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LIPIDS FROM *Mediasia macrophylla* LEAVES

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Lipids from Mediasia macrophylla *leaves were studied. Classes of neutral, glyco-, and phospholipids and their fatty-acid composition were determined. The content of chlorophyll and carotinoid pigments in the leaves and lipid extract was found. Essential oil was isolated by steam distillation and contained free and bound fatty acids.*

Key words: *Mediasia macrophylla*, neutral lipids, glyco- and phospholipids, pigments, essential oil.

Mediasia macrophylla (Regel et Schmalch.) M. Pimen (large-leaved mediasia) (Apiaceae) (celeries) is a perennial herbaceous plant that is used for food, spice, medicinal, and feed purposes [1, 2]. It is used to treat rheumatic abscesses, kidney inflammation, eczema, and herpes and to heal wounds and boils.

Flavonoids (quercetin and quercetin 7-O-β-D-galactopyranoside) were observed in fruit of mediasia [3].

Various organs of this plant contain essential oil (EO). The content is highest during bud formation. The EO content in freshly collected leaves was 0.89%; in stems and fruit, 0.46 and 0.42%, respectively [4, 5].

The composition of EO from *M. macrophylla* has been established [6]. A total of 33 compounds was found. The principal ones are *p*-cymene (27.2%), thymol (15.1%), and carvacrol (12.5%). Palmitic acid (2.9% of EO mass) was also found.

Lipids of mediasia leaves have not been previously studied. Only data on the fatty acid content of its seeds have been published [7].

We studied the lipids in leaves. Raw material of moisture content 11.2% was extracted by $CHCl_3$—CH_3OH (2:1, v/v). Nonlipid components were removed by treatment with aqueous $CaCl_2$ (0.04%). The remaining extract consisted of 5.7% of the absolute dry mass of leaves. Chlorophyll pigments were removed by passing the extract over a column packed with activated carbon and Celite [8]. The yield of lipids purified from pigments was 4.87% of the leaf mass. Lipids were separated into neutral (NL), glyco- (GL), and phospholipids (PL) by column chromatography over silica gel with elution successively by $CHCl_3$, $(CH_3)_2CO$, and CH_3OH. Their yield was 42.9, 44.1, and 13.0%, respectively, of the lipid mass. PL were analyzed by two-dimensional TLC on silica gel using systems 1 and 2. Four PL were observed: phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylcholine (PC), and phosphatidylethanolamine (PE), with PC and PA dominating.

The GL were separated by TLC using systems 3-5 and authentic sterolglucoside and digalactosyldiglyceride. The following GL were observed: sulfoquinovosyldiglycerides, digalactosyldiglycerides, cerebrosides, sterolglucosides, monogalactosylglycerides, and sterolglucoside esters. A spot with R_f 0.16, corresponding to galactose, was found after developing the glycolipid chromatograms with *o*-toluidinesalicylate in system 5.

The identification of the separate NL classes was difficult because of the presence of EO components. These had R_f values equal to those of NL. Therefore, the NL, EO, and unsaponifiable substances (US) isolated from the total lipids of mediasia were compared by TLC analysis on silica gel.

The US were isolated by the literature method [9]. Their content in the total lipids was 23.8%. The US contain 566.1 mg% carotinoids according to colorimetric analysis.

The following lipid classes were identified in the NL using TLC and systems 6-8: hydrocarbons, triacylglycerides, tocopherols, free fatty acids (FFA), triterpenes, and sterols.

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TABLE 1. Fatty-Acid Composition of Lipids from *M. macrophylla* Leaves, GC, % of Mass

Lipids	Fatty acids												
	12:0	13:0	14:0	15:0	16:0	17:0	18:0	16:1	18:1	18:2	18:3	Σ_{sat}	Σ_{unsat}
NL	0.5	Tr.	3.4	1.3	24.7	1.3	7.0	3.7	7.3	22.0	28.8	38.2	61.8
GL	0.6	1.1	10.4	1.4	36.6	1.7	5.2	1.1	7.7	11.5	22.7	57.0	43.0
PL	0.3	Tr.	0.9	0.6	43.1	2.1	2.3	2.9	6.7	15.6	25.5	49.3	50.7

TABLE 2. Pigment Content in *M. macrophylla* Leaves and Lipid Extract

Pigment content	Chlorophyll		Carotinoids
	"a"	"b"	
In leaves, mg/g	1.08	0.34	0.42
In lipid extract, mg/g	0.21	0.14	0.06
Transfered from leaves to lipid extract, %	19.4	41.2	14.28

TABLE 3. FFA and BFA Composition of *M. macrophylla* Essential Oil, GC, % of Mass

Acids												
10:0	11:0	12:0	13:0	14:0	15:0	16:0	17:0	18:0	16:1	18:1	$\sum_{sat}$	$\sum_{unsat}$
Free												
2.4	2.6	1.2	2.5	7.4	19.7	20.9	12.3	3.7	14.8	12.5	72.7	27.3
Bound												
5.2	10.1	2.5	2.9	4.3	10.6	6.2	18.1	7.4	14.0	18.7	67.3	32.7

The fatty-acid composition of NL, GL, and PL was determined using the methyl esters and GC (Table 1). It can be seen that saturated acids, mainly 16:0, dominate the GL and PL whereas 14:0 (10.4%) also appears in the GL. In NL, 16:0, 18:2, and 18:3 dominate.

The pigment composition of mediasia leaves was determined qualitatively by TLC on silica gel with $CaCO_3$ (1:1) using system 9 [10]. Two yellow spots with R_f values 0.39 and 0.99 were found. These correspond to α- and β,β'-carotenes. A green spot with R_f 0.46 and a grayish-blue one with R_f 0.71, which we assigned to chlorophylls "b" and "a", were also found.

The pigment content was determined spectrometrically both in leaves and the lipid extract after removing nonlipid components [11] (Table 2). Only part of the pigments transfers into the lipid extract of mediasia leaves. It should be noted that mainly chlorophyll "b" transfers into the extract although three times more chlorophyll "a" is present in the starting leaves.

The EO was isolated by steam distillation from mediasia leaves stored for six months. Its content during storage diminished from 0.83 to 0.30%. A light yellow oil with a pleasant characteristic aroma was obtained. The following properties were determined: acid number, 5.4 mg KOH; d_{20}, 0.9993; η_n^{20}, 1.5123.

The EO of mediasia leaves was treated with aqueous Na_2CO_3 (5%) in order to isolate FFA from it [12]. The yield of FFA was 2.1%. Bound fatty acids (BFA) were isolated from the remaining EO after extraction of FFA.

Low-molecular-weight acids of the FFA and BFA fractions were analyzed as ammonium salts by TLC on cellulose using system 10. Two acids were found: acetic and pelargonic. Table 3 gives the FFA and BFA composition of the EO and shows that fatty acids in the FFA are more saturated than in the BFA. The qualitative compositions of the FFA and BFA are identical. Of 11 fatty acids, 9 are saturated and only 2 unsaturated.

EXPERIMENTAL

GC was performed on a Chrom-5 instrument using a column packed with 5% Reoplex on N-AW, a thermostat temperature 190°C, and a N_2 flow rate 30 mL/min.

UV spectra were recorded on a Perkin—Elmer Lambda-16 instrument in hexane and acetone.

TLC was performed on silica gel and silufol plates using the following solvent systems: $CHCl_3$—CH_3OH—NH_3 (7 N) (65:30:4) (1), $CHCl_3$—CH_3OH—CH_3CO_2H—H_2O (170:25:25:6) (2), $CHCl_3$—$(CH_3)_2CO$—CH_3OH—CH_3CO_2H—H_2O (65:20:10:10:3) (3), $(CH_3)_2CO$—C_6H_6—H_2O (91:30:8) (4), $CHCl_3$—CH_3OH—CH_3O_2H—H_2O (170:30:20:7) (5), hexane—diethyl ether (7:3) (6) and (1:1) (7), heptane—MEK—CH_3CO_2H (43:7:1) (8), petroleum ether—$(CH_3)_2CO$—C_6H_6—*i*-PrOH (69.5:25:4:1.5) (9), and PrOH—NH_3 (7 N)—H_2O (9:1:2) (10).

EO from leaves was obtained by the Clevenger method in 3 h [13].

FFA and BFA were isolated from EO (0.8-1.5 g) by treating it three times with aqueous Na_2CO_3 (5%). After shaking and settling, the lower aqueous layer was removed. The aqueous fractions were combined and treated with H_2SO_4 solution (10%) to decompose fatty-acid salts. Fatty acids were isolated using diethyl ether, washing the solution with distilled water until neutral to methyl orange. The EO remaining after isolation of fatty acids was saponified with alcoholic KOH (10%). Unsaponifiable components were repeatedly extracted with ether. The products were treated and analyzed as FFA. The fatty-acid compositions of FFA and BFA were determined by GC.

Components of the isolated substances were identified using qualitative reactions, chromatographic mobilities in an adsorbent layer, and comparison with model specimens. The NL were developed with iodine vapor and H_2SO_4 (50%); GL, α-naphthalene and H_2SO_4 (50%); PL, Vaskovsky and Dragendorff's reagents and ninhydrin.

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