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Composition of biologically active lipids of *Lamiaceae* seed oils

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RESUMEN

Composición de lípidos biológicamente activos de aceites de semilla *Lamiaceae*.

Se estudia el contenido en aceite, fosfolípidos, esteroles y tocoferol de semillas pertenecientes a 7 especies de la Familia *Lamiaceae.* Se encontraron valores comprendidos entre 8.7-28.6% para los aceites en las semillas. El contenido en fosfolípidos para los aceites fue del 1.0-1.6%. Los principales componentes de la fracción fosfolípido fueron: fosfatidilcolina (35.5-63.1%), fosfatidilinositol (19.1-30.2%) y fosfatidiletanolamina (5.8-21.6%). En la fracción de esteroles (0.1-0.3% de esteroles totales en las muestras de aceites), 64.8-86.3% estaban presentes en forma libre y 13.7-35.2% como esteres de esteroles. El β-Sitosterol es el que predomina (48.8-87.9%), seguido de campesterol (1.5-22.5%) y stigmasterol (1.1-12.8%). Se encontró un contenido en tocoferoles de 6.3-649.7 mg/kg, principalmente α-tocopherol (48.5-99.9%).

PALABRAS-CLAVE: Aceite de semilla -- Composición lipídica –Lamiaceae.

SUMMARY

Composition of biologically active lipids of *lamiaceae* seed oils.

The content of glyceride oil, phospholipid, sterol and tocopherol composition of 7 species of fam. *Lamiaceae* seeds were investigated. 8.7-28.6% of glyceride oil in the seeds were determined. The content of phospholipids in the oils was found to be 1.0-1.6%. Phosphatidylcholine (35.5-63.1%), phosphatidylinositol (19.1-30.2%) and phosphatidylethanolamine (5.8-21.6%) were the main components in the phospholipid fraction. In the sterol fraction (0.1-0.3% total sterols in the oils) 64.8-86.3% are in free form and 13.7-35.2% as sterol esters. β-Sitosterol predominates in all glyceride oils (48.8-87.9%), followed by campesterol (1.5-22.5%) and stigmasterol (1.1-12.8%). 6.3-649.7 mg/kg tocopherols were found, mainly α-tocopherol (48.5-99.9%).

KEY-WORDS: Lamiaceae -- Lipid composition -- Seed oil.

1. INTRODUCTION

Botanical family *Lamiaceae (Labiatae*) includes many species of plants, which are widespread in the temperate areas of the Northern terrestrial globe. A characteristic feature of the representatives of this family is that, besides the glyceride oils, the seeds contain essential oils with condiment (Pamukov D. and C. Ahtaradjiev, 1990) or pharmacological (Sokolov S. and J. Zamotaev, 1984; Weiss R, 1974, Slavik B. and V. Choe, 1978) character. Some of them are cultivated and used as a source for obtaining honey, essential oils such as limonen, geraniol, menthol, citrol etc., for perfumery and cosmetics (Georgiev E., 1989). The seeds and leaves are used as food condiments in the production of beer, wine, liquors, for aromatization of sausages, meat, salads, cheese etc. (Stojanov and Kitanov, 1960). In the recent years investigations of the content and composition of the essential oils were carried out, but information about the composition of glyceride oil is scanty. It was not found information about content and composition of biological active substances such as phospholipids, sterols and tocopherols.

The data about the application of some representatives of family *Lamiaceae* in perfumery, confectionery and food industry directed our interest to some introduced but unstudied species. This work presents the results from comparative investigations of the content of glyceride oil in the seeds, sterol and tocopherol content and composition of phospholipid fraction of 7 species of *Lamiaceae* family which are:

1. *Leonurus cardiaca L.*
2. *Marrubium vulgare L.*
3. *Mentha spicata L.*
4. *Nepeta cataria L.*
5. *Salvia aethopis L.*
6. *Salvia nutans L.*
7. *Salvia verticalata L.*

2. MATERIAL AND METHODS

Fruit material. The investigated seeds were provided from the Plovdiv region in South Bulgaria. Botanical affiliation was established by routine methods (Gramatikov, 1992). The investigations were carried out on air dried seeds in technical ripeness.

Glyceride oil isolation. The oils were extracted in Soxhlet apparatus with fresh distilled n-hexane for 8 h. After rotation vacuum distillation of the solvent the extracted oils were dried and weighed (Ivanov and Aitzetmüller, 1995).

Phospholipid composition. Lipids were extracted from the seeds by Folch procedure (Beshkov and Ivanova, 1972). Polar lipids were separated from unpolar lipids by column chromatography. The

phospholipid constituents were separated by two - directional thin - layer chromatography on Silica gel 60 G "Merck", impregnated with 1% $(NH_4)_2SO_4$ water solution (Beshkov and Ivanova, 1972). The first direction was carried out in chloroform : methanol: ammonia 65: 25: 5 v/v/v and second in chloroform: methanol: ammonia: acetic acid : water 50:20:10:10:5 v/v/v/v/v. The spots of the separated individual phospholipids were identified by spraying with specific reagents (Kates, 1972). In addition, R_F and standard spots were used for definitive identification. The quantitative evaluation was carried out spectrophotometrically at 700 nm (Beshkov and Ivanova, 1972).

Sterol composition. The free sterols and sterol esters were separated from the other oil constituents by preparative TLC on Silica gel 60 G "Merck" and mobile phase n-hexane : diethyl ether 1:1. The sterol esters were saponified with ethanolic KOH, extracted and purified by TLC. The content and individual composition of sterols was carried out by gas chromatography using HP 5890 A unit with FID, 25 m 0.25 mm ID (internal diameter), 0.25m Fused Silica film thickness capillar column impregnated with 3% OV-17 and conditions as follows:

column temperature programme -260 to 300^{0}C, at 6^{0}C/min

detector temperature 320^{0}C, injector temperature 300^{0}C

gas carrier - nitrogen, 20 cm^3/min

Betulin was used as internal standard for quantitative evaluation of sterols (ISO 12228, 1999). Identification was confirmed by comparing the retention time of the individual constituents with that of standard solution containing cholesterol, campesterol, stigmasterol and sitosterol (Homberg and Bielefeld, 1989).

Tocopherol composition. Tocopherols and tocotrienols were analysed directly in the oils by HPLC with fluorescence detection (ISO, 1989, Ivanov and Aitzetmuller, 1995). "Merck-Hitachi" unit fitted with column "Nucleosil" Si 50 - 5μm, 250x4 mm and fluorescent detector "Merck-Hitachi" F 1000 was used. The operating conditions were as follows: excitation wavelength set at 295 nm, emission wavelength at 330 nm, mobile phase n-hexane : dioxane 94: 4, rate of mobile phase 1 cm^3/min. The peaks were identified by using authentic individual tocopherol standards provided by “Merck”.

3. RESULTS AND DISCUSSION

Data on the composition of the investigated plants are presented in Table I which shows that the seeds of *Lamiacae* family contain significant amounts of glyceride oil. Except for *Nepeta cataria L.* the investigated seeds of *Lamiacae* family contain more than 20.0% of glyceride oil. The oils also have a high content of phospholipids (1.0- 1.6 %).These values are closed to date about other wild plants (Ivanov and Aitzetmüller, 1995).

The amount of sterols and tocopherols in the oils of this family was lower then other plants used as condiments 0.1 - 0.3% and 14.7 - 649.7 mg/kg respectively, as compared with 0.2-0.7% sterols (Zlatanov and Ivanov,1995) and 234.1-1550.9 mg/kg tocopherols (Ivanov and Aitzetmuller, 1995) in Apiaceae seed oils. Relatively high levels of tocopherols were determined in *Mentha spicata L.* seed oil, 649.7 mg/kg.

The qualitative and quantitative phospholipid composition of the investigated oils is presented in Table II.

Table I

Content of glyceride oil in *Lamiaceae* seeds and phospholipids, sterols and tocopherols in the oils*

Botanical name	Content of oil in seeds, % wt	Content of phospholipids in oils, % wt	Content of sterols in oils, % wt	Content of tocopherols in oils, mg/kg
1. *Leonurus cardiaca L*.	26.5	1.0	0.1	176.9
2. *Marrubium vulgare L.*	24.4	1.3	0.3	14.7
3. *Mentha spicata L.*	21.9	1.4	0.2	649.7
4. *Nepeta cataria L.*	15.7	1.6	0.1	6.3
5.*Salvia aethopis L.*	28.6	1.3	0.1	18.2
6. *Salvia nutans L.*	27.4	1.2	0.1	97.8
7. *Salvia verticalata L.*	21.7	1.3	0.1	63.7

*Average of three determinations

Table II
Phospholipid composition of *Lamiaceae* seed oils*

Botanical name	Content (% wt)								
	PC	PE	PI	PA	LPC	LPE	MPG	DPG	Oth.
1. *Leonurus cardiaca L*.	55.5	6.6	27.9	2.2	0.3	2.2	3.6	0.3	1.4
2. *Marrubium vulgare L.*	47.3	18.0	27.2	3.3	0.9	1.1	0.7	0.5	1.0
3. *Mentha spicata L.*	45.0	24.6	19.1	4.7	1.3	2.0	2.1	1.2	-
4. *Nepeta cataria L.*	46.5	9.8	30.2	6.1	1.6	1.6	4.0	0.2	-
5. *Salvia aethopis L*.	50.0	16.1	29.4	2.1	-	0.3	0.9	0.6	0.6
6. *Salvia nutans L.*	35.5	5.8	27.0	16.8	1.6	3.1	3.1	3.9	3.2
7. *Salvia verticalata L.*	45.9	21.6	22.6	3.7	-	2.6	0.7	2.9	-

PC - Phosphatidylcholine, PE - Phosphatidylethanolamine, PI - Phosphatidylinositol, PA - Phophatidic acids, LPC -Lysophosphatidylcholine, LPE - Lysophosphatidylethanolamine, MPG - Monophosphatidylglycerol , DPG - Diphosphatidylglycerol, Oth. - Others.
* Average of three determinations

The obtained results show that the qualitative phospholipid composition is varied. All major classes were observed in the seeds. Phosphatidylcholine was the main component (35.5 - 55.5%) in all phospholipid fractions, followed by phosphatidylinositol (19.1 - 30.2%). In the four species of oils the content of phosphatidylcholine was found to be 50% above. It was significantly higher than in the other investigated oils, like *Apiaceae* (Ivanov et al, 1999) and *Rosaceae* family (Zlatanov et al, 1997) where 25.0 - 35.0% of phosphatidylcholine was established. On the other hand, the content of phosphatidylethanolamine (5.8 - 24.6%) was considerably lower in comparison with the quantity of above mentioned oils. In all phospholipid fractions were detected high percentages of phosphatidylinositol. The other phospholipids are presented in negligible quantities.

The content of the free and esterified sterols is given in Table III. The greatest part of them is in the free form (73.5 - 86.3%). Individual sterol composition was presented in Table IV. β-sitosterol predominates in all sterol fractions, followed by campesterol. Brassicasterol

Table III
Ratio of free and esterified sterols in *Lamiaceae* glyceride oils*

Botanical name	Content of free sterols in sterol fraction, % wt	Content of ester. sterols in sterol fraction, % wt
1. *Leonurus cardiaca L*.	79.7	20.3
2. *Marrubium vulgare L.*	80.2	19.8
3. *Mentha spicata L.*	74.5	25.5
4. *Nepeta cataria L.*	73.5	26.5
5. *Salvia aethopis L*.	68.6	31.4
6. *Salvia nutans L.*	64.8	35.2
7. *Salvia verticalata L.*	86.3	13.7

*Average of three determinations

Table IV
Sterol composition of *Lamiaceae* seed oils*

Botanical name	Content (% wt)																			
	Cholesterol		Brassicasterol		Campesterol		Stigmasterol		Δ^7 Campesterol		β-Sitosterol		Δ^5-Avenasterol		Δ^7-Stigmasterol		Δ^7-Avenasterol		$\Delta^{7,25}$-Stigmasterol	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1. *Leonurus car. L.*	0.7	2.1	1.1	2.7	1.5	12.3	6.7	6.2	-	1.1	76.2	53.3	6.5	16.0	3.1	4.9	4.2	1.6	-	-
2. *Marrubium vul. L.*	0.6	5.5	2.0	4.7	14.3	21.8	7.9	11.3	2.1	3.1	69.9	48.8	0.5	1.5	0.7	1.5	2.0	1.8	-	-
3. *Mentha spic. L.*	0.3	1.6	0.6	1.7	6.7	11.0	2.5	3.7	-	1.2	87.9	78.4	0.3	0.4	0.4	1.0	0.6	1.0	0.3	-
4. *Nepeta cat. L.*	0.6	3.6	11.2	12.7	4.5	6.8	2.3	2.3	-	3.1	73.9	56.4	7.3	8.4	-	4.2	-	1.4	0.2	1.5
5. *Salvia aeth. L.*	0.5	2.6	3.6	14.0	13.4	7.1	4.1	1.1	-	-	74.9	65.1	2.3	5.4	-	0.4	-	3.6	0.6	1.3
6. *Salvia nut. L.*	0.2	0.4	0.8	2.0	16.7	22.5	10.0	3.7		-	71.0	68.5	1.4	2.3	-	0.2	-	-	0.1	0.2
7. *Salvia ver. L.*	0.2	3.6	3.6	8.1	10.3	12.2	12.8	1.7	-	-	67.0	57.6	1.8	5.1	1.4	4.1	1.1	2.9	1.8	4.9

1. Free sterols
2. Esterified sterols
* Average of three determinations

Table V
Tocopherol composition of *Lamiaceae* seed oils*

Botanical name	Content (% wt)						
	α	α-3	β	γ	γ-3	δ	δ-3
1. *Leonurus cardiaca L.*	5.1	8.7	-	13.5	1.5	70.5	0.7
2. *Marrubium vulgare L.*	48.5	9.4	tr.	35.9	6.2	-	
3. *Mentha spicata L.*	98.5	-	-	-	-	1.5	-
4. *Nepeta cataria L .*	68.0	11.8	-	-	-	20.2	-
5.*Salvia aethopis L.*	99.9	0.1	-	-	-	-	-
6. *Salvia nutans L.*	51.6	-	0.6	24.9	17.1	15.8	-
7. *Salvia verticalata L.*	57.8	1.3	1.1	39.8	-	-	-

*Average of three determinations

was detected in significant amount in *Nepeta cataria L*. oil only (11.2% in free form and 12.7% in sterol esters). High level of stigmasterol derivatives (11.3%) was determined in esterified sterols of *Marrubium vulgare L*. and in free form in *Salvia nutans L.* and *Salvia verticalata L.* seed oils. High percentage of Δ^5-avenasterol (16.0%) was found in sterol esters of *Leonurus cardiaca L*. seed oil. A marked difference was detected between free and esterified sterols in the cholesterol content. In the fraction of sterol esters a significantly higher cholesterol percentage was observed (0.4 - 5.5% respectively) than in the free form (0.2 - 0.5%). Similar results were reported earlier about glyceride oils of *Apiaceae* (Zlatanov M., 1994, Zlatanov M. et al, 1997) and for tomato seed oils (Kiosseoglou and Boskou, 1989, Tiscornia et al, 1976). The content of β-sitosterol in free form (67.0-87.9%) is higher than in esterified form (48.8-74.4%). Other sterol constituents were presented in insignificant quantities or in traces in all investigated oils.

Tocopherol and tocotrienol composition of the oils is shown in Table V. Tocopherols were found to be the major part in all the tocopherol and tocotrienol fractions: the ratio tocopherols: tocotrienols varies from 100: 0,1 to 88.2: 11.8. α-tocopherol is the predominant component in almost seed oils (48.5 - 99.5%) followed by γ-tocopherol. Only in the *Leonurus cardiaca L.* oil δ-tocopherol was found to be the main component (70.5%). All other tocopherols and tocotrienols have been detected in negligible amounts.

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