Intraspecific variability of little-leaved linden (*Tilia cordata* Mill.) occurring in Eastern Poland

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INTRODUCTION

Little-leaved linden is considered as a valuable medicinal plant. In Poland, the species grows wild on natural sites in hail forests (*Tilio-Carpinetum*) but it is also often planted along the streets, in parks and gardens (1). Raw materials obtained from this plant (included in European Pharmacopoeia) are flowers rich in flavonoids and mucus (2), containing also a small amount of volatile compounds responsible for its specific flavor (3,4,5). Flowers reveal diaphoretic, anti-inflammatory and sedative activities and could be used in cold and respiratory diseases treatment (6). Due to increasing demand on this herbal raw material, the evaluation of its quality including chemical variability of wild growing populations seem to be important.

The aim of presented investigation was to determine intraspecific variability of little-leaved linden occurring on natural sites in Poland, concerning the composition of essential oil in flowers.

Photo 1. Little-leaved linden on natural site in Witryłów

Photo 2. Little-leaved linden on natural site in Strachocina

MATERIAL AND METHODS

In 2012, the raw material was collected from trees growing wild on 22 natural sites located in eastern area of Poland. Geographical location of these sites was determined using GPS apparatus (Table 1, Figure 1). The harvest of flowers took place in July, during its full blooming time. Collected raw material was dried in 35 °C and subjected to chemical analysis. The essential oil used for quantitative analysis was obtained by hydrodestilation method (with xylene addition), according to Polish Pharmacopeia VIII. Its composition was determined by GC and GC/MS methods. GC analyses were performed using a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and capillary, polar column HP 20M. The following temperature programme was used: oven temperature isotherm at 60 °C for 2 min., then it was programmed from 60 °C to 220°C at a rate of 4 °C min-1 and held isothermal at 220 °C for 5 min. Essential oil compounds identification was done by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (RI) relative to the series of n-hydrocarbons (C7-C30), under the same operating conditions. Retention indices of compounds were also compared with those reported in the literature. The GC/MS analyses were carried out using Shimadzu GC MS QP210S gas chromatograph equipped with Phenomenex Zebron ZBFFAP polar column. The operating conditions were as follows: oven temperature 2 min. isothermal at 60°C, then rising at 4°C/min to 210 °C and held isothermal for 5 min. Injector and detector temperatures: 210 °C and 280 °C, respectively. Ion source temperature -220°C, ionization voltage 70 eV. Mass spectra were scanned in the range 40-500 amu. Essential oil compounds identification was based on comparison of GC retention indices relative to retention times of a series of n-hydrocarbons (C7–C30) with those reported in literature and by comparison of mass spectra from the Mass Spectral Database, as following: NIST08, NIST27, NIST147, Wiley7N2, PAL 600K. The percentage composition of the oils was computed by the normalization method from the GC peak areas, without the use of correction factors.

RESULTS

Obtained results indicate that little-leaved linden flowers from investigated populations differed significantly in respect of essential oil composition. Among 23 compounds identified in the essentials oil, the dominants were: nonanoic acid, nonanal and acetophenone. The percentage of this compounds ranged, as following: nonanoic acid – from 2.57 population Lalin to 35.45% population Sobieszewska Wola, nonanal – from 1.30 population Zdory to 7.76% population Bykowce and acetophenone – from 3.05 population Sobieszewska Wola to 7.42% population Lalin. Other major compounds were higher alkanes i.e. heneicosane. tricosane and pentacosane, which percentages were up to 6.13; 20.73 and 6.05%. respectively. Terpenes fraction were represented mainly by monoterpenes: linalool, geraniol, alpha-terpineol and sesquiterpenes: nerolidol, farnesyl acetate and tetrahydrofarnesyl acetate. These results are closely related to those obtained by other authors. According to Niviskiene et al. (2007) and Prączko and Góra (2001) among main compounds present in *Tilia cordata* essential oil are: nonanal, nonanoic acid. hexahydrofarnesyl acetate as well as aliphatic hydroharbones. The composition of *Tilia* flowers essential oil could depend on the geographical location of *Tilia* trees, namely on the environmental pollution of these areas. Results obtained by Niviskiene et al. (2007) show that essential oil from flowers collected in polluted areas contain more oxygenated compounds (aliphatics monoterpenes and sesquiterpenes) than this one origin from ecologically clean habitats. Such differences in little-leaved linden flowers essential oil chemical composition (demonstrated in investigated study and by other authors) consequently may present different sensory profile and pharmacological activity of this raw material.

Table 1. Localization of little-leaved linden natural sites

No.	natural site	geographical location N	E
1.	Lalin	49 36.65	22 17.64
2.	Cerkiew	49 37.98	22 16.79
3.	Bykowce	49 32.84	22 16.25
4.	Wara	49 43.05	22 13.14
5.	Dydnia	49 42.14	22 08.63
6.	Trepcza	49 35.96	22 12.85
7.	Dynów	49 43.05	22 16.76
8.	Strachocina	49 36.43	22 05.60
9.	Witryłów	49 40.07	22 15.33
10.	Mińsk	52 16.99	21 56.98
11.	Wola Golkowska	52 04.95	20 93.18
12.	Zdory	53 42.76	21 46.18
13.	Wola Sobieszewska	50 94.66	22 69.96
14.	Siemiatycze	52 23.72	22 53.22
15.	Słochy Annopolskie	52 40.42	22 83 35
16.	Miłkowice	52 38.92	22 45.22
17.	Drohiczyn	52 23.91	22 38.07
18.	Rudka	52 42.20	22 31.55
19.	Jagodniki	52 71.23	23 52.16
20.	Dubicze	52 39.22	23 26.15
21.	Wygoda	53 08.30	22 16.55
22.	Postołowo	52 79.13	23 60.12

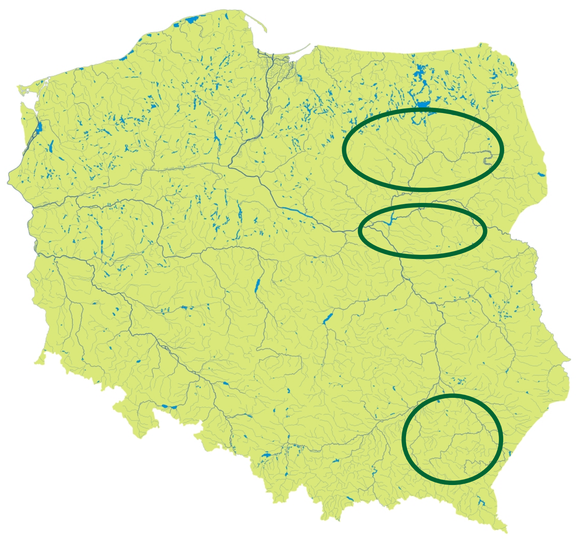

Figure 1. The distribution of investigated populations on area of Poland

Table 2. The chemical composition of essential oils from flowers of investigated little-leaved linden populations (%)

natural site/population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
compound	RI*–Retention Index. HP 20M polar column																						
hexanol	1391	-	0.22	0.69	0.33	0.18	0.43	0.18	0.44	0.18	0.83	0.25	0.52	0.26	0.36	0.49	0.58	0.30	0.49	-	0.45	0.19	0.70
nonanal	1399	**3.16**	**2.32**	**7.76**	**4.03**	**3.47**	**3.51**	**2.74**	**3.86**	**3.13**	**4.68**	**2.84**	**1.30**	**-**	**4.20**	**5.11**	**4.99**	**5.41**	**3.61**	**4.91**	**6.45**	**6.17**	**6.05**
tetradecane	1401	0.35	0.25	1.29	0.55	0.30	0.48	0.87	0.69	-	0.37	0.08	0.63	0.25	0.73	0.50	0.93	0.83	0.35	0.67	0.46	0.63	0.78
octen-3-ol	1463	-	0.94	-	0.94	0.61	0.77	0.70	0.95	0.44	0.40	-	0.42	0.47	0.46	0.54	0.78	0.67	0.44	1.52	0.93	1.72	-
octanal	1476	-	0.23	-	-	0.27	0.28	0.18	0.29	0.26	0.40	0.51	0.22	0.35	0.33	0.22	0.19	0.20	0.17	-	-	-	0.27
benzaldehyde	1516	2.62	0.71	0.85	0.98	0.90	0.93	0.82	0.95	0.71	0.91	0.78	1.59	0.45	0.88	0.47	0.65	0.51	0.49	0.45	0.65	0.38	0.71
trifluoroacetoxytridecane	1542	1.89	0.18		-	0.23	0.22	-	0.25	0.23	0.30	0.24	0.23	0.24	0.30	0.26	0.22	0.20	-	-	-	-	0.21
linalool	1544	-	0.35	0.94	0.66	0.60	0.51	0.34	0.81	0.43	1.07	0.65	0.22	0.93	0.86	1.16	0.66	0.88	0.64	0.49	0.58	0.52	0.78
terpinen-4-ol	1584	-	0.27	0.36	-	0.31	0.35	-	0.37	-	-	0.34	0.42	-	-	-	0.32	-	-	-	-	-	-
phenyloxirane	1646	7.16	0.68	0.62	-	0.90	1.02	-	-	1.42	-	-	2.38	-	-	-	0.63	-	-	-	0.80	-	5.50
acetophenone	1661	**7.42**	**4.25**	**-**	**6.46**	**4.09**	**5.05**	**4.21**	**5.68**	**5.05**	**6.11**	**3.80**	**6.13**	**3.05**	**6.90**	**4.33**	**5.42**	**4.13**	**3.50**	**5.24**	**6.45**	**4.79**	**-**
α terpineol	1681	-	0.70	0.74	1.91	0.38	-	6.06	1.08	0.35	0.56	0.77	0.46	0.20	1.57	1.66	0.80	3.11	0.52	0.82	1.55	1.43	2.29
nerol	1795	-	0.49	0.63	0.63	0.53	0.74	0.33	0.62	0.47	0.51	0.31	0.32	0.71	0.47	0.43	0.56	0.33	0.36	0.25	0.52	0.28	0.43
geraniol	1826	1.69	1.09	1.05	0.70	0.86	1.47	0.49	1.11	0.66	0.69	0.50	0.66	0.68	0.67	0.42	1.04	0.62	0.73	1.67	1.49	1.61	1.34
nerolidol	2024	1.76	1.72	0.47	4.77	2.18	3.18	0.68	3.66	5.71	1.16	0.68	2.61	1.09	0.70	3.40	4.49	6.50	6.90	0.46	0.43	1.90	0.64
heneicosane	2105	**2.21**	**6.13**	**1.43**	**2.65**	**3.58**	**3.66**	**7.06**	**3.52**	**4.27**	**2.66**	**1.18**	**2.50**	**-**	**2.24**	**2.90**	**2.18**	**2.27**	**1.10**	**4.41**	**3.49**	**5.85**	**2.67**
tetrahydrofarnesyl acetete	2124	**3.57**	**4.90**	**3.25**	**4.03**	**4.02**	**3.43**	**4.82**	**5.35**	**2.71**	**2.18**	**2.44**	**4.98**	**2.05**	**2.9**	**2.36**	**2.53**	**2.49**	**1.60**	**5.99**	**4.01**	**5.39**	**3.61**
nonanoic acid	2188	**2.57**	**6.23**	**9.70**	**7.28**	**15.55**	**11.12**	**3.87**	**11.06**	**12.96**	**18.24**	**11.98**	**4.55**	**35.45**	**13.37**	**15.43**	**15.18**	**13.36**	**11.87**	**6.77**	**10.82**	**6.91**	**16.17**
decanoic acid	2200	1.84	1.61	1.06	0.86	4.11	1.01	2.79	1.33	0.90	0.71	0.61	1.15	0.85	3.05	0.83	0.52	1.01	0.75	0.99	2.47	1.07	3.19
tricosane	2302	**5.91**	**13.95**	**7.69**	**11.61**	**12.38**	**9.54**	**20.73**	**11.78**	**9.03**	**9.41**	**5.66**	**11.31**	**5.57**	**11.05**	**7.35**	**7.95**	**7.92**	**7.52**	**13.34**	**8.35**	**13.85**	**6.44**
farnesyl acetate	2348	2.83	1.88	1.88	1.34	0.86	1.56	0.89	1.04	0.60	0.82	0.43	1.01	0.49	0.73	0.71	0.77	0.50	1.53	1.71	0.91	0.84	1.57
tetracosane	2402	-	0.92	0.73	0.98	0.87	0.67	1.25	0.82	0.58	0.88	1.28	1.03	0.47	0.93	0.58	0.49	0.68	0.61	0.97	-	1.01	0.47
pentacosane	2498	**3.08**	**3.38**	**4.32**	**4.85**	**4.34**	**3.56**	**5.90**	**4.02**	**3.11**	**6.05**	**3.84**	**3.87**	**0.14**	**4.67**	**3.19**	**3.45**	**3.62**	**3.55**	**4.80**	**-**	**5.40**	**3.01**

Photo 3. Little-leaved linden flowers

LITERATURE

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