-	-
18:3	-	-	-	-	0.38	-
18:2	0.14	1.08	0.02	0.06	0.12	-
18:2 conj.	0.48	0.62	0.31	0.08	1.05	-
9-18:1	0.48	1.34	0.09	0.53	0.41	-
11-18:1	0.21	0.47	0.21	0.46	-	-
18:0	0.1	0.1	0.09	0.22	0.03	-
24:0	0.08	0.23	0.13	0.13	-	-
26:0	0.1	0.19	0.14	0.14	-	-
28:0	0.15	0.43	0.24	0.27	-	-
Resin aldehydes						
Palustral	-	-	-	-	-	0.25
Levopimaral	-	tr	-	0.84	-	-
Dehydroabietal	0.11	1.06	0.1	0.37	-	-
Abietal	0.96	8.49	0.99	5.17	0.49	0.44
Neoabietal	0.06	0.11	0.05	0.08	-	0.09
Resin hydrocarbons						
Abieta-7,13-diene	0.04	tr	tr	0.13	-	*
Abieta-8,11,13-triene	-	-	-	-	-	0.47
Palustradiene	-	-	-	-	-	0.54
Neoabietadiene	-	-	-	-	-	0.62
Resin alcohols						
Dehyroabietol	0.27	1.53	0.18	0.98	0.21	0.21
Abietol	1.29	12.5	1.43	8.15	0.83	-
Neoabietol	0.35	3.37	0.24	2.01	-	0.33
Resin acids						
Sandaracopimaric acid	2.21	2.51	0.52	2.42	0.45	5.83
Isopimaric acid	-	-	-	-	-	1.80
Palustric acid	0.13	6.69	0.18	1.20	-	0.54
Levopimaric acid	0.71	6.50	0.35	8.67	-	0.33
Dehydroabietic acid	2.46	9.23	1.71	7.76	-	-
Abietic acid	7.95	50.2	8.38	42.3	2.72	5.22
Neoabietic acid	3.73	23.5	5.0	24.6	0.18	4.12
Isomeric dehydroabietic acid	0.78	1.67	0.66	2.05	0.11	0.16
Hydroxylated resin acids	6.06	12.6	5.81	16.7	2.37	0.18
Abietatetraenoic acid	0.56	2.03	0.53	1.69	0.03	-
Secodehydroabietic acid	0.06	0.21	0.07	0.11	-	-
Cupressic acid	-	-	-	-	-	0.10
Sterols						
Campestrol	0.20	0.35	0.25	0.34	-	-
Sitosterol	0.81	1.81	0.91	1.08	0.20	0.25
Sterlyesters	0.22	0.91	0.34	0.49	0.52	0.62
Triglycerides	1.38	1.84	0.62	1.17	14.8	11.6
Total identified amount	**32.7**	**154.6**	**30.2**	**131.8**	**25.1**	**36.0**

- : Not detected; tr: trace amount;*:overlapped;

DISCUSSION

The knowledge of the chemical composition of lipophilic components of cones will assist for the utilization of this natural product. In general terms, remarkable differences were detected among the cones of fir species. While, *A. cilicica* (154.6 mg g^{-1}) and *A.nordmanniana* (131.8 mg g^{-1}) have the highest amount of lipophilic compounds the two other fir species (*A.bornmülleriana* (32.7 mg g^{-1}), *A.equi-trojani 30.2* (mg g^{-1})) had similar results with Lebanon cedar (36 mg g^{-1}) and oriental spruce (25.1 mg g-1) cones.

Abietane type resin acids was the dominant group in all cones within different amounts. Hafızoglu and Reunanen (1994) found similar results for the cone resin of *A. nordmanniana* and oriental spruce. In that study, abietic acid was determined approximately 20 % and neoabietic acid 10 % . Nevertheless, in the wood part of *A. nordmanniana* both of these acids were found to be in trace amounts while in oriental spruce dehydroabietic, palustric and levopimaric acids were dominant (Ucar 2005). In Lebanon cedar cone sandaracopimaric acid, a pimarane type, was found to in high amounts (5.83 mg g-1) with abietic acid (5.22 mg g-1). Similar data was obtained in the oleoresin of the cedrus cone (Hafızoglu and Holmbom, 1987). In the wood part, beside these two compounds isopimaric acid also indicated as an important acid in Lebanon cedar (Hafızoglu 1987).

Resin acids, has antimicrobial and antifungal activities. Micales *et al.*, (1994) indicated that while abietane-type compounds (levopimaric, dehydroabietic, abietic and neoabietic acids) inhibited the mycelia growth in various fungi, the pimaric, isopimaric and sandracopimaric acids of the pimarane-type resin acids displayed only limited activity. In another study (Smith et al.2005) the diterpene isopimaric acid from the extract of immature pine cone was assayed against multidrug resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA). Kopper et al. (2005) also reported abietic and isopimaric acids as inhibitors for *Ophiostoma ips*, a conifer pathogenic fungus. A linear correlation between bioactivity against Gram-positive bacteria and lipophilicity degree was determined by San Feliciano et al. (1993). In the same study a reverse situation was observed for Gram-negative bacteria. Compared to common diterpen acids 7-oxo-dehydroabietic acid and 7-hydroxy-dehydroabietic acid, were found to be more active against inhibition of fungal growth (Savluchinke Feio et al.2006). From this point of view, extracts from the mature fresh cones especially from some fir species could be considered as a renewable resource for biological activities.

Acknowledgements
Special thanks to Prof. Bjarne Holmbom for his scientific supports, Laboratory of Wood and Paper Chemistry, Åbo Akademi, Turku, Finland. This work is a part of a project supported by Scientific and Technical Research Council of Turkey (TÜBITAK).

REFERENCES

- Ansin R, Ozkan C (1993) Tohumlu Bitkiler, Odunsu Taksonlar. Blacksea Technical University, Forestry Faculty, Yayın no:167/19, Trabzon.
- Ayrilmis N, Buyuksari U, Avci Koc E (2009) Ultikization of pine (*Pinus pinea* L.) cone in manufacture of wood based composite. Forest Ecology and Management, 259: 65-70.
- Barrero A F, Quilez del Moral J, Herrador M Mar, Arteaga JF, Akssira M, Benharref A, Dakir M (2005) Abietane diterpenes from the cones of Cedrus atlantica. Phytochemistry 66: 105-111.
- Eberhard TL, Han J, Micales JA, Young RA (1994) Decay Resistance in conifer seed cones: Role of Resin acids as inhibitors of decomposition. Holzforschung 48: 278-284.
- Eberhard T L, Young RA (1996) Characterization of conifer seed cone polysaccharides and lignin. Holzforschung 50: 401-407.
- Hafızoglu H (1987) Studies on the chemistry of Cedrus A.Rich. I.Wood extractives of Cedrus libani. Holzforschung 41:27-38.
- Hafızoglu H, Holmbom B (1987) Studies on the chemistry of Cedrus A.Rich. III.Oleoresin composition of Cones and Bark From Cedrus Libani. Holzforschung 41:141-145.

- Hafızoglu H, Reunanen M (1994) Composition of oleoresins from bark and cones of *Abies nordmanniana* and *Picea orientalis*. Holzforschung 48:7-11.
- Kilic A, Hafizoglu H, Dönmez IH, Tümen I, Sivrikaya H, Reunanen M, Hemming J (2011) Extractives in the cones of Pinus species. Eur J Wood Prod 69:37-40.
- Kusumoto N, Ashitani T, Hayasaka Y, Murayama T, Ogiyama K, Takahashi K (2009) Antitermic activities of abietane-type diterpenes from *Taxodium distichum* cones. J Chem Ecol 35: 635-642.
- Micales J A, Han J S, Davis J L, Young R A (1994) Chemical composition and fungitoxic activities of pine cone extractives. In: Biodeteriotation Research, (Ed. GC Llewellyn, W.V.Dashek, C.E. O'Rear), Plenum Press.
- San Feliciano A, Gordaliza M, Salinero M, Del Corral DJ (1993) Abietane acids: sources, biological activities and therapeutic uses. Planta Med 5: 485-490.
- Smith E, Williamson E, Zloh M, Gibbons S (2005) Isopimaric acid from *Pinus nigra* shows activity against Multidrug-resistant and EMRSA strains of *Staphylococcus aureus*. Phototherapy Research 19: 538-542.
- Savluchinke Feio S, Curto MJM, Gigante B, Roseiro JC (2006) Antimicrobial activity of resin acid derivatives. Appl Microbiol Biotechnol 72: 430-436.
- Sundberg A, Holmbom B (2005) Wood and Fiber Chemistry. Åbo Akademi, Laboratory of Wood and Paper Chemistry.pp:37-38.
- Ucar MB (2005) A comparative study on the chemical composition of the oriental spruce woods *Picea orientalis* from planted and natural forests. Chemistry of Natural Compounds. 41: 5.
- Unaldi UE, Toroglu S (2009) Studies on antimicrobial activity of pyramidal black pine: An endemic plant close to become extinct. J Environ Biol 30(2): 197-204.
- Villagomez HZ, Peterson DM, Herrin L, Young RA (2005) Antioxidant activity of different components of pine species. Holzforschung 59(2): 156-162.
- Yaltirik F (1993) Dendroloji Ders Kitabi I.Gymnospermae, 2nd ed. Istanbul University, Forestry Faculty, Yayın no: 3443/386.