Antifungal Activity of the Essential Oils from Some Species of the Genus *Pinus*

Mirosława Krauze-Baranowska[a,*], Marek Mardarowicz[b], Marian Wiwart[c], Loretta Pobłocka[a] and Maria Dynowska[d]

[a] Department of Pharmacognosy, Medical University of Gdańsk, Gen. J. Hallera 107 str., 80–416 Gdańsk, Poland. Fax: +48583493206. E-mail: krauze@farmacja.amg.gda.pl
[b] Department of Chemical Physics, Faculty of Chemistry, Maria Curie-Skłodowska University, M. Skłodowska-Curie Sq. 3, 20–031 Lublin, Poland
[c] Department of Plant Breeding and Seed Production, University of Warmia and Mazury, Łódzki Sq. 3, 10–724 Olsztyn, Poland
[d] Department of Botany, University of Warmia and Mazury, Łódzki Sq. 3, 10–724 Olsztyn, Poland

* Author for correspondence and reprint requests

Z. Naturforsch. **57c,** 478–482 (2002); received January 29/March 5, 2002

Essential Oils, *Pinus*, Antifungal Activity

The chemical composition of the essential oils from the needles of *Pinus ponderosa* (north american pine), *P. resinosa* (red pine) and *P. strobus* (eastern white pine) has been determined by GC/MS (FID). The essential oils from *P. resinosa* and *P. ponderosa* in comparison to *P. strobus* have been characterized by the higher content of β-pinene (42.4%, 45.7% and 7.9% respectively). On the other hand, α-pinene (17.7%) and germacrene D (12.2%) were dominant compounds of *P. strobus.* Moreover the essential oil from *P. resinosa* was more rich in myrcene-15.9%. Estragole and Δ-3-carene, each one in amount *ca* 8% were identified only in *P. ponderosa*. The content of essential oils in the needles slightly varied – 0.65% – *P. resinosa,* 0.4% – *P. strobus*, 0.3% – *P. ponderosa.* The antifungal activity has been investigated towards *Fusarium culmorum*, *F. solani* and *F. poae.* The strongest activity was observed for the essential oil from *P. ponderosa,* which fully inhibited the growth of fungi at the following concentrations – *F. culmorum, F. solani* at 2% and *F. poae* at 5%.

Introduction

From the chemotaxonomic point of view, regarding the composition of essential oils the genus *Pinus* is divided into two groups (Chalchat *et al*., 1994; Chalchat and Gorunovic, 1995). One group comprises the species rich in monoterpene hydrocarbons (α-,β-pinene, limonene, β-caryophyllene, germacrene D, Δ-3-carene) and the other is rich in the oxygenated monoterpenes (borneol, bornyl acetate) (Chalchat *et al*., 1994; Chalchat and Gorunovic, 1995; Orav *et al*., 1996; Sjodin *et al*., 1996; Yatagai and Hong, 1997). According to these data, it is worth to notice that the essential oils from the needles of *Pinus ponderosa* Dougl. ex Laws. (north american pine), *P. strobus* L. (eastern white pine) and *P. resinosa* Ait. (red pine) have not been studied in detail. In these species several groups of natural compounds mainly flavonoids (Niemann, 1988), piperidine alkaloids (Stermitz *et al*., 1994) and tannins (Harborne and Baxter, 2001) have been recognized and described in the literature. During last years most of the phytochemical studies on the above mentioned species focused on the terpene composition of oleoresins (Hanover, 1975) or on the volatile compounds emitted by conifer species and analysed by the head-space technique, in order to explain the relationships between plant-host and forest insects (Schäfer *et al*., 1995; Dormont *et al*., 1998).

The antimicrobial activity of essential oils from the family Pinaceae was investigated by many authors (Bagci and Digrak, 1996a, 1996b; Canillac and Monrey, 1996; Lis-Balchin *et al.*, 1998). It was reported, that essential oils with high monoterpene hydrocarbon levels, such as pine oil, were very active only against bacteria not against fungi, including *Fusarium culmorum* (Lis-Balchin *et al.*, 1998). However, the essential oils of the nine *Abies* species were more active against yeast species, including *Candida albicans,* than against bacteria (Bagci and Digrak, 1996b).

In this work, the composition of the essential oils isolated by hydrodistillation from the needles of *P. ponderosa, P. resinosa* and *P. strobus* is reported along with the determination of antifungal activity towards *Fusarium* spp.

Material and Methods

Plant material

The needles of *Pinus ponderosa* and *Pinus strobus* were collected from the Medicinal Plants Garden of Medical University of Gdańsk (Poland) (September 1999) but the needles of *Pinus resinosa* were obtained from the experimental forest of Arboretum in Rogów of Warsaw Agricultural University (Poland) (August 1999). Voucher speciments of these plants (99-010, 99-011, 99-012) have been deposited at the Herbarium of the Department of Pharmacognosy, Medical University of Gdańsk (Poland).

Isolation of essential oils

Dried and pulverized needles were hydrodistilled as described in the literature (Pharmacopeia Polonica V, 1995). The obtained essential oils were kept in sealed glass tube at 4 °C until analysis.

Gas chromatography

GC analysis was performed on a Carlo Erba GC 6000 (Italy) gas chromatograph equipped with a flame ionization detector (FID) and fitted with a DB-5 fused silica capillary column (30 × 0.25 mm, 0.25 μm thickness), the temperature programming was as follows: 35 °C (2 min.), 4 °C/min., 280 °C (15 min.); carrier gas helium, flow rate 1 ml/min. GC-MS – ITS-40 (Finningan MAT, USA), MS 70eV, 220 °C.

Antifungal activity

According to Krauze-Baranowska and Wiwart (2002) the essential oils were dissolved in MeOH in concentrations 2%, 5%, 10% (Morris *et al.*, 1979) and bioassayed towards *Fusarium culmorum* (W.G.Sm.) Sacc., *Fusarium poae* (Mart.) Appel. Wollenw. and *Fusarium solani* (Beck.) Wollenw. The last fungus from that group has been isolated from the suppurative and not healing wound of a shank of the patient who injured oneself a dry stem of dill (Dynowska, 1998). Inhibitory effect on growth of fungi was determined by measurements of the length of the germinating tubes (n-21) using a computer-aided image analysis (Oh *et al.*, 1996). For statistical analysis Student-Newman Kuels test was employed.

Results and Discussion

The chemical composition of the essential oils from three species of the genus *Pinus* – *P. ponderosa, P. resinosa* and *P. strobus* was elucidated employing GC with MS and FID detection. The identification of components was verified by comparison mass spectra with previously published spectra and confirmed by comparison of temperature retention indicies (Poole and Schütte, 1984) with published index data (NIST, Terpene Library of Finningan, MAT). The quantitative composition of oils was analyzed by GC (FID) by internal normalization assuming identical mass response factor for all compounds. In our studies, only those components which were present in the oils in amounts higher than 0.1% have been taken into consideration. About 61 constituents were determined, among them 50 identified in *P. strobus*, 49 in *P. ponderosa* and 43 in *P. resinosa*. Moreover 15 components occurred in amounts over 1% in *P. strobus,* 10 in *P. ponderosa* and 8 in *P. resinosa* (Table I).

The essential oil from *P. strobus* differed markedly from distillated *P. resinosa* and *P. ponderosa*, by low concentration of β-pinene –7.9% (Table I). On the contrary, in the eastern white pine oil germacrene D – 12.2% was the dominant compound next to α-pinene (17.7%). Moreover, constituents such as sesquiterpenes: δ-cadinene, γ-cadinene or oxygenated terpenes as α-cadinol and τ-cadinol/τ-muurol (not separated by GC) were found in higher amounts (7.7–2.8%) than in the previously studied *Pinus* species (Chalchat *et al.*, 1994; Chalchat and Gorunovic, 1995; Orav *et al.*, 1996; Sjodin *et al.*, 1996; Yatagai and Hong, 1997). In *P. strobus s*pathulenol (2.8%) and α-selinene/germacrene B (3.0%) were also identified in larger amounts in comparison to the essential oil from north american pine and other conifers oils (Chalchat *et al.*, 1994; Chalchat and Gorunovic, 1995; Orav *et al.*, 1996; Sjodin *et al.*, 1996; Yatagai and Hong, 1997). The content of monoterpenes: camphene, myrcene, limonene/phellandrene and sesquiterpenes- β-caryophyllene, the constituents characteristic for conifer essential oils, were very similar (*ca* 3.0%). The terpene composition of oil in the needles does not resemble those reported in the oleoresin and in the volatiles emitted by the foliage of eastern white pine (Dormont *et al.*, 1998;

Table I. Percentage composition of essential oils from *Pinus ponderosa, P. resinosa* and *P. strobus.*

Compound	RI	*Pinus strobus*	*Pinus ponderosa*	*Pinus resinosa*
Santene	884	0.2		
Tricyclene	920	0.2	tr	0.4
α-Pinene	933	17.7	10.2	23.3
Camphene	946	3.2	0.5	1.6
β-Pinene	977	7.9	45.7	42.4
Myrcene	992	3.6	1.4	14.5
α-Phellandrene	1004	0.4	tr	tr
3-Carene	1009	tr	8.4	0.5
α-Terpinene	1017	tr	0.1	tr
p-Cymene	1025	0.2	0.1	0.1
Limonene + β-phellandrene	1029	3.0	2.4	2.5
1,8-Cineole	1034	–	0.1	–
cis-ocimene	1041	tr	0.5	–
γ-Terpinene	1060	tr	0.2	tr
Terpinolene	1089	0.2	0.81	0.2
α-Thujone	1110	–	0.2	–
Endo-fenchol	1117	–	0.1	tr
trans-pinocarveol	1142	tr	0.3	0.3
Camphor	1149	–	0.1	tr
Pinocarvone	1167	tr	0.1	0.1
Borneol	1170	tr	0.1	0.1
4-Terpineol	1181	tr	0.3	0.1
α-Terpineol	1195	tr	1.4	0.8
Myrtenol	1201	tr	tr	0.2
Estragole = methyl chavicol	1204	–	8.0	
δ-Elemene	1341	0.3	0.1	–
α-Copaene	1379	0.3	tr	tr
β-Bourbonene	1390	0.9	–	tr
β-Cubebene	1395	0.2	–	tr
β-Elemene	1395	0.6	0.4	tr
Methyl eugenol	1413	–	0.6	–
β-Caryophyllene	1425	3.8	0.2	2.2
Thujopsene	1438	–	–	0.2
(*Z*)-*trans*-α-bergamotene	1441	–	0.6	–
α-Guaiene	1446	0.3	tr	
Aristolene	1450	–	0.3	
α-Humulene	1460	0.9	0.3	0.4
β-Cadinene	1481	0.2	0.1	tr
γ-Muurolene	1482	1.4	0.2	0.1
Germacrene D	1488	12.2	0.3	4.9
β-Selinene	1495	0.5	0.2	–
α-Selinene + germacrene B	1505	3.0	0.9	–
α-Muurolene	1506	1.5	0.5	0.1
γ-Cadinene	1521	2.8	0.9	0.1
δ-Cadinene	1530	7.5	3.1	0.4
α-Calacorene	1553	0.1		
trans-nerolidol	1569	1.0	0.1	tr
β-Calacorene	1574	0.5		
cis-3-hexenyl-benzoate	1581	0.2		
4-β-hydroxy-germacra-1(10),5-diene	1586	0.6		
Spathulenol	1588	2.8	0.5	tr
Caryophyllene oxide	1594	0.8		0.2
Veridiflorol	1604	0.1	0.6	tr
Cubenol	1638	0.3	0.2	tr
τ-Cadinol + τ-muurolol	1652	4.4	2.0	0.2
δ-Cadinol	1658	0.7	0.4	tr
α-Cadinol	1665	5.7	2.7	0.3
Unknown (M = 184)[a]	1894	1.0		
Manoyloxide	2009	0.5	–	0.5
Manool	2070		0.2	tr
Unknown (M = 289)[b]	2221		–	2.0

Mass spectral data of unknown compounds, number[a]: 184(1), 149(10), 147(12), 131(100), 103(75), 82(67), 77(41), 67(79), 51(11), 41(20), number[b]: 289(22), 271(15), 253(10), 243(69), 206(15), 201(15), 173(15), 163(18), 147(22), 133(14), 123(35), 107(42), 95(50), 79(43), 81(80), 67(52), 55(60), 43(100), 41(43).

Hanover, 1975). The results of our studies demonstrate that Δ-3-carene is present in needles as traces, what is in contrast to other reports. Dormont *et al*. (1998) and Hanover (1975) determined in the oleoresin as well as the foliage volatiles, the levels of Δ-3-carene as high as 10%-40%, while Schäfer *et al*. (1995) employing the SPME method were not successful to show the presence of this compound in volatiles.

The qualitative and quantitative profiles of the essential oils from *P. ponderosa* and *P. resinosa* were differentiated due to the presence of Δ-3-carene (8.4%) and estragole (8.0%) in *P. ponderosa* and the high level of myrcene (14.5%) in *P. resinosa*. Both essential oils may be classified as rich in monoterpene hydrocarbons (60%-80% of total oil), with a relatively large amount of β-pinene (45.7% and 42.4%, respectively).

The estimation of antifungal activity of the essential oils from *P. ponderosa*, *P. resinosa* and *P. strobus* was carried out towards three species of the genus *Fusarium*: *F. culmorum*, *F. poae* and *F. solani*. The latter fungus was isolated directly from the patient with confirmed hialohyphomycosis of the shank (Dynowska, 1998). Hialohyphomycoses are rare infections caused e.g. by fungi of *Fusarium* genus, which are mainly present in soils in different climatic zones. *Fusarium solani* may be the etiological agent of numerous skin lesions, inflammation of the internal structures in the eye and joints (Dynowska, 1998). The antimicrobial activity of essential oils has been determined for their 2%, 5% and 10% solutions (Morris *et al*., 1979). The oil from north american pine possesed the strongest activity, at lowest concentration inhibited the growth of two fungi: *F. culmorum* and *F. solani* (Table II). Fungicidal activity against *F. poae* was observed at 5% concentration for all essential oils. At this concentration the essential oils from *P. resinosa* and *P. strobus* were also active towards *F. solani* and *F. culmorum* (Table II). The obtained results confirm the antifungal activity of conifer essential oils but at higher concentrations than those reported by others (Lis-Balchin *et al*., 1998). The activity of oils from *P. ponderosa* and *P. resinosa* can be related to their chemical composition. It is shown that the high level of α- and β-pinene does not decrease the antifungal properties of the above oils, in opposite these compounds can be responsible for this activity. The essential oil from *P. strobus,* containing the lowest amount of hydrocarbon monoterpenes showed the weakest antifungal activity (Table II). Above observations are in contrast to data published by Lis-Balchin *et al*. (1998), who correlated lack of the antifungal activity of the needle pine oil with the high content of α- and β-pinenes. On the other hand our results are in good agreement with data reported by Magiatis *et al*. (1999). The antifungal activity of oil from *Pistacia lentiscus* (Anacardiaceae) was found to be due to the high concentration of α-pinene.

Table II. The antifungal activity of the essential oils from *Pinus ponderosa, P. resinosa* and *P. strobus.*

Essential oil	Antifungal activity[a]		
	Fusarium culmorum	*Fusarium poae*	*Fusarium solani*
Pinus ponderosa	100	78 ± 5	100
Pinus resinosa	32 ± 7	92 ± 4	84 ± 8
Pinus strobus	38 ± 6	70 ± 5	58 ± 4

[a] Antifungal activity is estimated as% inhibition of growth at a 2% concentration, mean ± SEM (n = 21) and its significant difference from the control (methanol) $p < 0.01$.

Acknowledgments

The authors kindly thank Prof. dr hab. Jerzy Tumiłowicz, Director of the experimental forest of Arboretum in Rogów of Warsaw Agricultural University (Poland) who afforded the possibility for collecting the plant material.

Bagci E. and Digrak M. (1996a), Antimicrobial activity of essential oils from trees. Turk. J. Biol. **20**, 191–198.

Bagci E. and Digrak M. (1996b), Antimicrobial activity of essential oils of some *Abies* species from Turkey. Flavour Fragr. J. **11**, 251–256.

Canillac N. and Monrey A. (1996), Sensitivity of *Listeria* to silver fir and maritime pine essential oils. Sci. Aliments **14**, 403–411

Chalchat J. C., Garry R. P. and Gorunovic M. S. (1994), Chemotaxonomy of Pines native to the Balkans: Composition of the essential oil of *Pinus heldreichii* Christ. Pharmazie **49**, 852–854.

Chalchat J. C. and Gorunovic M. S. (1995), Chemotaxonomy of pines native to the Balkans (IV): Variations in the composition of essential oils of *Pinus omorika* Paneie according to plant part and age of specimens. Pharmazie **50**, 640–641.

Dynowska M. (1998), Hialohyphomycosis of the shank caused by *Fusarium solani*. Mikol. Lek. **5**, 241–245

Dormont L., Roquest A. and Malosse C. (1998), Cone and foliage volatiles emitted by *Pinus cembra* and some related conifer species. Phytochemistry **49**, 1269–1277.

Hanover J. W. (1975), Comparative physiology of eastern and western white pines. Oleoresin composition and viscosity. For. Sci. **21**, 214–221.

Harborne J. B. and Baxter W. (2001), The Chemical Dictionary of Economic Plants. Wiley and Sons, Chichester, p. 582.

Krauze-Baranowska M. and Wiwart M. (2002), Antifungal activity of biflavones from *Taxus baccata* and *Ginkgo biloba.* Planta Med., submitted

Lis-Balchin M., Deans S. G. and Eaglesham E. (1998), Relationship between bioactivity and chemical composition of commercial essential oils. Flavour Fragr. J. **13**, 98–104.

Magiatis P., Melliou E., Skaltsounis A. L., Chinou I. and Mitaku S. (1999), Composition and antimicrobial activity of the essential oils of *Pistacia lentiscus var. chia.* In: Book of Abstracts: 2000 Years of Natural Products Research-Past, Present and Future. Leiden University, Amsterdam, p. 622.

Morris J., Khettry A. and Seitz E. W. (1979), Antimicrobial activity of aroma chemicals and essential oils. J. Am. Oil Chem. Soc. **56**, 595–603.

Niemann G. J. (1988), Distribution and evolution of the flavonoids in gymnosperms. In: The Flavonoids. Advances in Research since 1980 (Harborne J. B., ed.). Chapman and Hall, London, pp. 469–478.

Oh K. B., Chen Y., Matsuoka H., Yamamoto A. and Kurata H. (1996) Morphological recognition of fungal spore germination by a computer-aided image analysis and its application to antifungal activity evaluation. J. Biotechnol. **45**, 71–79.

Orav A., Kailas T. and Liiv M. (1996), Analysis of terpenoic composition of conifer needle oils by steam distillation/extraction, gas chromatography and gas chromatography-mass spectrometry. Chromatographia **43**, 215–219.

Pharmacopeia Polonica V, supplement I, Polskie Towarzystwo Farmaceutyczne, Warszawa (1995), p. 150.

Poole C. F. and Schütte S. A. (1984), Contemporary practice of chromatography. Elsevier, Amsterdam, p. 25.

Schäfer B., Henning P. and Engewald W. (1995), Analysis of monoterpenes from conifer needles using solid phase microextraction. J. High Res. Chromatogr. **18**, 587- 592.

Sjodin K., Persson M., Borg-Kalson A.-K. and Norin T. (1996), Enantiomeric compositions of monoterpene hydrocarbons in different tissues of four individuals of *Pinus silvestris.* Phytochemistry **41**, 439–445.

Stermitz F. R., Tawara J. N., Boeckl M., Pomeroy M., Foderaro T. A. and Todd F. G. (1994), Piperidine alkaloid content of *Picea* (spruce) and *Pinus* (pine). Phytochemistry **35**, 951–953.

Yatagai M. and Hong Y. (1997), Chemical composition of the essential oil of *Pinus massoniana* Lamb. J. Essent. Oil Res. **9**, 485–487.