

The Chemical Composition of *Microbiota decussata*

Mirosława Krauze-Baranowska^{a*}, Marek Mardarowicz^b and Marian Wiwart^c

^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

* Author for correspondence and reprint requests

Z. Naturforsch. **57c**, 998–1003 (2002); received April 30/July 15, 2002

Biflavones, Essential Oils, Antifungal Activity

From the leaves of *Microbiota decussata* (Cupressaceae) biflavones: cupressuflavone, amentoflavone and 7-O-methylamentoflavone were isolated and identified. The amount of cupressuflavone in *Microbiota decussata* and *xCupressocyparis leylandii* (Leyland cypress) (Cupressaceae) was determined by HPLC (1.82% and 0.83%, respectively). The chemical composition of essential oils from bark and leaves of *Microbiota decussata* was established by GC-MS (GC-FID) analysis. As a major component thujopsene (39.2% and 45.9%, respectively) was identified. Wiridiflorol (3.0%) and τ -muurolol (0.3%) were present only in leaves but globulol (1.5%) exclusively in bark. The content of essential oils in *M. decussata* was high – 5.4% in bark and 12.6% in leaves. The essential oils from *M. decussata* and *xC. leylandii* were bioassayed towards different fungi of the genus *Fusarium*. Leyland cypress essential oil at 2% concentration fully inhibited the growth of all fungi.

Introduction

As a part of our research on the family Cupressaceae (Krauze-Baranowska *et al.*, 1999; Mardarowicz *et al.*, 1998), biflavones and essential oils were investigated in the leaves and the bark of *Microbiota decussata* Kom. This species is a monoecious evergreen bush, which in natural habitat grows on the Sikhote-Alin mountain ridge (Russia) (Raldugin *et al.*, 1981; Tkachev *et al.*, 1991). *M. decussata* represents a monotypic genus of the Cupressaceae (Raldugin *et al.*, 1981; Tkachev *et al.*, 1991). The chemical composition of *M. decussata* is not entirely known. Tkachev *et al.* (1991) and Raldugin *et al.* (1981) isolated from the needles of *M. decussata* a number of sesquiterpene alcohols, among them microbiotol – a rarely occurring secondary metabolite in the plant kingdom. Besides the above mentioned sesquiterpene alcohol as a major constituent, in the essential oil from needles Melching *et al.* (1998) showed the presence of the sesquiterpene hydrocarbon – α -microbiotene.

Continuing the study on antifungal activity of essential oils (Krauze-Baranowska *et al.*, 2002) we have also bioassayed the oils hydrodistilled from *M. decussata* and *xCupressocyparis leylandii* (Dall. et Jacks.) (Leyland cypress) (Cupressaceae) (Mar-

darowicz *et al.*, 1999). The chemical composition of the latter was determined by Mardarowicz *et al.* (1999). Δ -3-Carene, sabinene and limonene were indicated as the main components in quantities higher than 15%.

Material and Methods

Plant material

The terminal branches of *Microbiota decussata* Kom. (Cupressaceae) were collected in the Medicinal Plant Garden of Medical University of Gdańsk (Poland) in October 1999. A voucher specimen of the plant (99–013) has been deposited at the Herbarium of the Department of Pharmacognosy, Medical University of Gdańsk (Poland).

Extraction and isolation of biflavones

The dried and pulverized leaves (60 g) were extracted successively with petroleum ether, chloroform and methanol in a Soxhlet apparatus. The isolation of biflavones was performed from the methanol extract by preparative column chromatography on Sephadex LH-20 column (25 g, 45 $\times$ 1.5 cm, MeOH) giving compound **1** (eluates 10–

15, 4 ml each) (150 mg). Compound **2** (8 mg) and compound **3** (5 mg) were purified by re-chromatography on Sephadex LH-20 column (10 g, 20 × 1.5 cm, MeOH) from eluates 16–19 and eluates 21–23, respectively.

Compound **1**: cupressuflavone. TLC R_f (1) – 0.28, HPTLC R_f (2) – 0.29, HPLC t_R – 13.9 min. UV λ_{max} MeOH nm: 276, 328; +AlCl₃: 281, 352; +AlCl₃/HCl: 281, 345; +CH₃ONa: 233, 285, 393; +CH₃COONa : 285, 342, 381; + CH₃COONa/H₃BO₃ 282, 332. LSI-MS (–) DTE/DTT (matrix) m/z (rel. int.): 537 [M-H][–] (11). ¹³C NMR (DMSO-d₆) δ : 182.4 (C-4,4''), 163.9 (C-2,2''), 162.8 (C-7,7''), 161.4 (C-5,5''), 161.2 (C-4,4'''), 155.2 (C-9,9''), 128.3 (C-2',6'2'',6'''), 121.5 (C-1',1'''), 116.2 (C-3',5', 3'',5'''), 104.1 (C-10,10''), 103.0 (C-3,3''), 98.8 (C-6,6''), 98.6 (C-8,8''). The tertiary carbons were revealed by a DEPT experiment.

Compound **2**: 7-O-methylamentoflavone TLC R_f (1) – 0.15, HPTLC R_f (2) – 0.10, HPLC t_R – 30.8 min. UV λ_{max} MeOH nm: 273, 326; +AlCl₃: 282, 303; +AlCl₃/HCl: 274, 301sh, 347, 380sh; +CH₃ONa: 288, 398; +CH₃COONa : 270, 287sh, 350; +CH₃COONa/H₃BO₃ 279, 331. EI-MS m/z (rel. int.): 552 [M]⁺ (100), 537 [M-15]⁺ (8), 167 (8), 121 (3). ¹H NMR (Table I). The position of the methoxyl group was elucidated by correlation spectra ¹H-¹H COSY and ROESY and C/H signals assignments were done by HMBC, HSQC experiments. ¹³C NMR (DMSO-d₆) δ : 182.7 (C-4''), 182.6 (C-4), 165.8 (C-7), 164.7 (C-2), 164.3 (C-2''), 162.7 (C-7''), 161.8 (C-4'''), 161.7 (C-5), 161.2 (C-5''), 160.4 (C-4'), 157.9 (C-9), 155.1 (C-9''), 132.1 (C-2'), 128.8 (C-2'',6'''), 128.6 (C-6'), 122.0 (C-1''), 121.4 (C-1'), 120.7 (C-3'), 116.9 (C-5'), 116.4 (C-3'',5'''), 105.3 (C-10), 104.7 (C-8''), 104.2 (C-10''), 103.7 (C-3), 103.2 (C-3''), 99.3 (C-6''), 98.7 (C-6), 93.3 (C-8), 56.7 (OCH₃).

Compound **3**: amentoflavone TLC R_f (1) – 0.06, HPTLC R_f (2) – 0.23, HPLC t_R – 20.7 min. UV λ_{max} MeOH nm: 270, 334; +AlCl₃: 281, 344, 381; +AlCl₃/HCl: 280, 350, 383; +CH₃ONa: 276, 382; +CH₃COONa : 275, 373; + CH₃COONa/H₃BO₃ 276, 325. EI-MS m/z (rel. int.): 538 [M]⁺ (100). ¹H NMR (Table I). The values of chemical shifts of protons H-3 and H-3'' were established using ¹H-¹H COSY (Krauze-Baranowska *et al.*, 1999) and ROESY experiments. ¹³C NMR data are in agreement with the literature data (Markham, 1982).

TLC was performed on polyamide 11 F₂₅₄ (Merck) and HPTLC RP-18 F₂₅₄ (Merck) plates using different mobile phases: 1-chloroform:methanol:methylethylketone (4:2:3 v/v/v) and 2-methanol:water:formic acid (70:30:6 v/v/v) (Krauze-Baranowska and Malinowska, 2002).

HPLC analysis was carried out on Spherisorb ODS II column (250 × 4 mm, 5 μ m, Merck) with mobile phase tetrahydrofuran:water:phosphoric acid (40:60:1 v/v/v), flow rate 0.8 ml/min., UV-330 nm (Krauze-Baranowska *et al.*, 1999). For the quantitative analysis of cupressuflavone, the leaves of *Microbiota decussata* (0.5 g) and *xCupressocyparis leylandii* (1.0 g) were preliminary purification with petroleum ether and chloroform in a Soxhlet apparatus. Biflavones were extracted from the plant material with methanol (100 ml) and injected.

Isolation of essential oils

Dried and pulverized leaves and bark (10 g) were hydrodistilled as described in the literature (Pharmacopea Polonica V, 1995). The obtained essential oils were kept in a sealed glass tube at 4 °C until analysis. The essential oil from leaves of *xCupressocyparis leylandii* was hydrodistilled by Mardarowicz *et al.* (1999) and kept under the above conditions until antifungal assay.

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 μ m 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 the procedure previously described by Krauze-Baranowska and Wiwart (2002) the essential oils were dissolved in MeOH and bioassayed in concentrations 2%, 5%, 10% (Morris *et al.*, 1979) towards *Fusarium culmorum* (W. G. Sm.) Sacc., *Fusarium poae* (Mart.) Appel. Wollenw. and *Fusarium solani* (Beck.) Wollenw. The last fungus was isolated directly from a patient with confirmed

hialohyphomycosis of the shank (Dynowska, 1998; Krauze-Baranowska *et al.*, 2002). Inhibitory effect on the growth of fungi was determined by measurements of the length of 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

From the methanolic extract from the leaves of *Microbiota decussata* the following biflavones, unknown earlier in this species, have been isolated and identified: cupressuflavone (**1**), 7-O-methylamentoflavone (**2**) and amentoflavone (**3**). Their structures were elucidated on the basis of UV, EI-MS, LSI-MS and by comparison of 1D, 2D NMR spectra- DEPT, ^1H - ^1H COSY, ROESY, HMBC, HSQC (Table I) with the literature data (Geiger and Markham, 1996; Krauze-Baranowska *et al.*, 1999; Markham, 1982; Wollenweber *et al.*, 1998). In the ^{13}C NMR spectrum of compound **1** two carbon signals with very similar values of chemical shifts at 98.8 and 98.6 ppm characteristic for non-substituted carbon in position C-6 of biflavone (Markham, 1982) were observed, that confirmed the presence of C8-C8' bond of biapigenin skeleton. Regarding the data of DEPT spectrum the above signals have been distinguished as not substituted carbon C-6 (98.8 ppm) and substituted

carbon in position C-8 (98.6 ppm). This observation is a new one against earlier reports (Markham, 1982; Wollenweber *et al.*, 1998), that demonstrated the downfield shift of the carbons C-8/C-8' of cupressuflavone below 100 ppm (between 102–104 ppm). On the other hand this is in agreement with values of chemical shifts of parallel carbon C-8/C-8' of 4'-O-methylcupressuflavone (Krauze-Baranowska *et al.*, 1999). According to Geiger and Markham (1996) the H-6 and H-8 signals in ^1H NMR spectrum of compound **2**, were relatively downfield shifted, what suggested the methylation in position C-7 of this biflavone. The structure of compound **2** was established as 7-O-methylamentoflavone by ^1H - ^1H COSY, ROESY spectra and heterocorrelation experiments HMBC, HSQC. The spectral data of compound **3** were identical with described previously (Krauze-Baranowska *et al.*, 1999; Markham, 1982; Wollenweber *et al.*, 1998) and confirmed its structure as amentoflavone.

The results of quantitative analysis by employing HPLC indicated, that high concentration of cupressuflavone in *M. decussata* – 1.82% and *Cupressocyparis leylandii* – 0.83% in contrary to a small amount of amentoflavone characterizes these both species. This interesting fact is contradictory to previous statements of Gadek and Quinn (1986), who describe amentoflavone as the basic chemotaxonomic marker for most species of the family Cupressaceae. Furthermore, it was shown that analyzed plants can be considered as a very rich source of cupressuflavone in plant kingdom.

The content of essential oils in the bark and the leaves of *M. decussata* was high and considerably variable from 5.4% to 12.6%, respectively. The chemical composition of the oils from *M. decussata* was elucidated employing GC with MS and FID detection. The identification of components was verified by comparison the mass spectra with previously published spectra and confirmed by comparison of temperature retention indices (Poole and Schütte, 1984) with the 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%

Table I. ^1H NMR data of the biflavones **1–3**, recorded in DMSO- d_6 at ambient temperature at 500 MHz. Assignments were made according to Markham (1982), and Wollenweber *et al.* (1999) and by results from ^1H - ^1H COSY, ROESY, HMBC, HSQC.

H	1	2	3
H-3	6.82s	6.91s	6.83s
H-6	6.48s	6.37d (2.2)	6.19s
H-8	–	6.77d (2.2)	6.45s
H-2'	7.51d (8.8)	8.04dd (8.7/2.4)	8.04s
H-3'	6.75d (8.8)	–	–
H-5'	6.75d (8.8)	7.14d (8.7)	7.12d (7.9)
H-6'	7.51d (8.8)	8.04dd (8.7/2.4)	7.99d (8.3)
H-3''	6.82s	6.80s	6.79s
H-6''	6.48s	6.39s	6.36s
H-8''	–	–	–
H-2''',6'''	7.51d (8.8)	7.56d (8.8)	7.58d (7.4)
H-3''',5'''	6.75d (8.8)	6.72d (8.8)	6.70d (7.3)
OH-4',4'''	10.32s	10.25s	10.25s
OH-5,5'''	13.18s	12.98, 13.10s	13.00, 13.12s
OH-7,7'''	10.85s	–, 10.60brs	10.80s
7-OMe	–	3.82s	–

Compound	RI	Essential oil	
		Leaves	Bark
α -Pinene	933	0.2	1.4
Camphene	946	tr	0.2
Sabinene	974	0.5	0.4
β -Pinene	976	tr	0.1
Myrcene	994	0.1	0.2
Δ^3 -Carene	1011	2.0	7.3
Limonene	1030	1.4	0.6
<i>Cis</i> -ocimene	1042	0.2	tr
<i>Trans</i> -ocimene	1051	0.1	tr
Terpinolene	1090	0.2	0.5
α -Terpinenyl acetate	1356	0.1	0.3
Isolongifolene*	1394	0.3	0.2
Italicene	1404	0.2	0.1
α -Cedrene	1420	1.3	1.1
β -Cedrene	1428	1.1	1.0
Thujopsene	1440	39.2	45.9
<i>Cis</i> - β -farnesene	1461	1.3	0.9
Thujopsadiene	1472	0.1	0.2
Similar with beta-chamigrene	1474	0.2	0.2
β -Chamigrene	1486	2.0	2.5
Unknown (M = 204) ^a	1488	4.2	1.8
Germacrene B	1503	0.1	0.1
α -Longipinene	1509	3.8	4.1
Unknown (M = 204) ^b	1513	1.0	1.2
Cuparene	1515	0.5	0.9
Unknown (M = 204) ^c	1523	0.6	0.6
δ -Cadinene	1531	0.8	0.9
<i>Trans</i> - γ -bisabolene	1539	1.4	1.0
Unknown (M = 204) ^d	1542	2.4	2.4
Hedycaryol*	1559	2.1	0.6
<i>Trans</i> -nerolidol	1570	0.2	0.2
4- β -Hydroxygermacra-1(10),5-diene	1586	2.1	0.3
Caryophyllene oxide	1592	1.7	0.5
Globulol	1596		1.5
Wiridiflorol	1599	3.0	
Unknown (M = 205) ^e	1599	1.0	1.4
Widdrol	1613	3.8	6.7
Cedrol	1615	2.6	2.2
Nerolidyl acetate*	1637	2.4	0.7
τ -Cadinol	1653	0.1	0.4
τ -Muurolol	1654	0.3	
δ -Cadinol	1659	0.1	tr
β -Eudesmol	1664	tr	0.2
α -Cadinol	1667	0.8	1.1
Unknown (M = 204) ^f	1678	0.6	0.5
Epi- α -bisabolol	1694	1.5	1.3
Abietatriene	2075	tr	0.2
<i>Trans</i> -totarol	2334	0.1	0.9

Table II. The percentage composition of essential oils from the leaves and bark of *Microbiota decussata*.

* Not confirmed by the values of RI; tr, trace
Mass spectral data of unknown compounds, number^a: 204(2), 189(10), 175(4), 161(15), 148(6), 147(7), 133(33), 123(49), 119(40), 111(56), 105(89), 95(55), 94(69), 93(62), 91(100), 81(69), 79(70), 69(84), 55(47), 41(82), number^b: 204(8), 189(1), 175(4), 161(2), 148(2), 136(50), 121(100), 105(33), 93(50), 91(40), 79(23), 77(16), 55(10), 41(29), number^c: 204 (15), 189 (5), 175(2), 161(39), 148(4), 147(4), 133(23), 123(43), 121(100), 119(36), 107(47), 105(61), 95(27), 93(48), 91(54), 81(36), 79(46), 67(30), 55(18), 41(46), number^d: 204 (9), 189 (3), 161(8), 148(5), 147(6), 133(19), 119(100), 107(23), 105(53), 93(45), 91(50), 79(17), 77 (18), 69(16), 55(26), 41(36), number^e: 205 (3), 161(4), 147(6), 140(100), 133(17), 123(30), 125(41), 109(31), 107(24), 105(23), 95(38), 93(26), 91(23), 81(41), 79(27), 67(30), 55(25), 43(67), 41(41), number^f: 204 (13), 189 (4), 161(10), 148(9), 147(6), 133(46), 121(37), 119(100), 105(66), 93(66), 91(53), 81 (16), 79(32), 77 (18), 69(11), 59(22), 55(20), 43(37), 41(33).

have been taken into consideration. About 48 constituents were determined, among them 41 were identified in the essential oil from leaves of *M. decussata* and 40 in the essential oil from bark. Moreover 18 components occurred in amounts over 1% in leaves and 15 in bark. The qualitative composition of both essential oils was similar, with

some exceptions-wiridiflorol (3.0%) and τ -muurolol (0.3%) were present only in leaves but globulol (1.5%) was revealed only in bark. The quantitative differences have been also observed in the following compounds: α -pinene (0.2% in leaves, 1.4% in bark), Δ^3 -carene (2.0% to 7.3%), widdrol (3.8% to 6.7%) were presented in higher concen-

trations in bark but limonene (1.4% to 0.6%), hedyacryol (2.1% to 0.6%), 4- β -hydroxygermacra-1(10),5-diene (2.1% to 0.3%), caryophyllene oxide (1.7% to 0.5%) and nerolidyl acetate (2.4% to 0.7%) were dominated in leaves. Sesquiterpene-thujopsene was identified as a major constituent of both essential oils in concentrations – 39.2% in leaves and 45.9% in bark (Table II). In contrary to previously reported data, an occurrence of microbitene (Melching *et al.*, 1998) and microbitol (Raldugin *et al.*, 1981; Tkachev *et al.*, 1991) in the analyzed essential oils from *M. decussata* have not been confirmed. Otherwise, in the investigated essential oils have been detected such sesquiterpene alcohols as α -bisabolol, hedyacryol and β -eudesmol, which were isolated earlier together with thujopsan-2 α -ol by Raldugin *et al.* (1981) from the needles of *M. decussata*, in the following concentrations: 3%, 47%, 0.5%, 35%. The contents determined by us of hedyacryol and α -bisabolol in epi-form varied between 2.1%–0.6% and 1.5%–1.2% respectively, but β -eudesmol appeared in a 0.2% concentration in the bark and as trace in leaves (Table II). It is worth to notice that Raldugin *et al.* (1981) suggested the possibility of a partial nonenzymatic dehydration of thujopsan-2 α -ol to thujopsene, what could explain the high content of the latter in the essential oils from *M. decussata*. Nevertheless, the obtained results can also lead to the conclusion, that different chemical types occur within the genus *Microbiota*.

The antifungal activity of the essential oils from *M. decussata* and *xC. leylandii* was compared. Inhibitory effect of the investigated oils on the growth of fungi in liquid medium was estimated using a computer-aided image analysis coupled to

a microscope (Oh *et al.*, 1996). Bioassays were performed towards three genus of fungi from the species *Fusarium-F. solani*, *F. poae* and *F. culmorum*. Surprisingly, the analyzed essential oils showed significant diversity regarding antifungal activity. The oil from the leaves of *xC. leylandii* at concentration 2% fully inhibited the growth of germinating tubes of all fungi (Table III). The two essential oils from *M. decussata*, at the same concentration, showed poor-moderate (30–50% inhibition), moderate (50–70% inhibition) or good (above 70% inhibition) antifungal activity, depending on the species of fungus (Table III). It is interesting, that such different effects of fungicidal action of oils from *M. decussata* and *xC. leylandii* can be explained by significant differences in chemical composition. In the oil from *xC. leylandii* monoterpene hydrocarbons (ca. 58%) were present as the main components: Δ -3-carene, limonene, sabinene and α -pinene (Mardarowicz *et al.*, 1998) whereas in the oils of *M. decussata* sesquiterpene hydrocarbone- thujopsene was determined. The above results are in agreement with our earlier report (Krauze-Baranowska *et al.*, 2002), and confirm the antifungal activity of monoterpene hydrocarbons in contrast to less active sesquiterpene. Previously, Lis-Balchin *et al.* (1998) reported that sesquiterpenes of essential oils are inactive against fungi. In spite of the very close content of thujopsene (39.2% to 45.9%) some differences in antifungal activity of essential oils from leaves and bark of *M. decussata* have been revealed. The antifungal activity was similar towards *Fusarium poae* (79.8%, 72.4% inhibition) but different towards *F. solani* and *F. culmorum*. The essential oil from leaves was stronger towards *F. solani* (95.8% inhi-

Table III. Antifungal activity of essential oils from *Microbiota decussata* and *Cupressocyparis leylandii* towards fungi from the genus *Fusarium*.

Essential oil	Antifungal activity		
	<i>Fusarium solani</i>	<i>Fusarium poae</i>	<i>Fusarium culmorum</i>
<i>Microbiota decussata</i>			
leaves	95.8 $\pm$ 7.6	79.8 $\pm$ 4.5	37.4 $\pm$ 5.2
bark	68.7 $\pm$ 8.2	72.4 $\pm$ 6.3	50.4 $\pm$ 12.6*
<i>Cupressocyparis leylandii</i>			
leaves	100	100	100

Antifungal activity is estimated as % inhibition of growth of germ tubes, mean $\pm$ SEM at a 2% concentration of essential oil in the mixture methanol/liquid medium (18:82 v/v) and significant difference from the control (methanol/liquid medium 20:80 v/v) is $p < 0.01$, * $p < 0.05$.

bition) than hydrodistillated oil from the bark (68.7% inhibition) (Table III). Nevertheless, the latter had slightly higher activity (50.4% inhibition) against *F. culmorum* than the essential oil from leaves (37.4% inhibition). The above difference can indicate that specific sensitivity of the genus of fungus towards thujopsene is possible. On the other hand, the observed diversity can be linked to the presence wiridiflorol in the oil from leaves

or globulol in the oil from bark, although the effect of some other constituents present in amounts comparatively higher in the oil from leaves or in the oil from bark can not be excluded (Table III).

Acknowledgments

This study was partially supported by KBN grant No 4P05F00918.

- Dynowska M. (1998), Hialohyphomycosis of the shank caused by *Fusarium solani*. Mikol. Lek. **5**, 241–245.
- Gadek A. P. and Quinn C. J. (1986), Biflavones from the family Cupressoideae, Cupressaceae. Phytochemistry **24**, 267–272.
- Geiger H. and Markham K. R. (1996), The ¹H-NMR spectra of the biflavones isocryptomerin and cryptomerin B – a critical comment on two recent publications on the biflavone patterns of *Selaginella selaginoides* and *S. denticulata*. Z. Naturforsch. **51c**, 757–758.
- Krauze-Baranowska M., Cisowski W., Wiwart M. and Madziar B. (1999), Antifungal biflavones from *Cupressocyparis leylandii*. Planta Med. **60**, 572–574.
- Krauze-Baranowska M., Mardarowicz M., Wiwart M., Pobłocka L. and Dynowska M. (2002), Antifungal activity of the essential oils from some species of the genus *Pinus*. Z. Naturforsch. **57c**, in press.
- Krauze-Baranowska M. and Malinowska I. (2002), TLC analysis of flavonoids from some species of Gymnospermae. J. Planar Chromatogr. 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.
- Markham K. R. (1982), Techniques of Identification of Flavonoids. Academic Press, London.
- Mardarowicz M., Cisowski W., Krauze-Baranowska M. and Migas P. (1999), Chemical composition of Leyland Cypress essential oils. J. Essent. Oil Res. **11**, 9–12.
- Melching S., Blume A., König W. A. and Mühle H. (1998), Natural occurrence of α - and β -microbiotene. Phytochemistry **48**, 661–664.
- 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.
- 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.
- Pharmacopea 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.
- Raldugin V. A., Storochenko V. G., Resvuchin A. I., Pentegova V. A., Gorovoj P. G. and Baranov V. I. (1981), Terpenoidy iz rastenij sem. Cupressaceae. Khim. Prir. Soedin. **2**, 163–169.
- Tkachev A. V., Sharikov M. M. and Raldugin V. A. (1991), Structure of microbitol, a new sesquiterpene alcohol from needles of *Microbiota decussata*. J. Nat. Prod. **54**, 849–853.
- Wollenweber E., Kraut L. and Mues R. (1998), External accumulation of biflavonoids on gymnosperm leaves. Z. Naturforsch. **53c**, 946–950.